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Chiral discrimination by high-performance liquid chromatography with joint use of two cyclodextrin additives

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Abstract

The diversity of adsorption properties of native and permethylated cyclodextrins enabled us to propose a new advantageous chromatographic system with two chiral selectors working jointly (system III). Fundamental information regarding the retention mechanism in such a system with two chiral additives is presented. Depending on the localisation of the chiral complexation process three chromatographic systems have been designed and experimentally compared under the same chromatographic conditions (solvent composition, temperature). Phenomenological and theoretical models of the above mentioned systems, where complexation occurs in the mobile phase (system I), in the stationary phase (system II) or in both phases together (system III), have been studied and experimentally confirmed. Species of biological interest were used as model compounds. It has been found that almost always system III offers the best results, although all the investigated substances display different chromatographic behaviour, i.e., adsorption on RP18 phase and complexation by native and permethylated cyclodextrin. The final enantioselectivity coefficient (α_3) may by predicted as a product of partial coefficients (α_1, α_2) according to the equation $\alpha_3=\alpha_1\cdot\alpha_2$. © 1997 Elsevier Science B.V.

Keywords: Chiral discrimination; Enantioselectivity; Chiral selectors; Cyclodextrins

1. Introduction

The most characteristic property of cyclodextrins (CDs) is their ability to form inclusion complexes with a variety of substances. CDs as natural substances, built of D-glucose units, are homochiral hosts and can be widely used for discrimination of enantiomers. There are essentially two approaches for applying CDs in liquid chromatography (LC): the first one involves the use of CDs bonded to silica gel [1] while the second one applies CDs as a mobile phase additive [2].

Unfortunately, the differentiation of many enantiomers is usually poor, i.e., the enantioselectivity (α) reaches values very close to 1.00. To enhance resolution many trials have been attempted, mainly using various modifications of CDs. It is worth noting that large numbers of CDs derivatives which are now commercially available exhibit significantly diverse physicochemical properties, including adsorption properties on chromatographic sorbents [3]. As a consequence, there are many possible appplications for CDs in chromatography as new chiral agents in both phases are now possible [4]. Moreover, the improvement of selectivity in chiral systems by the use of more complicated retention mechanisms with more than one chiral selector seems to be practically possible and promising for

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the future. In this paper we consider the role of CDs as mobile phase additives. Our investigation is focused on the system with folded retention mechanism in which two CD agents work jointly but in different ways: the first one as a chiral selector in the mobile phase, and the second one as a chiral selector dynamically generated on the stationary phase. In our previous work [5] it has been shown that simultaneous use of two chiral additives: native β-cyclodextrin (β-CD) and its derivative (2,3,6-tri-O-methyl)-β-cyclodextrin (TM-β-CD), in appropriate conditions can result in better enantioselectivity with a shorter analysis time. In this work we try to present the fundamental information regarding the retention mechanism in such a system and to answer the question: when does the improvement of selectivity and synergetic effect of chiral recognition occur?

Depending on the localisation of chiral complexation process three chromatographic systems have been designed and experimentally compared under the same chromatographic conditions (solvent, temperature): (a) system I with CD selector in the mobile phase; in system I the process responsible for chiral recognition occurs in the mobile phase; (b) system II with dynamically generated stationary phase; in system II the process responsible for chiral recognition occurs at the stationary phase; (c) system III with joint action of two CD selectors; in system III the processes responsible for chiral recognition occur in both phases together.

Phenomenological and theoretical models of the mentioned above systems have been studied and experimentally confirmed.

2. Experimental

2.1. Reagents

α-Cyclodextrin (α-CD), β-cyclodextrin (β-CD), (2,3,6-tri-O-methyl)-α-cyclodextrin (TM-α-CD) and (2,3,6-tri-O-methyl)-β-cyclodextrin (TM-β-CD) were supplied by Chinoin (Budapest, Hungary). All other reagents and solvents were of analytical reagent grade and were used as received.

The model compounds were substances of pharmaceutical interest: barbiturate, mephenytoin, camphor, fenchone, *trans*-sobrerol. Their formulae and sources are quoted in Table 1.

2.2. Apparatus and procedures

Chromatographic experiments were performed using a Waters (Vienna, Austria) Model 590 chromatograph.

The following equipment was used for analytical purposes: two columns: 250×4.6 mm I.D. packed with 10 μm LiChrosorb RP18 and 250×1 mm I.D. packed with 5 μm LiChrosorb RP18; a Rheodyne type injector and a Waters UV–Vis detector Model 490.

The mobile phases were aqueous ethanolic solutions: (i) without any CD (system 0); (ii) with single native cyclodextrin, α-CD or β-CD (system I); (iii) with their permethyl derivatives (system II), or (iv) with a mixture of native CD and its permethyl derivative (system III).

In systems II and III measurements were made after elution of an amount of mobile phase which reached constant values of retention and enantioselectivity [2]. The temperature was kept constant using cryostat Model MK 70 (MLW, Germany).

2.3. Determination of adsorption isotherms

The adsorption isotherms of β-CD and TM-β-CD were calculated by the elution by characteristic points (ECP) method [6,7]. This dynamic method of isotherm determination enables us to calculate isotherms point-by-point from the shape of a single overloaded peak.

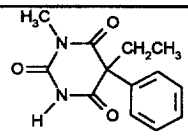
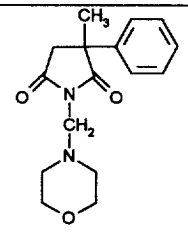
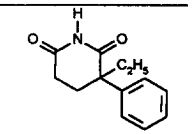
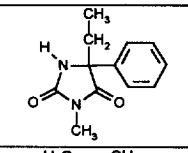
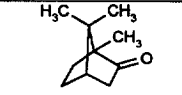
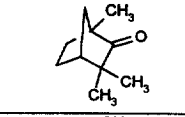
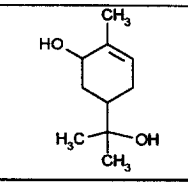
The following equipment was used for adsorption study: column: 250×4.6 mm I.D. packed with 10 μm LiChrosorb RP18, detector: refractive index detector Type 198 Knauer (Berlin), injector: Rheodyne type 7125 equipped with 20 μl loop.

The concentrations of samples in the eluent were: $4 \cdot 10^{-1}$ *M* for TM-β-CD and $5 \cdot 10^{-2}$ *M* for β-CD.

The mobile phases were aqueous ethanolic solutions: 5%, 7.5%, 10% EtOH for β-CD and 40%, 45%, 50% EtOH for TM-β-CD.

In the case of TM-β-CD additional measurements of adsorption by the static method were performed.

Table 1
Structural formulae of the investigated compounds

Compound	Formula	Origin
Methylphenobarbital (racemate)		donated by Prof. J. Bojarski (Kraków, Poland)
Morsuximide (racemate)		donated by Prof. J. Bojarski (Kraków, Poland)
Glutethimide (racemate)		donated by Prof. J. Bojarski (Kraków, Poland)
Mephenytoin (racemate)		donated by Prof. J. Bojarski (Kraków, Poland)
(+) and (-)-Camphor		Fluka (Buchs, Switzerland)
(+) and (-)-Fenchone		Fluka (Buchs, Switzerland)
trans-sobrerol (racemate)		Aldrich-Chemie (Steinheim, Germany)

The column was equilibrated with an eluent containing various concentrations of TM-β-CD in 20% EtOH (10^{-4}, $5\cdot10^{-4}$, 10^{-3} and 10^{-2} *M*). In each case the generated chiral stationary phases were eluted out by 200 ml of 80% MeOH and the solutions were evaporated. Each point of isotherm, corresponding to the fixed TM-β-CD concentration in the eluent, was determined by weight of the eluted TM-β-CD.

3. Theoretical considerations of retention mechanism

3.1. System I – with CD selector in the mobile phase

This well known and widely applied retention mechanism [2,8] describes a chromatographic system where the CD is either not at all or very weakly

adsorbed on the stationary phase, for example in the case of native CD used in a reversed-phase (RP) system with mobile phase containing higher concentrations of organic solvent. Since the capacity factor of CD is then close to zero, the adsorption of CD and its influence on the properties of the stationary phase may be neglected.

Besides the above-mentioned statement, the system I model has been derived assuming that: (a) the guest molecule is adsorbed on the stationary phase and complexed by CD in the mobile phase, (b) complexes of 1:1 stoichiometry are formed exclusively and (c) the adsorption of the complex at the stationary phase is very small and may be neglected.

In system I, the chiral recognition is realised by complexation in the mobile phase.

By introducing equations for the distribution and complexation constants for the definition of capacity factor k' we obtain the final formula describing retention in system I as follows [2,8–10]:

$$k'_1 = \varphi\frac{S}{C} = \varphi\frac{S_C + S_F}{C_C + C_F} = \varphi\frac{K_0 + K_C K_{CD}[CD]}{1 + K_{CD}[CD]} \qquad (1)$$

where subscript 1 refers to system I, φ is the phase ratio of the column, S and C are the total concentrations of solute at the stationary and in the mobile phase, respectively, S_F is the concentration of free guest at the stationary phase and C_F is the concentration of free guest in the mobile phase, S_C and C_C are the concentrations of complex in the stationary phase and mobile phase, respectively, K_0 and K_C are distribution constants of the free guest molecules and of the inclusion complex, respectively, K_{CD} is the formation constant for the inclusion complex and [CD] is concentration of free CD in the mobile phase.

When the adsorption of the complex on RP phase is negligible, a simpler formula is obtained:

$$k'_1 = \varphi\frac{K_0}{1 + K_{CD}[CD]} \qquad (2)$$

Since $k'_0 = \varphi K_0$, where k'_0 is the capacity factor with no CD present, the final equation for capacity factor in system I may be written as:

$$k'_1 = \frac{k'_0}{1 + K_{CD}[CD]} \qquad (3)$$

3.2. System II – with dynamically generated chiral stationary phase

This mechanism is dominated by strong adsorption of the CD at the stationary phase, as for example in the case of permethylated CD in an RP system with aqueous mobile phase containing low concentration of organic solvent. Since the capacity factor of CD is very high ($k' > 100$), the concentration of CD on the stationary phase is several orders of magnitude higher than CD concentration in the mobile phase. That is true only for low concentrations of CD in the mobile phase (the concentration in the linear range of isotherm). It was experimentally confirmed that as long as concentration of TM-β-CD in the system with dynamically generated stationary phase is equal to or lower than $5 \cdot 10^{-4}$ M, a change in the capacity factor of investigated compounds is not significant while going from the system containing TM-β-CD to the same system following removal of TM-β-CD from the eluent. Thus CD action in the mobile phase in such a system may be neglected. Besides the abovementioned statement, the system II model has been derived assuming that: (a) the guest molecule is adsorbed on the RP stationary phase and complexed by molecules of CD derivative immobilised on the stationary phase and (b) complexes of 1:1 stoichiometry are formed exclusively.

Chiral recognition in such a system is realised only on the dynamically generated stationary phase.

The total concentrations of solute in the mobile phase and in the stationary phase in system II are given by Eqs. (4) and (5), respectively:

$$C = C_F \qquad (4)$$

$$S = (1 - \gamma)S_F + \gamma S_C \qquad (5)$$

where γ is the degree of coverage of the stationary surface by TM-CD.

The capacity factor in system II (k'_2) may be expressed by Eq. (6)

$$k'_2 = \varphi\frac{S}{C} = \varphi\frac{(1 - \gamma)S_F + \gamma S_C}{C_F} \qquad (6)$$

where subscript 2 refers to system II.

If

$$K_0 = \frac{S_F}{C_F}$$

and complexation constant for GTMCD complex

$$K_{TCD} = \frac{S_C}{C_F[TCD]}$$

where [TCD] is the concentration of TM-CD, then Eq. (6) may be transformed into Eq. (7)

$$k_2' = (1-\gamma)k_0' + \varphi\gamma K_{TCD}[TCD] \quad (7)$$

3.3. System III– with joint action of two CD selectors

Since both described systems (I and II) can exist under the same chromatographic conditions (sorbent, solvent composition, temperature), the retention mechanism of mutual action of two CD selectors can be proposed and applied. The chiral separation may occur in both phases, with native CD dissolved in the mobile phase and being supported by chiral stationary phase, dynamically generated by permethylated CD.

System III has been derived assuming that: (a) native CD does not influence the properties of the RP stationary phase, (b) the permethylated derivative of CD is strongly adsorbed on the stationary phase, (c) the guest molecules may be adsorbed on the stationary phase, complexed by CD in the mobile phase or complexed by CD derivative immobilised on the stationary phase, (d) only 1:1 complexes of guest with native CD and TM-CD are formed and (e) the adsorption of the native CD complex at the stationary phase is negligible.

Using the description of systems I and II, the capacity factor in system III may be written as:

$$k_3' = \varphi\frac{S}{C} = \varphi\frac{(1-\gamma)S_F + \gamma S_C}{C_F + C_C} = \frac{(1-\gamma)k_0' + \varphi\gamma K_{TCD}[TCD]}{1 + K_{CD}[CD]} \quad (8)$$

where subscript 3 refers to system III.

Considering Eqs. (3) and (7), Eq. (8) can be expressed as:

$$k_3' = \frac{k_1'k_2'}{k_0'} \quad (9)$$

3.3.1.1. Optimisation of enantioselectivity

The enantioselectivity (α) in system III can be written as:

$$\alpha_3 = \frac{k_3'(2)}{k_3'(1)} \quad (10)$$

where numbers 2 and 1 in parentheses refer to enantiomers eluted from the column as the second and the first one, respectively.

The final equation for joint enantioselectivity results from combination of Eqs. (9) and (10):

$$\alpha_3 = \alpha_1\alpha_2 \quad (11)$$

where subscripts 1, 2, 3 refer to the systems I, II and III, respectively.

It is important to notice that improvement of enantioselectivity may occur only if the elution order of enantiomers in both systems with native CD and its permethylated derivative is the same (α_1 and α_2 are greater than 1). The main requirement for this is: chiral additives should interact with enantiomers showing inverse selectivity. Only then is one enantiomer bound more strongly by the chiral selector immobilised on the stationary phase whereas the second enantiomer is predominantly associated with the chiral additive in the mobile phase.

4. Results and discussion

4.1. Adsorption of β-CD and TM-β-CD

The overloaded peaks obtained for β-CD and TM-β-CD on the RP18 support using binary (ethanol–water) mobile phases with various percentages of ethanol show a tailing shape [11,12]. For both CDs the experimental data fit the Langmuir isotherm. Assuming a monomolecular adsorbed layer, the Langmuir isotherm can be expressed by Eq. (12) [13]:

$$S = \frac{C}{\frac{k_2}{k_1} + \frac{C}{Ql}} \quad (12)$$

Table 2
Parameters of adsorption isotherms of β-CD and TM-β-CD obtained for various percentages of ethanol in aqueous binary mobile phase

CD	%EtOH	k_1/k_2 k'	Ql (mM)
β-CD	5	11.90	9.33
	7.5	3.28	8.35
	10	1.39	4.06
	20	0.018[a]	
	30	0.002[a]	
TM-β-CD	20	960[a]	
	30	176[a]	
	40	32.20	43
	45	13.84	33
	50	5.86	30

[a] The values of capacity factor for 20 and 30% EtOH were calculated by linear extrapolation.

where C and S are concentrations of the substance in the mobile phase and stationary phase at the equilibrium state, k_1 and k_2 the rate constants for adsorption and desorption, respectively and Ql is the capacity of the monomolecular adsorption layer.

This formula can be used to determine important parameters for the characterisation of the isotherm in a given chromatographic system: the ratio of the rate constants of the sorption process k_1/k_2, which corresponds to the capacity factor k' under analytical conditions, and the capacities of the stationary phase Ql. The values of discussed parameters for each isotherm were determined by a two-step procedure: first, isotherm points were calculated from overloaded chromatographic peaks by the ECP method [6,7] and second, received points were fitted by the Langmuir function, Eq. (12). The best fitting parameters are reported in Table 2.

For both substances the ratio of the rate constants of the sorption processes, and consequently, capacity factor distinctly decreases with increasing concentration of ethanol in the mobile phase. Similarly, the capacity of stationary phase decreases with increasing EtOH concentration in the eluent which is logical when we interpret parameter Ql as a "effective capacity" taking into account competitive adsorption of organic solvent.

An additional isotherm of TM-β-CD determined by static measurements is presented in Fig. 1.

For concentrations greater than 10^{-3} M (in 20% EtOH) the amount of adsorbed TM-β-CD does not change. A rough estimation of the capacity factor from this isotherm give the value (967) which is compatible with the results obtained by linear extrapolation (960, see Table 2).

The linear extrapolation shows essential differences between adsorption of two discussed CDs. Adsorption of TM-β-CD is much stronger and for concentration of EtOH<30% this additive cannot be

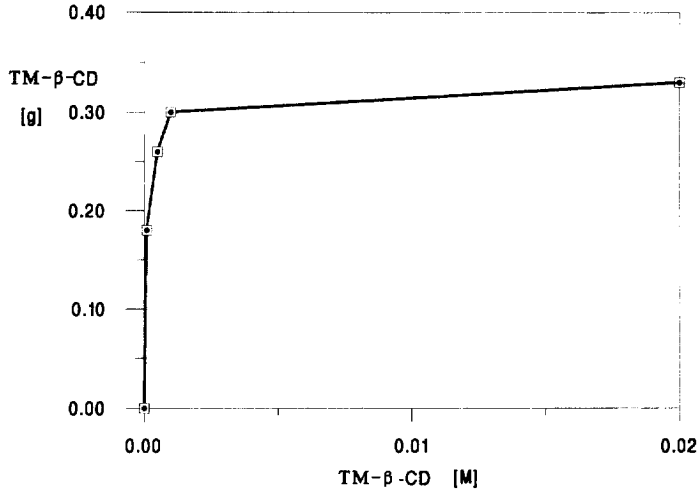


Fig. 1. Isotherm of TM-β-CD determined by the static method (quantity of TM-β-CD in grams adsorbed on the column packed with about 2.2 g of 10 μm LiChrosorb RP18 versus concentration of TM-β-CD in the eluent, aqueous 20% EtOH).

eluted ($k' > 100$). On the contrary, β-CD under these conditions is not retained at all, and the corresponding capacity factor reaches values less than 0.01.

This difference in the behaviour of the two CDs formed the basis for our investigations, making it possible to project a new retention mechanism with the joint action of two chiral selectors (system III).

4.2. Experimental verification of theoretical models

The retention and enantioselectivity of seven model chiral compounds were studied in systems 0, I, II and III. The results obtained experimentally in system III were compared with those from theoretical calculations (Eqs. (9) and (11)).

Table 3 lists capacity factors k' and enantioselectivities α of investigated compounds found in all systems; the numbers in parentheses refer to the data calculated theoretically from Eqs. (9) and (11).

4.3. Capacity factor in relation to chiral additives

As one can see in Table 3, the k' values generally decrease with addition of chiral additives to the mobile phase, this effect concerns both native CD and its permethylated derivative.

In system I decrease of k' values can be directly related to the degree of complexation. Increase of complexation is followed by a reduction in capacity factor and vice versa (Eq. (3)).

In system II both processes, adsorption and complexation, occur at the stationary phase. From the experimental data it can be seen that the complexation process retains solute on the chiral stationary phase for less time than the adsorption process on the RP system. Except for camphor with TM-β-CD, retention times of all investigated substances are shorter in system II than in the system without any CD (system 0).

An examination of retention times in systems I and II leads to the observation that sobrerol and mephenytoin (with β-CD and TM-β-CD) and fenchone (with α-CD and TM-α-CD) belong to compounds eluted in system II earliest and in system I latest. Such behaviour suggests that their ability to form complexes should be relatively weak both in the case of native CD and its permethylated derivative. However, this supposition needs further confirmation by more exact independent measurements of stability constant values.

The schematic relation between capacity factor in system III and the data found in systems I and II is presented in Fig. 2. Generally, k' values of investigated substances in system III are smaller than those observed in systems I and II.

Comparing the experimental values of capacity factor found in system III with those theoretically calculated according to Eq. (9) (Table 3) one should note a discordance of up to 10% for all the investigated substances. The origin of the discrepancy may come from various simplifications applied to the models. Although, the error analysis needs further future investigation, the magnitude of this discordance seems to be small enough to allow us to recommend this simple model for prediction of experimental results in system III.

4.4. Dependence of enantioselectivity on chiral additives

All the investigated substances may be divided into three groups A, B, C (see Fig. 3) according to their behaviour observed in system III.

4.4.1. Group A

Consisting of methylphenobarbital and morsuximide, for which chiral discrimination can be observed in both systems with native CD ($\alpha_1 > 1$) and its permethylated derivatives ($\alpha_2 > 1$). In system III improvement of enantioselectivity occurs according to Eq. (11). E.g., for methylphenobarbital the enantioselectivity is equal to 1.08 in system I, 1.16 in system II, while in system III it increases to 1.24 (Table 3). As mentioned earlier, these data indicate reverse enantioselectivity of inclusion by two CDs, i.e., one enantiomer is bound more strongly by a chiral selector immobilised on the stationary phase, while the second one is predominantly associated with a chiral selector in the mobile phase.

4.4.2. Group B

Substances for which enantioselectivity is observed only either in system I or in system II belong to this group, i.e., mephenytoin and camphor ($\alpha_1 > 1$ and $\alpha_2 = 1$) and glutethimide ($\alpha_1 = 1$ and $\alpha_2 > 1$).

Table 3
Retention data for tested compounds in studied systems (see Section 3 for description)

Compound	Conditions	EtOH–water (v/v)	-CD	System 0		System I		System II		System III	
				k'	α	k'	α	k'	α	k'	α
Methylphenobarbital	[a]	20/80	β-CD	27.4		8.6		17.4		5.4 (5.5)	
			and		1.00		1.08		1.16		1.24 (1.25)
			TM-β-CD	27.4		9.3		20.1		6.7 (6.8)	
Morsuximide	[b]	20/80	β-CD	11.7		6.2		7.9		3.8 (4.2)	
			and		1.00		1.00		1.07		1.10 (1.07)
			TM-β-CD	11.7		6.2		8.4		4.2(4.5)	
	[b]	10/90	β-CD	34.3		9.4		11.6		3.4 (3.2)	
			and		1.00		1.03		1.11		1.15 (1.14)
			TM-β-CD	34.3		9.7		12.8		3.9 (3.6)	
Glutethimide	[a]	20/80	β-CD	24.0		3.4		20.7		2.6 (2.9)	
			and		1.00		1.00		1.08		1.12 (1.08)
			TM-β-CD	24.0		3.4		22.4		2.9 (3.2)	
Mephenytoin	[b]	20/80	β-CD	22.8		17.7		10.7		7.9 (8.3)	
			and		1.00		1.07		1.00		1.06 (1.07)
			TM-β-CD	22.8		19.0		10.7		8.4 (8.9)	
	[b]	10/90	β-CD	73.5		35.0		17.0		9.1 (8.1)	
			and		1.00		1.18		1.00		1.18 (1.18)
			TM-β-CD	73.5		41.3		17.0		10.7 (9.6)	
Camphor	[a]	35/65	α-CD	15.1		9.5		12.3		7.9 (7.7)	
			and		1.00		1.16		1.00		1.18 (1.16)
			TM-α-CD	15.1		11.0		12.3		9.3 (9.0)	
	[b]	35/65	β-CD	15.7		6.8		16.3		7.4 (7.1)	
			and		1.00		1.00		1.00		1.00
			TM-β-CD	15.7		6.8		16.3		7.4 (7.1)	
Fenchone	[a]	35/65	α-CD	18.4		17.3		15.0		(14.1)	
			and		1.00		1.00		1.00		(1.00)
			TM-α-CD	18.4		17.3		15.0		(14.1)	
	[b]	35/65	β-CD	18.0		11.2		15.7		10.9 (9.8)	
			and		1.00		1.00		1.00		1.00
			TM-β-CD	18.0		11.2		15.7		10.9 (9.8)	
trans-Sobrerol	[b]	20/80	β-CD	13.4		11.7		4.1		3.4 (3.6)	
			and		1.00		1.00		1.00		1.00
			TM-β-CD	13.4		11.7		4.1		3.4 (3.6)	

Chromatographic conditions: columns: [a] 250×1 mm packed with 5 μm LiChrosorb RP18, [b] 250×4.6 mm packed with 10 μm LiChrosorb RP18. Flow-rate: [a] 0.04 ml/min, [b] 0.95 ml/min. Detector: UV–Vis at 254 nm except for camphor, fenchone 280 nm, and for *trans*-sobrerol 210 nm.

Eluents: system 0 aqueous ethanolic solution at appropriate concentration given in table (EtOH–water). System I [a] $2 \cdot 10^{-2}$ *M* of α- or β-CD, respectively, in the same eluent as in system 0, [b] 10^{-2} *M* of β-CD in the same eluent as in system 0. system II [a] and [b] $5 \cdot 10^{-4}$ *M* of TM-α- or TM-β-CD, respectively, eluent as in system 0. System III [a] $2 \cdot 10^{-2}$ *M* of α- or β-CD/$5 \cdot 10^{-4}$ *M* TM-α- or TM-β-CD, [b] 10^{-2} *M* of β-CD/$5 \cdot 10^{-4}$ *M* TM-β-CD eluent as in system 0.

Temperature was 25°C except for glutethimide and fenchone with α-CD/TM-α-CD, where it was 20°C.

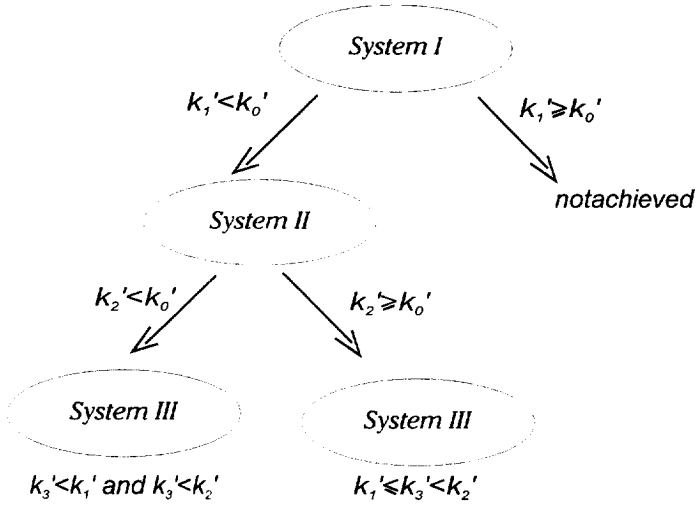


Fig. 2. Schematic relation between capacity factor in system III and the results obtained in systems I and II.

According to Eq. (11), in this case, in system III the enantioselectivity should be the same value as in the single system enabling chiral discrimination. As can be seen for group B in system III it is possible to obtain the same enatioselectivity as in system I or system II but with a shorter analysis time. This phenomenon is also advantageous for enatioselectivity. Shorter analysis time allows us to decrease the concentration of organic solvent (ethanol) which usually restricts separation. Organic solvent competes with the solute in occupying the CD cavity [14]. Therefore, lower concentrations of ethanol results in better enantioselectivity (see data for mephenytoin in Table 3).

4.4.3. Group C

Substances for which enantioselectivity is not observed in both systems, i.e., neither with native Cd nor with its permethylated derivative constitute this

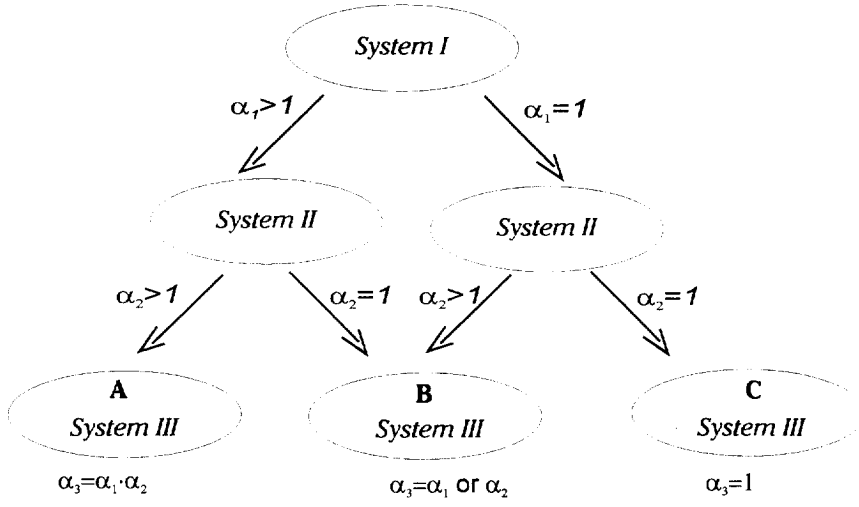
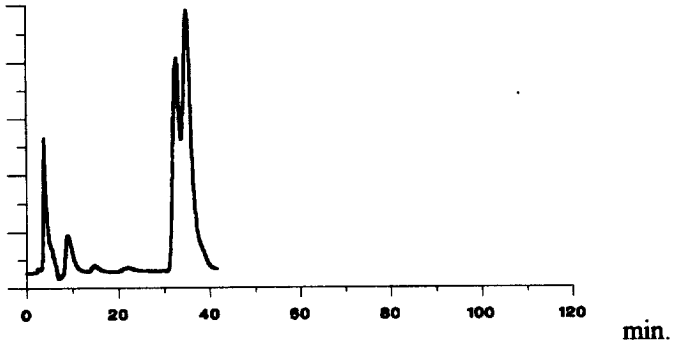
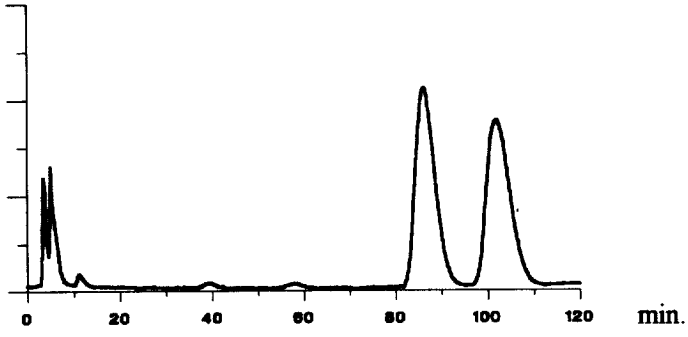


Fig. 3. Schematic relation between enantioselectivity in system III and the results determined in systems I and II.

System I



System II



System III

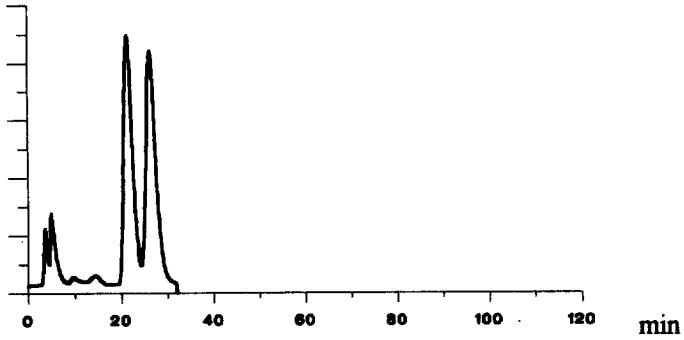


Fig. 4. Chromatograms of methylphenobarbital obtained in systems I, II and III. Chromatographic conditions: column (250×1 mm) packed with 5 μm LiChrosorb RP18; flow-rate, 0.04 ml/min; detector, UV–Vis 254 nm; eluent: 20 vol.% EtOH in water with $2 \cdot 10^{-2}$ β-CD for system I, with $5 \cdot 10^{-4}$ TM-β-CD for system II, with $2 \cdot 10^{-2}$ β-CD and $5 \cdot 10^{-4}$ TM-β-CD for system III, temperature 15°C.

group. For group C (*trans*-sobrerol, fenchone), there is no enantioselectivity in system III either.

5. Conclusions

The following conclusions should be pointed out:

The diverse adsorption properties of native and permethylated CD enable us to propose a new, advantageous chromatographic system with two chiral selectors working jointly but in different ways: the native CD as chiral selector in the mobile phase, and permethylated CD as a chiral selector of the dynamically generated stationary phase.

Although all the investigated substances display different physicochemical properties, i.e., adsorption on RP18 phase, complexation by native and permethylated CD, system III can offer the best results under convenient conditions: (i) for group A (methylphenobarbital, morsuximide) in system III a decrease of retention time is followed by increase of enantioselectivity, these phenomena are demonstrated by chromatograms in Fig. 4. (It should be pointed out that at present the diverse enantioselectivity of chiral selectors can not be predicted a priori.); (ii) and for group B (glutethimide, camphor, mephenytoin) the same enantioselectivity is obtained with a shorter analysis time.

Decrease of retention time in the system with two chiral additives (system III) makes it possible to reduce the concentration of ethanol and hence obtain better enantioselectivity. E.g., for mephenytoin, varying the concentration of ethanol from 20 to 10% v/v EtOH–water raises the enantioselectivity from 1.06 to 1.18, while the change of retention time is rather small (7.9 to 9.1 and 8.4 to 10.7, respectively); see Table 3.

The existing discrepancy between experimental values of k' and α and those calculated from Eqs. (9) and (11) seems to be sufficiently small to use the described model for prediction of experimental results in practice.

In the theoretical model, the influence of mutual interactions between two CDs such as formation of mixed complexes or competition between CDs in mass transport between phases has not been taken into account. Nevertheless, the experimental data indicate that their influence on chromatographic results should be relatively small (less then 10%).

To investigate and exploit the observed phenomena in practice a single RP column may be used several times with many varieties of the mobile phase containing various chiral additives.

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