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Joint use of cyclodextrin additives in chiral discrimination by reversed-phase high-performance liquid chromatography: temperature effects

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Abstract

The temperature dependence of chiral separations was investigated in combined system of reversed-phase (RP) liquid chromatography using two chiral additives: single α or β native cyclodextrins and their permethylated derivatives. The model tested compounds of pharmaceutical interest were: methylphenobarbital, mephenytoin, morsuximide and camphor. Taking the localization of a complexation process as a criterion – the combined system with two selectors has been rationalized as occurring in three stages. The influence of temperature (in narrow range of 20°C) on retention and enantioselectivity was studied in; System I (complexation occurs in the mobile phase), in System II (complexation on the stationary phase) and in System III (complexation in both phases together). In System III (as for System I) it has been found that the model compounds could be classified into three groups based on their retention dependence on temperature: retention decrease with temperature decrease, retention increase with temperature decrease or no influence of temperature on retention. For all the compounds investigated, decrease in temperature increases the selectivity. Standard enthalpy (ΔH^0) and entropy (ΔS^0) changes of solute transfer between the mobile and the stationary phase and standard enthalpy (ΔH^0_{CD}) and entropy (ΔS^0_{CD}) changes of complex formation were also calculated. In Systems I and III, if the complexation in the mobile phase is favored process compared with interaction with the stationary phases (RP or covered by permethylated cyclodextrin), the shortest retention time and the best selectivity is observed at low temperature.

Keywords: Enantiomer separations; Temperature effects; Cyclodextrins; Methylphenobarbital; Mephenytoin; Morsuximide; Camphor

1. Introduction

Although chiral discrimination has been one of the main topics of chromatographic practice for over fifteen years, the baseline separation of enantiomers still presents many difficulties and numerous analytical and preparative problems remain to be solved. Thus, new chromatographic systems demonstrating better performance, i.e., better stereoselectivity and/or better efficiency are still being searched for with great interest and various ideas have been attempted. Among such, combined chromatographic systems have been recently proposed [1–5]. Generally, the combination relies on jointly using two appropriate chiral selectors; one in stationary- and the second in mobile-phase solution. It is worth noting that sys-

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tems with two chiral additives were also recently studied in capillary electrophoresis [6–8].

We have designed a modified procedure in this area which exploits the diversity of inclusion and adsorption properties of native and permethylated cyclodextrins. This allows us to propose a new, advantageous, kind of chromatographic procedure in which two additives work simultaneously.

Fundamental information regarding the retention mechanism and a summary of enantioselectivity coefficients in such a system have been presented [9]. We have found under fixed conditions of temperature and solvent, enantioselectivity in such a system may be synergically affected only when one enantiomer binds to the first additive adsorbed on a reversed-phase (RP) stationary phase, whereas the other enantiomer predominantly associates with the second chiral additive dealing inside the mobile phase solution. In sum, two chiral additives should work in a RP high-performance liquid chromatography (HPLC) column by different mechanisms and with inverse direction towards enantiomers. It is worth noting that to explore and exploit these phenomena in practice only a single RP column with many varieties of the mobile phase solution containing two chiral additives is required. In the present paper we attempt to answer the question: how is this combined system influenced by temperature? To the best of our knowledge, the influence of temperature on a combined system has not yet been investigated.

The main goal of our study was to obtain insight into the mechanisms of resolution and complexation of the above mentioned combined processes by thermodynamic means.

In the classical reversed-phase systems of chromatography, plots of the logarithms of capacity factors against the reciprocal of absolute temperature are linear and are known as Van 't Hoff plots. This fundamental relation arises because of the assumption that the retention mechanism is the same over the temperature range being investigated and all parameters of the Van 't Hoff plots are independent of temperature.

However, any reversible process which alters the entropy and enthalpy of sorption leads to a nonlinearity in the Van 't Hoff relation, due for example to complexation, change of conformation or multiple retention mechanisms. The course of such nonlinearity may then be very instructive in providing knowledge of the nature of the altering process itself.

Although many papers deal with the use of cyclodextrins (CDs) in various kinds of chromatography, few report studies of temperature variation and only rarely has chiral discrimination been a concern [10–14].

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-grade and were used as received.

The model tested compounds were substances of pharmaceutical interest: methylphenobarbital, morsuximide, mephenytoin and camphor.

2.2. Apparatus and procedures

Chromatographic experiments were performed using a Waters (Vienna, Austria) Model 590 pump, a Rheodyne type injector and a Waters Model 490 UV–Vis detector.

Aqueous ethanolic solutions without any cyclodextrin (System 0 and System IIA) with single native cyclodextrin, α-CD or β-CD (System I) or their permethyl derivatives (System IIB) or with a mixture of native CD and its permethyl derivative (System III) were the mobile phase. In Systems IIB and III measurements were made not earlier than after elution a certain amount of mobile phase solution (i.e., after generation of the chiral stationary phase) to reach constant values of retention and enantioselectivity [15]. The temperature was kept constant using a Model MK 70 (MLW, Germany) cryostat.

The column used in Systems 0, I, IIB and III for methylphenobarbital, mephenytoin and camphor was of 250×1 mm I.D. packed with 5 μm LiChrosorb RP18 and for morsuximide a 250×4 mm I.D. column packed with 10 μm LiChrosorb RP18. A Cyclobond I 2000 Astec (Whippany, USA) column was used for System IIA. A 250×4 mm I.D. column

packed with 10 μm LiChrosorb RP18 and a Chiralyser Knauer (Berlin) polarimetric detector was used in the adsorption study of TM-β-CD. The column was first equilibrated with an eluent containing $5 \cdot 10^{-4}$ *M* of TM-β-CD in 20% EtOH at two different temperatures: 15 and 35°C. Additionally, the generation of chiral stationary phase was controlled utilizing a polarimetric detector. In each case, the chiral stationary phases generated were eluted out by 200 ml of 80% MeOH and the solutions were evaporated. The quantity of adsorbed cyclodextrin was determined by weight. For both temperatures the mass of adsorbed TM-β-CD was 0.20 g.

The experimentally determined dead volume of this column was 2.4 ml, i.e., the concentration of TM-β-CD-[TCD] was $6 \cdot 10^{-2}$ *M*/l, and ln [TCD]= −2.8.

3. Theoretical considerations

In our earlier paper [9] we rationalized the combined system having two chiral additives as occurring in three stages, taking the localization of a complexation process as the criterion (in the mobile phase System I, on the stationary phase System II and in both phases together System III). The same reasoning has been applied here.

3.1. System 0

In System 0, i.e., that without chiral selector, the capacity factor of the solute G is dependent on the partitioning process between mobile and stationary phase alone.

Mobile phase: G^m
$\uparrow\downarrow$
Stationary phase: G^s

The Van 't Hoff expression for such a chromatographic system is [16]:

$$\ln k_0' = \frac{-\Delta H^0}{RT} + \frac{\Delta S^0}{R} + \ln \varphi \qquad (1)$$

where: k' is the capacity factor, ΔH^0 and ΔS^0 represent standard enthalpy and entropy changes of transfer of the solute between the mobile and the stationary phase, R is the gas constant, T the absolute temperature and φ is the volume phase ratio of the stationary to the mobile phase.

In all text the letters m and s refer to the mobile and stationary phase, respectively.

3.2. System I – CD selector in the mobile phase

In System I, the adsorption of CD and its influence on the stationary phase is neglected [17]. The equilibrium is then as follows:

Mobile phase: $G^m + CD^m \leftrightarrow GCD^m$
$\uparrow\downarrow$
Stationary phase: G^s

In this system the capacity factor is dependent not only on partitioning process but also on the complex formation constant K_{CDm}. The solute capacity factor in a system with native CD in the mobile phase can be defined by Eq. 2 [4,9,18–20]

$$k_1' = \frac{k_0'}{1 + K_{CDm}[CD]} \qquad (2)$$

where subscripts 1 and 0 refer to Systems I and 0, respectively and [CD] is the concentration of free cyclodextrin in the mobile phase.

Taking the natural logarithm of both sides of this equation, Eq. 2 may be transformed into Eq. 3

$$\ln k_1' = \ln k_0' - \ln K_{CDm} - \ln\left(\frac{1}{K_{CDm}} + [CD]\right) \qquad (3)$$

leading to a final Eq. 4 [13]

$$\ln k_1' = \frac{-(\Delta H^0 - \Delta H^0_{CDm})}{RT} + \frac{\Delta S^0 - \Delta S^0_{CDm}}{R} + \ln \varphi - \ln\left(\frac{1}{K_{CDm}} + [CD]\right) \qquad (4)$$

where:

$$\ln K_{CDm} = -\Delta H^0_{CDm}/RT + \Delta S^0_{CDm}/R \qquad (5)$$

and ΔH^0_{CDm} and ΔS^0_{CDm} are standard enthalpy and entropy changes of complex formation in the mobile phase, respectively.

According to the definition, the enantioselectivity (α) can be written as:

$$\alpha = \frac{k'(2)}{k'(1)} \tag{6}$$

where (2) and (1) refer to enantiomers eluted from the column as the second and the first one, respectively.

Again taking the logarithm of both sides of Eq. 6 and introducing Eq. 5 for both enantiomers, leads to a final formula for enantioselectivity in System I, which can be written as:

$$\ln \alpha_1 = \frac{-\left(\Delta H^0_{\mathrm{CD1m}} - \Delta H^0_{\mathrm{CD2m}}\right)}{RT} + \frac{\Delta S^0_{\mathrm{CD1m}} - \Delta S^0_{\mathrm{CD2m}}}{R} + \ln \frac{1/K_{\mathrm{CD1m}} + [\mathrm{CD}]}{1/K_{\mathrm{CD2m}} + [\mathrm{CD}]} \tag{7}$$

where subscript 1 refers to System I.

This formula underlines that the enantioselectivity is dependent on the complexation process alone.

3.3. System II – with CD on the stationary phase (dynamically generated by permethyl derivatives of β-CD)

If the equilibrium concentration of CD on the stationary phase is a few orders of magnitude higher than CD concentration in the mobile phase (for the 250×4 mm column the amount of cyclodextrin immobilized on the stationary phase is 0.20 g and the amount of cyclodextrin in the mobile phase present in the column is less than 0.0002 g) the complexation in mobile phase may be neglected [9,17] and equilibrium can be demonstrated as:

Mobile phase: G^m

$\uparrow\downarrow \quad +$

Stationary phase: $G^s \quad TCD^s \leftrightarrow GTCD^s$

In System II the capacity factor is dependent on the partitioning process between mobile and uncovered stationary phase and complexation with CD immobilized on the stationary phase, i.e., [9]

$$k'_2 = (1-\gamma)k'_0 + \gamma \cdot k'_{\mathrm{TCDs}} \tag{8}$$

where γ is the degree of coverage of the stationary surface by CD and, k'_{TCDs} is responsible for retention due complexation processes on the stationary phase.

Finally, the capacity factor in System IIB is given by Eq. 9

$$k'_2 = (1-\gamma)k'_0 + \gamma\varphi K_{\mathrm{TCDs}}[\mathrm{TCD}] \tag{9}$$

where K_{TCDs} complexation constant for the G–TCD complex and [TCD] is the concentration of CD on the stationary phase.

If CD completely covers the stationary phase, then $\gamma = 1$, and a simpler formula

$$k'_2 = \varphi K_{\mathrm{TCDs}}[\mathrm{TCD}] \tag{10}$$

is obtained or in logarithmic form:

$$\ln k'_2 = \ln \varphi + \ln K_{\mathrm{TCDs}} + \ln [\mathrm{TCD}] \tag{11}$$

Combining Eqs. 11 and 12:

$$\ln K_{\mathrm{TCDs}} = -\Delta H^0_{\mathrm{TCDs}}/RT + \Delta S^0_{\mathrm{TCDs}}/R \tag{12}$$

where $\Delta H^0_{\mathrm{TCDs}}$ and $\Delta S^0_{\mathrm{TCDs}}$ are standard enthalpy and entropy changes of complex formation in the stationary phase, leads to Eq. 13:

$$\ln k'_2 = \frac{\Delta H^0_{\mathrm{TCDs}}}{RT} + \frac{\Delta S^0_{\mathrm{TCDs}}}{R} + \ln \varphi + \ln [\mathrm{TCD}] \tag{13}$$

Finally, substituting Eq. 13 in the definition of enantioselectivity (Eq. 6), provides the equation

$$\ln \alpha_2 = \frac{-\left(\Delta H^0_{\mathrm{TCD2s}} - \Delta H^0_{\mathrm{TCD1s}}\right)}{RT} + \frac{\Delta S^0_{\mathrm{TCD2s}} - \Delta S^0_{\mathrm{TCD1s}}}{R} \tag{14}$$

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

Combining Systems I and II, gives rise to System III, in which: (i) native CD does not influence the properties of the RP stationary phase, (ii) the permethylated derivative of CD is strongly adsorbed on the stationary phase, (iii) the guest molecules may be complexed by CD in the mobile phase or complexed by CD derivative immobilized on the stationary phase, (iv) only 1:1 complexes of guest with native CD and TM-CD are formed, (v) the adsorption of the native CD complex on the stationary phase is negligible and (vi) TM-CD completely covers the stationary phase.

Now:

Mobile phase: $G^m + CD^m \leftrightarrow GCD^m$

$+$

Stationary phase: $TCD^s \leftrightarrow GTCD^s$

The capacity factor in System III may then be written as [9]

$$k_3' = \frac{k_1' k_2'}{k_0'} \quad (15)$$

and

$$\ln k_3' = \ln k_1' + \ln k_2' - \ln k_0' \quad (16)$$

By analogy with the systems above:

$$\ln k_3' = \frac{-(\Delta H^0_{TCDs} - \Delta H^0_{CDm})}{RT} + \frac{\Delta S^0_{TCDs} - \Delta S^0_{CDm}}{R} + \ln \varphi - \ln (1/K_{CDm} + [CD]) + \ln [TCD] \quad (17)$$

and Eq. 18

$$\ln \alpha_3 = \frac{-(\Delta H^0_{CD1m} - \Delta H^0_{CD2m}) - (\Delta H^0_{TCD2s} - \Delta H^0_{TCD1s})}{RT} + \frac{(\Delta S^0_{CD1m} - \Delta S^0_{CD2m}) + (\Delta S^0_{TCD2s} - \Delta S^0_{TCD1s})}{R} + \ln \frac{1/K_{CD1m} + [CD]}{1/K_{CD2m} + [CD]} \quad (18)$$

The equation for joint enantioselectivity in System III may be written as [9]

$$\alpha_3 = \alpha_1 \alpha_2 \quad (19)$$

where subscripts 1, 2 and 3 refer to the Systems I, IIB and III, in that order.

Now:

$$\Delta\Delta G = -RT \ln \alpha \quad (20)$$

hence

$$\Delta\Delta G_3 = \Delta\Delta G_1 + \Delta\Delta G_2 \quad (21)$$

4. Results and discussion

The retention and enantioselectivity of four model compounds were studied on the same column with different eluent (System 0, System I, System IIB and System III) in the temperature range between; from 10 to 30°C and 15 to 35°C (or 50°C). The range of temperature was corresponded to the system under study. Temperatures lower than 10°C were not studied because of pressure problems on the column. At temperatures higher than 35°C no resolution of enantiomers was achieved, except for methylphenobarbital, where up to 50°C the resolution was still distinguishable.

For comparison, for mephenytoin, methylphenobarbital and morsuximide the influence of temperature was studied on a commercially available column with covalently bonded β-CD – System IIA.

The results obtained are listed in Tables 1–4.

The thermodynamic parameters were calculated from examination of Van 't Hoff expression.

To simplify, it was assumed that ln φ and ln $(1/K_{CDm} + [CD])$ do not change in the temperature range used (20°C) and the slope of a ln k' against $1/T$ plot provides a value for the difference in enthalpy for each system. These assumptions are supported by the linear plots of ln k' versus $1/T$ in this range of temperature. All thermodynamic parameters obtained from Van 't Hoff plots are collected in Tables 5 and 6.

4.1. Capacity factor in relation to temperature

In System 0, the retention times for all the compounds investigated decrease with increase in column temperature and Van 't Hoff plots show linear behavior in all ranges of temperature studied.

In System I, as one can see from Eq. 4, the capacity factor is dependent on two processes which take place in two different phases. Three situations now arise: (1) if the enthalpy of complexation in the mobile phase is *less negative* than the enthalpy of interaction with the RP phase, the ''normal'' Van 't Hoff plot is observed (increasing temperature–decreasing retention e.g., as in mephenytoin), (2) if the enthalpy of complexation in the mobile phase is *equal* to the enthalpy of interaction with the RP phase, no temperature dependence should be observed (morsuximide) and (3) if the enthalpy of complexation in the mobile phase is *more negative*

Table 1
Chromatographic data for methylphenobarbital in studied systems (description in text)[a]

Temperature (°C)	System 0	System I		System IIA*		System IIB		System III	
	k_1'	k_1' k_2'	α	k_1' k_2'	α	k_1' k_2'	α	k_1' k_2'	α
		7.63				21.72		4.64	
15.5			1.080				1.191		1.282
		8.24				25.86		5.95	
		8.42		7.00					
20	31.5		1.078		1.114				
		9.08		7.80					
		8.58		5.10				5.36	
25	27.4		1.079		1.108				1.259
		9.26		5.65				6.75	
		8.87		3.77		14.01		5.57	
30	23.7		1.074		1.106		1.157		1.233
		9.53		4.17		16.21		6.87	
		8.95		2.87		12.23		5.67	
35	20.6		1.065		1.108		1.143		1.220
		9.53		3.18		13.98		6.92	
		9.00		2.27		10.89		5.72	
40			1.059		1.093		1.131		1.198
		9.53		2.48		12.32		6.85	
		8.53							
50			1.041						
		8.88							

[a] Chromatographic conditions: columns: *System IIA Cyclobond I 2000, flow-rate 0.9 ml/min. Systems 0, I, IIB and III (250×1 mm) packed with 5 μm LiChrosorb RP18, flow-rate 0.04 ml/min, detection UV–Vis 254 nm. Eluent: 20% (v/v) EtOH in water for System 0 and System IIA, 20% (v/v) EtOH in water with $2\cdot10^{-2}$ *M* β-CD for System I, 20% (v/v) EtOH in water with $5\cdot10^{-4}$ *M* TM-β-CD for System IIB, 20% (v/v) EtOH in water with $2\cdot10^{-2}$ *M* β-CD and $5\cdot10^{-4}$ *M* TM-β-CD for System III.

than the enthalpy of interaction with the RP phase, the Van 't Hoff plots should have negative slopes (increasing temperature–increasing retention time e.g., methylphenylbarbital, camphor).

As for System 0, in Systems IIA and IIB an increase in temperature is always followed by a decrease in retention time for all the compounds investigated. Comparing the enthalpy values for System 0 and System IIB (the experiments in System 0 and System IIB were performed on the same column) a more negative value for systems with no chiral additives is obtained for all the compounds investigated. The more negative enthalpy indicates that transformation to the stationary phase is more effective. The capacity factors in these two systems also indicates that all solute remains on the RP phase longer (System 0) than on the CDs immobilized on the stationary phase (System IIB). The change in entropy in these systems can be considered to be a change in ordering of the solute between mobile and stationary phase. Further, negative values of entropy in these systems indicate a better ordering of these compounds on the stationary phase, due to interaction with RP phase and complexation by permethyl CD derivatives relative to the mobile phase. For RP phase, more negative values of entropy for all the compounds investigated were obtained than for dynamically generated chiral stationary phase.

In System III (as for System I) the capacity factor is dependent on two processes, but this time there is both complexation in the mobile phase and complexation on the stationary phase (see Eq. 17). (It has

Table 2
Chromatographic data for mephenytoin in studied systems (description in text)[a]

Temperature (°C)	System 0	System I		System IIA*		System IIB		System III	
	k_1'	k_1' k_2'	α	k_1' k_2'	α	k_1' k_2'	α	k_1' k_2'	α
		11.79				15.00		6.04	
10			1.198				1.00		1.207
		14.13				15.00		7.29	
		11.14		2.15		13.30		5.75	
15	24.44		1.165		1.34		1.00		1.162
		12.98		2.89		13.30		6.68	
		10.30		1.78				5.51	
20	20.61		1.128		1.29				1.129
		11.62		2.30				6.22	
		9.77		1.48		10.78		5.22	
25	17.97		1.088		1.26		1.00		1.115
		10.63		1.87		10.78		5.82	
		8.96		1.29		8.15			
30	15.18		1.081		1.22		1.00		
		9.69		1.57		8.15			
				1.14					
35					1.18				
				1.35					

[a] Chromatographic conditions: columns: *System IIA Cyclobond I 2000, flow-rate 0.85 ml/min, Systems 0, I, IIB and III (250×1 mm) packed with 5 μm LiChrosorb RP18, flow-rate 0.04 ml/min, detection UV–Vis 254 nm. Euent: 20% (v/v) EtOH in water for System 0 and System IIA, 20% (v/v) EtOH in water with $2\cdot10^{-2}$ *M* β-CD for System I, 20% (v/v) EtOH in water with $5\cdot10^{-4}$ *M* TM-β-CD for System IIB, 20% (v/v) EtOH in water with $2\cdot10^{-2}$ *M* β-CD and $5\cdot10^{-4}$ *M* TM-β-CD for System III.

been confirmed experimentally that the concentration of permethyl derivative does not change at 15 and 35°C).

Depending on which process is more favorable, three situations are observed. (1) For mephenytoin, an increase in temperature is followed by decreases in capacity factor, (2) for methylphenobarbital and camphor the capacity factor decreases with decrease in temperature and (3) for morsuximide the capacity factor is hardly influenced by temperature

The estimation of enthalpy of complexation in the mobile phase, ΔH^0_{CDm}, from results in Systems I and III are listed in Table 5.

The enthalpy of complexation in the mobile phase (as compared with enthalpy of interaction with the RP phase and complexation on the stationary phase) is the most negative for methylphenobarbital and camphor. For these two compounds the shortest retention time for Systems I and III is observed at low temperature. This means that complexation in the mobile phase is an enthalpy-favored process compared with both interaction with the RP phase and complexation with permethyl derivatives immobilized on the stationary phase.

For mephenytoin, the enthalpy of complexation in the mobile phase (System I) is less negative than the enthalpies of interaction with stationary phases (Systems 0 and IIB). This means that processes on the stationary phases are more favorable than complexation in the mobile phase.

Conversely, almost equal enthalpies are obtained for morsuximide, the capacity factor of which is hardly changed by change in temperature.

The non-linear $\ln k'$ vs. $1/T$ plots for methylphenobarbital, morsuximide and camphor at temperature above 35°C suggest that the retention mechanism undergoes a change above this temperature.

Table 3
Chromatographic data for morsuximide in studied systems (description in text)[a]

Temperature (°C)	System 0	System I		System IIA*		System IIB		System III	
	k_1'	k_1' k_2'	α	k_1' k_2'	α	k_1' k_2'	α	k_1' k_2'	α
		6.2				9.6		3.8	
15	15.6		1.00				1.094		1.132
		6.2				10.5		4.3	
				6.35					
20					1.106				
				7.02					
		6.2		4.79		7.9		3.8	
25	11.7		1.00		1.092		1.063		1.105
		6.2		5.23		8.4		4.2	
				3.57					
30					1.088				
				3.88					
		5.7		2.90		6.3		3.8	
35	9.1		1.00		1.079		1.016		1.053
		5.7		3.13		6.4		4.0	

[a] Chromatographic conditions: columns: *System IIA Cyclobond I 2000, flow-rate 0.9 ml/min, Systems 0, I, IIB and III (250×4 mm) packed with 10 μm LiChrosorb RP18, flow-rate 0.9 ml/min, detection UV–Vis 254 nm. Eluent: 20% (v/v) EtOH in water for System 0 and System IIA, 20% (v/v) EtOH in water with 10^{-2} *M* β-CD for System I, 20% (v/v) EtOH in water with $5 \cdot 10^{-4}$ *M* TM-β-CD for System IIB, 20% (v/v) EtOH in water with 10^{-2} *M* β-CD and $5 \cdot 10^{-4}$ *M* TM-β-CD for System III.

Table 4
Chromatographic data for camphor in studied systems (description in text)[a]

Temperature (°C)	System 0	System I		System IIA		System IIB		System III	
	k_1'	k_1' k_2'	α	k_1' k_2'	α	k_1' k_2'	α	k_1' k_2'	α
		7.9				14.4		6.2	
15	18.8		1.316				1.00		1.355
		10.4				14.4		8.4	
		8.9				13.4		7.2	
20	17.0		1.225				1.00		1.250
		10.9				13.4		9.0	
		9.5				12.3		7.9	
25	15.1		1.158				1.00		1.177
		11.0				12.3		9.3	
		9.7				10.8		8.6	
30	13.4		1.103				1.00		1.093
		10.7				10.8		9.4	

[a] Chromatographic conditions: column (250×1 mm) packed with 5 μm LiChrosorb RP18, flow-rate 0.04 ml/min, detection UV–Vis 280 nm. Eluent: 35% (v/v) EtOH in water for System 0, 35% (v/v) EtOH in water with $2 \cdot 10^{-2}$ *M* α-CD for System I, 35% (v/v) EtOH in water with $5 \cdot 10^{-4}$ *M* TM-α-CD for System IIB, 35% (v/v) EtOH in water with $2 \cdot 10^{-2}$ *M* α-CD and $5 \cdot 10^{-4}$ *M* TM-α-CD for System III.

Table 5
Thermodynamic parameters for investigated compounds (description in text)[a]

Compound	$-(\Delta H^0/R)$		$(\Delta S^0/R)+\ln\varphi$	$-(\Delta H^0_{TCDs})/R$		$(\Delta S^0_{TCDs})/R+\ln\varphi$	$-(\Delta H^0_{CDm})/R$		$(\Delta S^0_{CDm})/R+\ln(1/K_{CDm}+[CD])$	
	(1)		(1)	(1)		(1)	System I	System III	System I	System III
		r^2			r^2		(1)	(1)	(1)	(1)
	(2)		(2)	(2)		(2)	(2)	(2)	(2)	(2)
	2781±189		−6.46±0.63	2445±331		−3.11±1.13	1616±248	1635±262	−4.82±0.83	−4.85±1.24
Mephenytoin		0.999			0.975					
	2781±189		−6.46±0.63	2445±331		−3.11±1.13	1145±227	1185±275	−3.33±0.76	−3.44±1.28
	2832±120		−6.18±0.40	2566±54		−3.02±0.18	3504±287	3507±219	−10.57±0.96	−10.63±0.73
Methylphenobarbital		0.989			0.999					
	2832±120		−6.18±0.40	2754±48		−3.50±0.16	3446±299	3464±232	−10.45±1.0	−10.56±0.77
					0.999					
	2391±47		−5.56±0.16	1865±116		−1.41±0.39	2022±269	1865±116	−6.12±0.91	−5.57±0.39
Morsuximide		0.999			0.996					
	2391±47		−5.56±0.16	2192±167		−2.45±0.56	2022±269	1873±238	−6.12±0.91	−5.60±0.80
					0.994					
	1978±76		−3.93±0.25	1652±181		−0.25±0.62	3172±335	3531±350	−10.17±1.12	−11.41±1.19
Camphor		0.997			0.976					
	1978±76		−3.93±0.25	1652±181		−0.25±0.62	2147±280	2274±335	−6.87±0.94	−7.45±1.15

[a] The numbers (1) and (2) refer to enantiomers eluted as the first and second, respectively. Systems I and III refer to the system from which results were calculated.

Table 6
Difference in free energy ($-\Delta\Delta G/R$) obtained for investigated enantiomers by complexation with cyclodextrins in different systems[a]

Compound	System I			System IIA*			System IIB			System III		
	$-\Delta\Delta H/R$; $T\Delta\Delta S/R$	$-\Delta\Delta G/R$	$-\Delta\Delta G/R=T\ln\alpha$	$-\Delta\Delta H/R$; $T\Delta\Delta S/R$	$-\Delta\Delta G/R$	$-\Delta\Delta G/R=T\ln\alpha$	$-\Delta\Delta H/R$; $T\Delta\Delta S/R$	$-\Delta\Delta G/R$	$-\Delta\Delta G/R=T\ln\alpha$	$-\Delta\Delta H/R$; $T\Delta\Delta S/R$	$-\Delta\Delta G/R$	$-\Delta\Delta G/R=T\ln\alpha$
	471			556						451		
Mephenytoin		42	44		84	85					44	44
	−429			−472						−407		
	58			72			187			231		
Methylphenobarbital		23	22		32	33		52	50		73	72
	−35			−40			−135			−158		
				139			327			319		
Morsuximide					32	29		27	26		37	36
				−107			−300			−282		
	1025									1229		
Camphor		77	79								86	87
	−948									−1143		

[a] $T=288$ K. Chromatographic conditions as in Tables 1–4.

4.2. Enantioselectivity in relation to temperature

Although the retention can increase or decrease with rise in temperature, the increase in temperature for all the compounds investigated in all the systems studied led to deterioration in enantioselectivity. As seen from Eqs. 7, 14 and 18 the enantioselectivity is dependent only on the discrimination process, i.e., here complexation with CDs or its derivatives.

The slope of the plots of ln α as a function of $1/T$ represents the difference in enthalpy of complexation changes ($\Delta\Delta H$) between two enantiomers. The term

$$\ln \frac{1/K_{CD1m} + [CD]}{1/K_{CD2m} + [CD]}$$

is small (indeed, it is close to 0) and if it is neglected, the intercepts are related to the entropy of complexation changes ($\Delta\Delta S$) between enantiomers (see Table 6). From these intercept values the difference in free energy ($\Delta\Delta G$) can then be obtained as

$$\Delta\Delta G = \Delta\Delta H - \Delta\Delta T\Delta\Delta S \quad (22)$$

The difference in free energy can also be expressed as:

$$-\frac{\Delta\Delta G}{R} = -\frac{\Delta\Delta H}{R} + T\frac{\Delta\Delta S}{R} \quad (23)$$

and

$$-\frac{\Delta\Delta G}{R} = T \ln \alpha \quad (24)$$

A comparison of results obtained from Eqs. 23 and 24 is given in Table 6

As seen in Eqs. 18 and 21, differences in enthalpy, entropy and free energy between enantiomers in System III are equal to the sums of differences of enthalpy, entropy and free energy in Systems I and IIB. For methylphenobarbital in Systems I and IIB, the calculated sum for enthalpy changes is −2043 J/mol (System I −483J/mol+System IIB −1560 J/mol) and the experimentally obtained value in System III is −1928 J/mol. Entropy changes for methylphenobarbital gave the following results: $T\Delta S$ in System I is −292 J/mol, in System IIB −1126 J/mol, System I+System IIB −1418 J/mol and in System III −1318 J/mol (see Table 6). The differences between theoretical and experimental results for methylphenobarbital and mephenytoin are less than 10%. The biggest difference between theoretical and experimental values was obtained for camphor. We recall that our recent study [21] suggested that camphor and α-CD form 1:2 complexes which was not assumed in the model here.

It is worth noting that for methylphenobarbital, mephenytoin and morsuximide in Systems I and IIA, discrimination is obtained by complexation with the same CD but in different phase. In System I, β-CD is present in the mobile phase whereas in System IIA it is covalently bonded to the stationary phase.

By comparing $\Delta\Delta H/R$ and $\Delta\Delta S/R$ in Systems I and IIA in Table 6 one can estimate the complexing ability of CD in the free and bonded states. For all three studied compounds more negative vale of $\Delta\Delta G$ was achieved for covalently bonded cyclodextrin than for cyclodextrin in the mobile phase.

5. Conclusions

As was pointed out in our previous publication [9] System III with joint use of two chiral additives under convenient conditions can offer the best results, i.e., the shortest time of retention with the same or better enantioselectivity. Additionally if the complexation in the mobile phase is an enthalpy favored process compared with complexation on the stationary phase the shortest retention time and the best selectivity is obtained at low temperature. On the other hand if the complexation on the stationary phase is more favorable there is an increase in enantioselectivity but retention time increase with decreasing in column temperature. The good agreement between enthalpy and entropy changes of complex formation in the mobile phase calculated from results in Systems I and III (see Table 5) and the comparable enthalpy, entropy and free energy changes obtained experimentally and calculated in the System III show that the described model can be useful for prediction of experimental results.

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