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Influence of organic solvent on the behaviour of camphor and α-pinene enantiomers in reversed-phase liquid chromatography systems with α-cyclodextrin as chiral additive

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Abstract

Reversed-phase liquid chromatography has been applied in order to gain insight into the α-cyclodextrin (α-CD)–solute complexation process, which occurs in the aqueous mobile phases containing a secondary achiral modifier. The model compounds tested were (±)-camphor and (±)-α-pinene. Methanol, ethanol, and 1 or 2-propanol were used as secondary modifiers. Retention factors and enantioseparation factors have been determined on a RP 18 stationary phase as a function of the α-CD concentration, secondary modifier content, and temperature changes. The shortest retention and the best separation of studied compounds were achieved for aqueous–methanol eluents. Apparent stability constants in various binary aqueous–organic solvent mixtures have been evaluated for α-CD complexes of camphor enantiomers. Using the competition concept, values for the stability constants in pure water have been calculated. It has been found that: (1) the quotient of the stability constants for both enantiomers, denoted as absolute enantioselectivity E, always remains constant at a fixed value ($E \cong 1.9$), which may indicate that the complex composition does not change, (2) only the first step in the complexation process is altered by changing the solvent, which does not seem to affect the separation of the enantiomers, (3) the remarkable enantioselectivity that is observed results from the second step in the complexation process, (4) enthalpy changes are much more favourable for camphor–α-cyclodextrin complex formation than for the transfer of camphor to the stationary phase, which means that complexation dominates over adsorption and retention is shorter at lower temperatures, (5) the difference in free energy changes of complexation ($\Delta\Delta G$) between the enantiomers of camphor is about 1.5 kJ/mol at 20°C.

Keywords: Enantiomer separation; Stability constants; Camphor; α-Pinene; Cyclodextrins

1. Introduction

The influence of the achiral microenvironment on the chiral properties of chromatographic systems is far from being understood, although some information and suggestions have already been published. It has been pointed out by Davankov [1], that achiral sorbent matrices could play a significant role in the chiral discrimination of ligand-exchanging systems with bifunctional amino acids as chiral dopants to the eluent. Dynamic modification of the chiral bonding properties of CHIRAL-AGP columns by achiral organic and inorganic additives has been studied by Hermansson et al. [2]. The explanation for the

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observed change in enantioselectivity induced by the modifier on the glycoprotein stationary phase could be that the addition of organic solvent to the mobile phase can reversibly affect the secondary structure of the AGP molecule. There is also evidence that the nature of the solvent may influence or control the structure of the cyclodextrin complexes [3].

The usual solvent effect consists of a decrease in stability of the complex relative to water. However, some reports have described an increase in the stability of cyclodextrin (CD) complexes in mixed solvents [4,5] relative to purely aqueous systems. Several authors, applying spectroscopic methods to investigate the ternary complexes of cyclodextrins (CDs), suggest that joint inclusion of a given alcohol may optimize the fitting of the solute in the CD cavity, thus increasing their stability. In some cases these complexes seem to have a 1:1:1 stoichiometry [5,6].

Reversed-phase liquid chromatography (RPLC) has been applied to investigate the retention behaviour of β-CD–pyrene complexes in the presence of alcohol modifiers [7] and some tert-butyl compounds [8]. The remarkable reduction of the retention factor has been attributed to the formation of a ternary β-CD–pyrene–tert-butyl alcohol complex. Improved chiral separations with achiral modifiers in capillary electrophoresis with β-CD have been recently reported [9].

In his review, Connors [3] proposed five different hypotheses to account for the solvent effect in cyclodextrin complex stability. (1) The idea that hydrophobic interactions are the major contributor to complex stability in water, and that an increase in the organic solvent content decreases the hydrophobic driving force, is very popular. (2) A second hypothesis suggests that complex destabilization by addition of organic solvents results from the greater dispersion interaction between the substrate and solvent. (3) A third hypothesis invokes a stoichiometry equilibrium that includes water in the complex. (4) Another idea, commonly used in chromatographic studies [10,11], is that the organic solvent competes with the solute in occupying the CD cavity. (5) The fifth hypothesis supposes that the organic co-solvent undergoes inclusion together with the substrate. Some authors suggest that combination of several effects may occur.

During our work with α-CD as mobile phase additive we have found that achiral organic solvents may sometimes contribute substantially to chiral recognition. To the best of our knowledge, studies on the participation of organic achiral solvent in the chiral recognition of solute by α-CD in RPLC systems have not yet been reported.

In this paper we have focused our attention on the behaviour of camphor and α-pinene in RPLC systems with aqueous mobile phase modified with α-CD and some alcohols. The aim was to gain a better understanding of the mechanisms by which the achiral modifier changes the enantioselectivity and retention factor.

2. Theoretical considerations

Our experimental set-up consists of RPLC systems with aqueous–alcohol mobile phase, either with CDs (System I) or without CDs (System 0). The apparent stability constants of the α-CD complexes for various mobile phase modifiers were measured according to the model equilibria described below.

2.1. System 0

In System 0, without chiral selector, the retention factor of the solute G is only dependent on the partitioning process between mobile and stationary phase:

$$\begin{array}{ll} \text{Mobile phase} & G_m \\ & \uparrow\downarrow K \\ \text{Stationary phase} & G_s \end{array}$$

$$K = \frac{[G]_s}{[G]_m} \tag{1}$$

where K is the distribution constant of solute G between mobile and stationary phase, $[G]_s$ and $[G]_m$ being the concentrations of solute G in the stationary and the mobile phase, respectively. The distribution constant for a given stationary phase depends on the solute, mobile phase composition, i.e. the concentration of organic modifier, and temperature. The van't Hoff expression for such a chromatographic system is [12]:

$$\ln k_0 = -\frac{\Delta H^0}{RT} + \frac{\Delta S^0}{R} + \ln \varphi \quad (2)$$

where k_0 is the retention factor in System 0, ΔH^0 and ΔS^0 represent standard enthalpy and entropy changes of transfer of the solute between the mobile and stationary phase, R is the gas constant, T is the absolute temperature, and ϕ is the volume phase ratio of stationary to mobile phase.

2.2. System I (CD selector in the mobile phase)

Considering System I, it has been assumed that both adsorption of CD and solute–CD complex can be neglected and do not influence the stationary phase behaviour [13]. Furthermore, the degree of complexation of solute by CD may be determined by following the decrease of retention factor k. As known from previous results, both camphor and α-pinene form 1:2 complexes with α-CD [14–17]. The equilibria are then as follows:

Mobile phase $G_m + CD_m \overset{K_1}{=} GCD_m + CD_m \overset{K_2}{=} G(CD_m)_2$

$\uparrow\downarrow$

Stationary phase G_s

The complexation process can be summarized as:

$$G_m + 2CD_m \overset{K_1K_2}{=} G(CD_m)_2$$

In this system the retention factor is not only dependent on the partitioning process, but also on the stability constants K_1 and K_2. The solute retention factor k_1 in System I can be now defined by Eq. (3) [17–19]:

$$k_1 = \frac{k_0}{1 + K_1[\mathrm{CD}] + K_1K_2[\mathrm{CD}]^2} \quad (3)$$

where [CD] is the concentration of α-CD in the mobile phase.

Eq. (3) can be transformed into Eq. (4) in the following manner:

$$\ln k_1 = \ln k_0 - \ln K_1K_2 - \ln\left(\frac{1}{K_1K_2} + \frac{[\mathrm{CD}]}{K_2} + [\mathrm{CD}]^2\right) \quad (4)$$

leading finally to Eq. (5):

$$\ln k_1 = \frac{-(\Delta H^0 - \Delta H^0{}_{\mathrm{CD_m}})}{RT} + \frac{\Delta S^0 - \Delta S^0{}_{\mathrm{CD_m}}}{R} + \ln \varphi - \ln\left(\frac{1}{K_1K_2} + \frac{[\mathrm{CD}]}{K_2} + [\mathrm{CD}]^2\right) \quad (5)$$

where the product K_1K_2 corresponds to the overall complexation process of solute by α-CD in System I, and

$$\ln K_1K_2 = -\Delta H^0{}_{\mathrm{CD_m}}/RT + \Delta S^0{}_{\mathrm{CD_m}}/R \quad (6)$$

where $\Delta H^0{}_{\mathrm{CD_m}}$ and $\Delta S^0{}_{\mathrm{CD_m}}$ are standard enthalpy and entropy changes of complexes formation in the mobile phase, respectively.

The solvent influence on guest–CD inclusion has most frequently been explained using the competition model. This model treats the inclusion of guest and solvent molecules in CD as two separate events. Competitive inclusion of solvent may be expressed simply using the following equation [10,11]:

$$[\mathrm{CD}]_M = \frac{[\mathrm{CD}]_T}{1 + K_{solv}[\mathrm{solv}]} \quad (7)$$

where $[CD]_T$ and $[CD]_M$ are the total and equilibrium molar concentrations of CD, [solv] is the initial molar concentration of organic solvent, and K_{solv} is the stability constant of the 1:1 solvent–CD inclusion complex. Since in this experiment [solv] ≫ [CD], it is assumed that [solv] is equal to the equilibrium concentration of organic solvent. Thus, the real CD concentration available for solute molecules is smaller than the overall concentration due to solvent inclusion. Therefore, the CD guest complexations in water/organic solvent mixtures are weaker than in pure water and consequently the apparent stability constants are lower.

3. Experimental

3.1. Reagents

α-Cyclodextrin (α-CD) and β-cyclodextrin (β-CD) were supplied by Chinoin (Budapest, Hungary). The model compounds (+)-camphor, (−)-camphor, (+)-α-pinene and (−)-α-pinene were supplied by Fluka (Buchs, Switzerland). All other reagents and

solvents were of analytical grade and were used as received.

3.2. Apparatus and procedures

Chromatographic experiments were performed using a Waters (Vienna, Austria) Model 590 pump, a Rheodyne type injector and a Waters UV–Vis detector Model 490 (detection: 280 nm for camphor and 220 nm for α-pinene). The mobile phase were aqueous solutions with organic modifier (methanol, ethanol, 1-propanol and 2-propanol) without cyclodextrin (System 0) and with α-CD (System I). The column used for camphor was: 250×1 mm I.D. packed with 5 μm LiChrosorb RP 18 and for α-pinene: 250×1 mm I.D. packed with 10 μm LiChrosorb RP 18. Flow rates were 0.04 and 0.08 ml/min, respectively. All chromatographic measurements, except for thermodynamic study were done at ambient temperature of the air-conditioned room (20°C). For thermodynamic studies, the temperature was controlled using a Model MK 70 (MLW, Germany) cryostat.

The binding constants were fitted by non-linear least square procedures according to the 1:2 stoichiometry model using Eq. (3). For the determination of apparent stability constants total concentrations of CD were used, while for the stability constant in pure water the equilibrium molar concentration of CD was applied according to Eq. (7). As K_{solv} in Eq. (7), the values of stability constants of α-CD–alcohol complexes were taken from Matsui's and Mochida's paper [20].

4. Results and discussion

4.1. Retention and selectivity

The retention and enantioselectivity parameters for camphor and α-pinene determined using various alcohols as the additive to the mobile phase are collected in Table 1.

Considering System 0 it has been observed that, in compliance with the polarity scale, less polar alcohols, like ethanol and various propanols, make the eluent much stronger than methanol. In systems with β-CD, the retention time of camphor is shorter for ethanol than for methanol, just like for System 0. This indicates that both alcohols influence the complexation properties of β-CD in a similar manner, which is confirmed by the small and comparable association constants of β-CD for methanol and ethanol, i.e. 0.32 and 0.93, respectively [20].

But in systems with α-CD the retention time of both enantiomers of camphor and the first eluted enantiomer of α-pinene is shorter for methanol than for other alcohols, which suggests a higher degree of solute complexation in methanolic solutions. These results correspond well with the stability constants observed for the α-CD–alcohol complexes as published by Matsui et al., also listed in Table 1.

It is quite obvious that the very weak association of methanol by α-CD ($K_{solv}=0.93$) makes it a more favourable medium for solute–α-CD complexation than ethanol ($K_{solv}=5.62$). Moreover, using α-CD in aqueous methanol a much better enantioselectivity factor for camphor may be achieved in comparison with the other alcohols investigated. Of special interest is the separation of α-pinene enantiomers, when α is equal to 2.01 in case of methanol solution, while no separation occurs with ethanol and 1- or 2-propanol. These data demonstrate that solvent may promote or destroy the chiral recognition of solute depending on both solvent and solute nature.

For more details, the relations between retention and selectivity factors of camphor versus methanol concentration at constant α-CD concentration over a temperature range of 15–45°C are presented in Table 2 and Fig. 1.

The retention decreases with decreasing temperature and increasing methanol concentration. The shortest retention time was found at low temperatures and high methanol concentrations. These two effects are due to two different phenomena. First of all, retention decreases with decreasing temperature, because it is accompanied by a higher degree of complexation. Consequently, the concentration of free solute molecules that can be adsorbed on the stationary phase gets smaller. On the other hand, retention decreases with increasing methanol concentration owing to the weaker adsorption of solute molecules on RP phase as the result of solvation phenomena.

The changes in separation factor are more complicated and therefore more difficult to explain. It was

Table 1
Chromatographic parameters of camphor and α-pinene enantiomers with various alcohols as organic modifiers without chiral selector and with α- and β-cyclodextrin as additives to the mobile phase determined at ambient temperature

Camphor[a]					
Alcohol	%	Without CD k	β-CD 1.5×10^{-2} M k . . . . α	α-CD 2×10^{-2} M k_2/k_1 . . . . α	Stability constant K_{solv} for α-CD with alcohols*
MeOH	20	128.3	7.0 1.00	8.5/5.8 1.46	0.93
	35	31.9		7.4/4.9 1.53	
EtOH	20	55.3	5.8 1.00	21.3/14.4 1.48	5.62
	35	13.9		10.9/8.9 1.22	
1-PrOH	20	13.3		13.9/13.5 1.03	23.44
2-PrOH	20	26.5		12.6/9.2 1.37	4.90
α-Pinene[b]					
Alcohol	%	Without CD k	α-CD 2×10^{-2} M k_2/k_1 . . . . α		
MeOH	50	316.0	105.4/52.6 2.01		
EtOH	50	125.7	73.5 1.00		
1-PrOH	50	6.3			
	30	64.2	63.3 1.00		
2-PrOH	50	11.1			
	40	45.0	39.3 1.00		

[a] Camphor: column: 250×1 mm packed with LiChrosorb RP 18 5 μm, flow-rate 0.04 ml/min.
[b] α-Pinene: column: 250×1 mm packed with LiChrosorb RP 18 10 μm, flow-rate 0.08 ml/min.
* Stability constants for alcohols and α-CD (K_{solv}) taken from Matsui's et al. [20].

observed that at temperatures below 25°C the separation factor has reached a maximum value with 30% methanol, while for 20% methanol the maximum value arises at 35°C. The chromatograms in Fig. 2 clearly illustrate the influence of temperature on the retention and selectivity of camphor enantiomers at 20 and 40% methanol and 20% ethanol (the concentration of α-CD was 2×10^{-2} M in all cases).

When complexation of camphor by α-CD dominates over adsorption of camphor on RP-phase, the retention should become longer and the separation worse with growing temperature. Camphor enantiomers follow this rule with 20% ethanol (Fig. 2A) and 40% methanol (Fig. 2C). However, with 20% methanol at 40°C the enantioseparation factor is remarkably greater than at 20°C. This phenomenon may suggest some changes of equilibria including complex composition (inner or outer), which improve the enantioseparation.

4.2. Stability constants

In order to measure the solvent effect on the stability of the camphor–α-CD complex the influence of the α-CD concentration on the retention factor has been studied in eluents containing 20 and 35% methanol or ethanol. The apparent stability constants and the stability constants in pure water when competition effects are taken into account are presented in Table 3. The binding constants have

Table 2
Chromatographic parameters of camphor enantiomers for various eluents and temperatures[a]

Temp °C	System 0		System I with MeOH												System I with EtOH			
	35% MeOH	35% EtOH	20%		26.5%		30%		35%		40%		50%		20%		35%	
	k	k	k_1	α	k_1	α	k_1	α	k_1	α	k_1	α	k_1	α	k_1	α	k_1	α
			k_2		k_2		k_2		k_2		k_2		k_2		k_2		k_2	
15			7.5		5.1		3.5		3.3		3.2		2.6		11.3		7.9	
		18.8		1.30		1.53		2.03		1.60		1.45		1.45		1.52		1.32
			9.7		7.8		7.1		5.3		4.6		3.8		17.2		10.4	
20																	8.9	
	37.9	17.0																1.22
																	10.9	
25			9.5		6.8		5.8		6.5		4.9		4.0		19.4		9.5	
	34.3	15.1		1.40		1.46		1.52		1.55		1.44		1.28		1.38		1.16
			13.4		9.9		8.8		10.0		7.0		5.1		26.8		11.0	
30																	9.7	
		13.4																1.10
																	10.7	
35			14.2		11.3		10.0		11.5		7.4		5.1		26.1			
	28.0			1.47		1.44		1.43		1.40		1.29		1.17		1.23		
			21.0		16.3		14.3		16.1		9.5		5.9		32.0			
40									14.9									
	25.7	10.9								1.31								
									19.4									
45			22.7		17.2		14.7				9.1		5.4		28.0			
	23.5			1.43		1.33		1.29				1.16		1.09		1.11		
			32.3		22.9		19.0				10.6		6.0		31.1			

[a] Chromatographic conditions: column: 250×1 mm I.D. packed with LiChrosorb RP 18, flow-rates 0.04 ml/min, mobile phase were aqueous solutions with organic modifier (methanol or ethanol) without α-CD (System 0) and with 2×10^{-2} *M* α-CD (System I).

been determined following the relations *k* vs. [CD]. For the determination of apparent stability constants total concentrations of CD were used, while for the stability constant in pure water the equilibrium molar concentration of CD was applied (see procedure in Experimental section and in footnotes of Table 3).

The experimental and calculated chromatographic parameters are collected in Table 4. From these data it is evident that experimental and calculated values for retention and enantioselectivity factors are in very good agreement, except for the values measured in 20% methanol at higher concentration of α-CD. The observed discrepancies in more diluted solutions of methanol with higher concentration of α-CD seem to confirm the afore mentioned view, suggesting changes in the complex composition. Anyway, the close agreement between experimental and calculated values as observed in most cases testifies the correctness of the applied procedure and the 1:2 stoichiometry model that is being used.

At the same concentration of alcohol, it is seen that the apparent overall constant K_1K_2 is almost one order of magnitude larger for methanol than for ethanol. This behaviour conforms to the degree of association of alcohols by α-CD. However, when the individual constants for two alcohols are being compared, one can see that the K_1 values are much more diversified than the corresponding K_2 values.

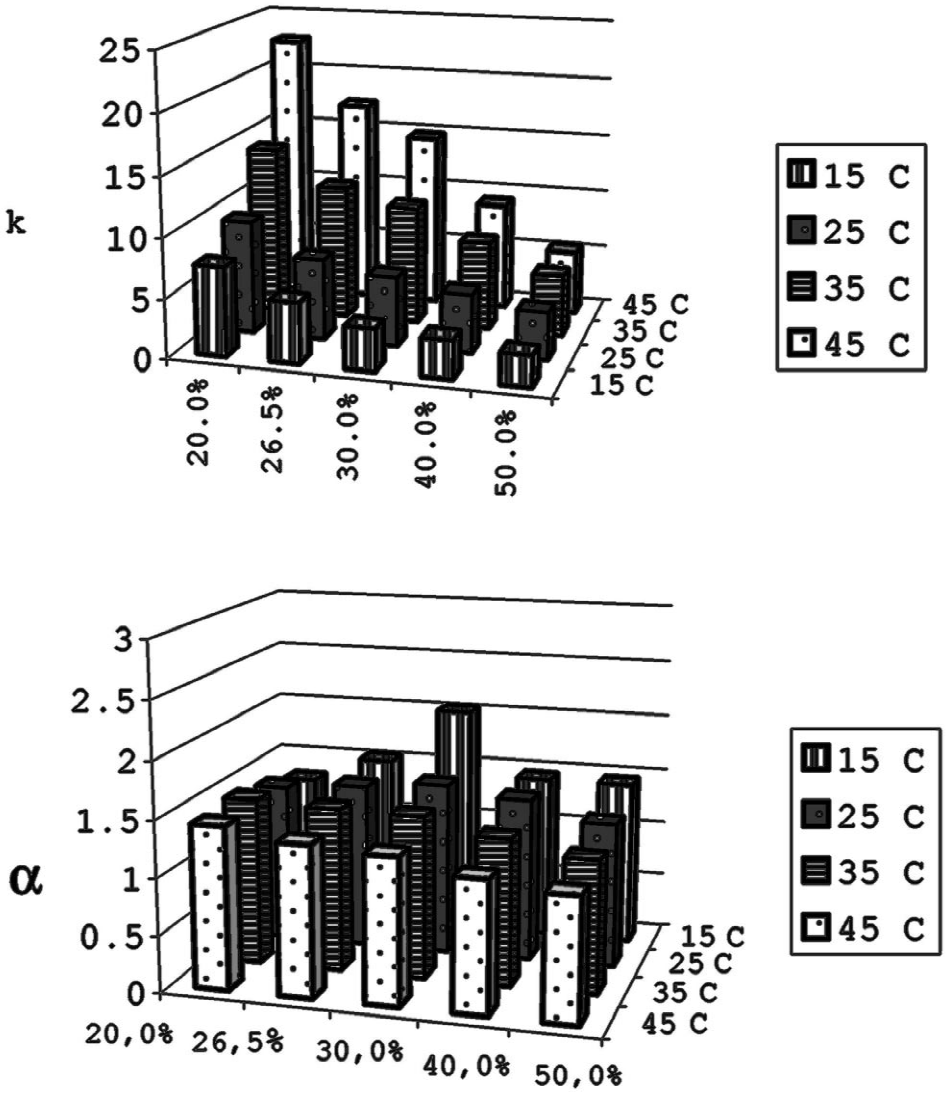

Fig. 1. Relation between chromatographic parameters (A) retention and (B) enantioselectivity of camphor enantiomers and methanol concentration at temperature range 15–45°C. Concentration of α-CD 2×10^{-2} *M*. Chromatographic conditions as in Table 1.

So it seems that both the alcohol and the solute compete in occupying the α-CD cavity, as far as formation of the 1:1 complex is concerned. The second binding constants K_2 are similar for methanol and ethanol and are much larger than those of the first complexation step.

According to the competition model, the values of the stability constants in pure water should be larger than the apparent values and should be constant. As one can see in Table 3 the stability constants in pure water are in fact much larger than the apparent ones (in 35% ethanol even a factor of 10^3). However, they are not constant and they differ depending on the experimental data used for the calculations. In case of lower concentrations of alcohol (20%) the overall stability constants K_1K_2 in pure water are about two times larger than those in 35% alcohol. The values of K_1K_2 in pure water for both camphor enantiomers calculated from the data in 35% methanol, i.e. 8.8×10^5 and 4.8×10^5, correspond very well to the data

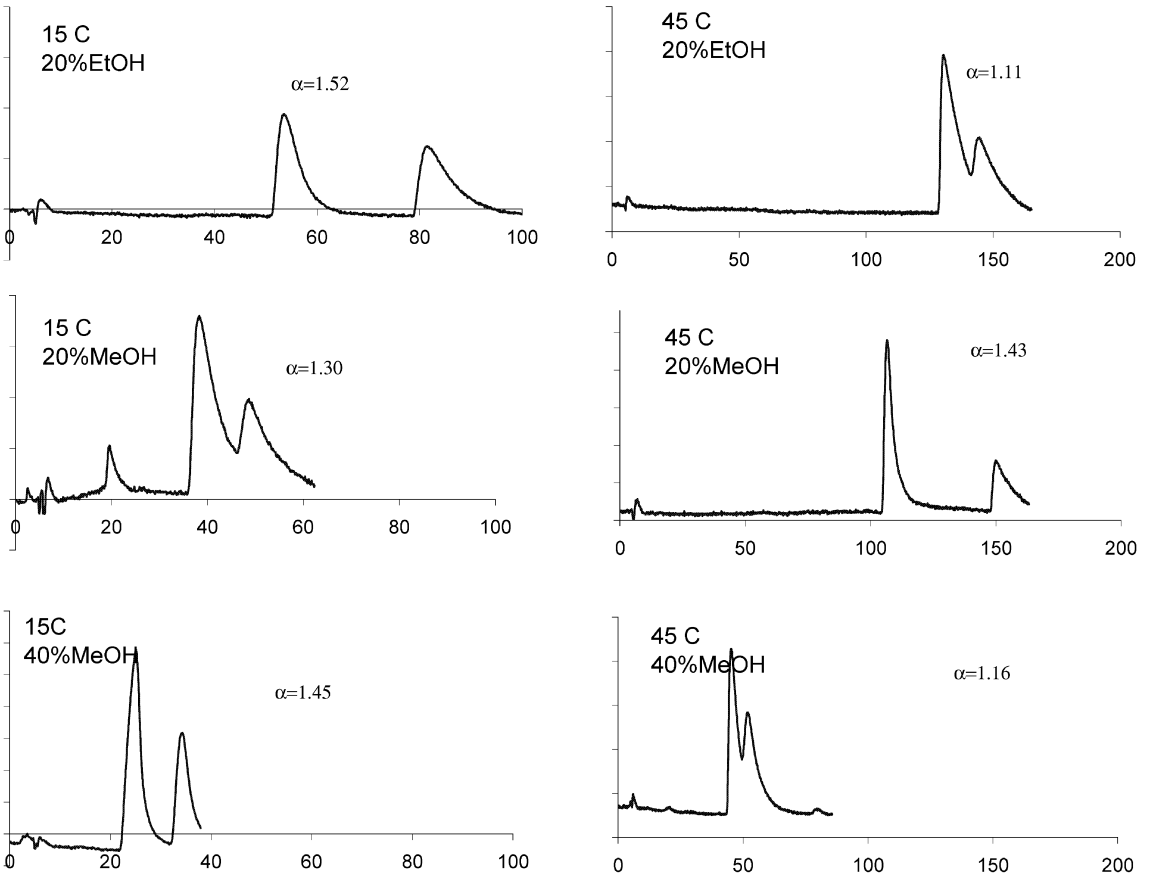

Fig. 2. Chromatograms of non-racemic mixtures of camphor enantiomers (in all cases excess of (+)-enantiomer) obtained at temperature 15 and 45°C. The mobile phases were aqueous solutions with (A) 20% ethanol, (B) 20% methanol and (C) 40% methanol, containing 2×10^{-2} *M* α-CD; chromatographic conditions as in Table 1.

calculated from ^{1}H NMR titration, i.e. 6.7×10^{5} and 3.7×10^{5} [16]. For 35% ethanol a relatively large standard deviation was obtained; this error can be due to the small range of α-CD equilibrium concentrations used. However, larger concentrations are experimentally not accessible, because of solubility problems.

4.3. Enantioselectivity

Considering the separation of enantiomers, two parameters will be taken into account. Namely:

(1) Chromatographic enantioseparation factor (α)

By definition, it can be written as:

$$\alpha = \frac{k(\mathrm{II})}{k(\mathrm{I})} \tag{8}$$

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

Combination of Eqs. (3) and (8) gives:

$$\alpha = \frac{1 + K_{1\mathrm{I}}[\mathrm{CD}] + K_{1\mathrm{I}}K_{2\mathrm{I}}[\mathrm{CD}]^2}{1 + K_{1\mathrm{II}}[\mathrm{CD}] + K_{1\mathrm{II}}K_{2\mathrm{II}}[\mathrm{CD}]^2} \tag{9}$$

α changes asymptotically with the CD concentration, from 1.0 (CD=0) to the constant value $K_{1\mathrm{I}}K_{2\mathrm{I}}/K_{1\mathrm{II}}K_{2\mathrm{II}}$

Table 3
The stability constants of camphor enantiomers with α-cyclodextrin[a]

(A) Apparent stability constants for a given eluent

Compound	20% MeOH			20% EtOH		
	K_1	K_2	K_1K_2	K_1	K_2	K_1K_2
(+)-Camphor	147±44	417±170	61 299	*	*	6950
(−)-Camphor	170±24	194±46	32 980	*	*	3700
			$E=1.86$			$E=1.88$
	35% MeOH			35% EtOH		
(+)-Camphor	39±5	277±45	10 803	7.0±2.5	200±87	1400
(−)-Camphor	31±3	189±27	5859	5.8±1.8	126±50	731
			$E=1.84$			$E=1.92$

(B) Stability constants in pure water when competition effect is taken into account

	20% MeOH			20% EtOH		
	K_1	K_2	K_1K_2	K_1	K_2	K_1K_2
(+)-Camphor	822±248	2328±957	19.1×10^5	*	*	29.0×10^5
(−)-Camphor	950±132	1082±255	10.3×10^5	*	*	15.6×10^5
			$E=1.85$			$E=1.86$
	35% MeOH	35% EtOH				
(+)-Camphor	356±43	2488±408	8.8×10^5	212±95	8191±4402	17.3×10^5
(−)-Camphor	283±28	1698±243	4.8×10^5	183±72	4927±2427	9.0×10^5
			$E=1.83$			$E=1.92$

[a] The binding constants were fitted by non-linear least square procedures according to the 1:2 stoichiometry model using Eq. (3). For the determination of apparent stability constants total concentrations of CD were used, while for the stability constant in pure water the equilibrium molar concentration of CD was applied according to Eq. (7). As K_{solv} in Eq. (7), the values of stability constants of α-CD–alcohol complexes were taken from Matsui's and Mochida's paper [20]. E is the absolute enantioselectivity calculated according to Eq. (10). * Stepwise constants cannot be separately determined.

(2) The absolute enantioselectivity E for 1:2 stoichiometry (introduced by us):

$$E = \frac{K_{1I}K_{2I}}{K_{1II}K_{2II}} \quad (10)$$

that should be constant and independent from the CD concentration.

As was mentioned before, the stability constants in pure water were found not to be constant. However, the absolute enantioselectivity factor E as determined from both the apparent constants and from constants in pure water, remains stable at constant value $\cong 1.9$, as can be seen from Table 3. It seems that some important effects have been overlooked in the calculation of the stability constants. Until now they are unknown, but should be equal for both enantiomers, because the absolute enantioselectivity factor E remains constant.

When comparing the absolute enantioselectivity factor E separately for both complexation steps, it was found that the quotient of K_{1I}/K_{1II}, corresponding to the first complexation step, varies around 1.0, while the quotient of the stability constant of the second step of complexation, i.e. K_{2I}/K_{2II}, gives values between 1.6 and 1.9. Thus, the observed enantioselectivity is the result of the second complexation process.

4.4. Thermodynamics

We have followed many authors, who applied temperature studies to recognize the mechanisms of chromatographic processes including chiral resolution undergoing at various conditions [21–26].

4.4.1. System 0

For camphor, the thermodynamic changes of

Table 4
Experimental and calculated retention and selectivity factors of camphor enantiomers in eluents with 20 and 35% of methanol and ethanol[a]

20% MeOH						
CD	k_1	k_2	α	k_1 CALC	k_2 CALC	α CALC
0	128.3	128.3	1	128.3	128.3	1
0.005	40.0	49.0	1.22	39.2	48.0	1.22
0.008	19.2	26.8	1.40	21.0	28.7	1.37
0.01	14.2	20.7	1.45	14.9	21.4	1.44
0.015	8.0	11.9	1.49	7.5	11.7	1.55
0.02	5.8	8.5	1.46	4.5	7.3	1.62
0.023	4.8	7.0	1.45	3.5	5.7	1.65
0.025	4.5	6.1	1.37	3.0	5.0	1.66
20% EtOH						
0	55.3	55.3	1	55.3	55.3	1
0.005	47.2	51.3	1.09	47.0	50.6	1.08
0.008	38.1	45. 8	1.20	38. 2	44.7	1.17
0.01	33.0	41.3	1.25	32.5	40.3	1.24
0.015	21.2	29.4	1.39	21.5	30.2	1.40
0.02	14.4	21.3	1.48	14.6	22.3	1.53
35% MeOH						
0	31. 9	31.9	1.00	31.9	31.9	1
0.001	30.7	30.7	1.00	30.4	30.8	1.01
0.005	21.7	24.5	1.13	21.7	24.5	1.13
0.008	15.9	19.5	1.23	15.9	19.6	1.23
0.01	12.6	16.8	1.33	12.9	16.8	1.30
0.015	8.3	12.0	1.45	7.9	11.4	1.44
0.02	4.9	7.4	1.53	5.2	8.0	1.54
0.023	4.1	6.3	1.54	4.2	6.6	1.58
35% EtOH						
0	15.2	15.2	1.00	15.2	15.2	1.00
0.001	14.5	14.5	1.00	15.1	15.1	1.00
0.005	13.9	13.9	1.00	14.2	14.5	1.02
0.01	12.9	13.5	1.05	12.6	13.4	1.07
0.015	10.6	12.1	1.14	10.7	12.2	1.13
0.02	8.9	10.9	1.22	8.9	10.8	1.21
0.023	8.0	10.1	1.27	8.0	10.0	1.25
0.025	7.6	9.9	1.30	7.4	9.5	1.28
0.03	6.2	8.4	1.34	6.2	8.3	1.35
0.04	3.9	5.7	1.47	4.3	6.3	1.47

[a] k_1, k_2, α are the experimental results; k_1 CALC, k_2 CALC, α CALC are the results obtained by simulation procedure for the apparent stability constants presented in Table 3 according to Eq. (3).

transfer between mobile and stationary phase were obtained according to Eq. (2). The retention factors were studied with 35% ethanol and 35% methanol as the eluents. The slope of a plot of ln k against $1/T$ (see Fig. 3) provides information about the difference in enthalpy, whereas the intercept is related to $\Delta S/R + \ln \varphi$. The results are presented in Table 5.

If one compares the results for methanol and ethanol, it can be seen that the enthalpy changes for both alcohols are similar. The transfer of solute to the stationary phase is even somewhat more favourable for ethanol than for methanol. The entropy changes for both alcohols indicate that the solute is more organized on the stationary phase than in the mobile phase. The entropy changes for the transfer of solute to the stationary phase is more negative for

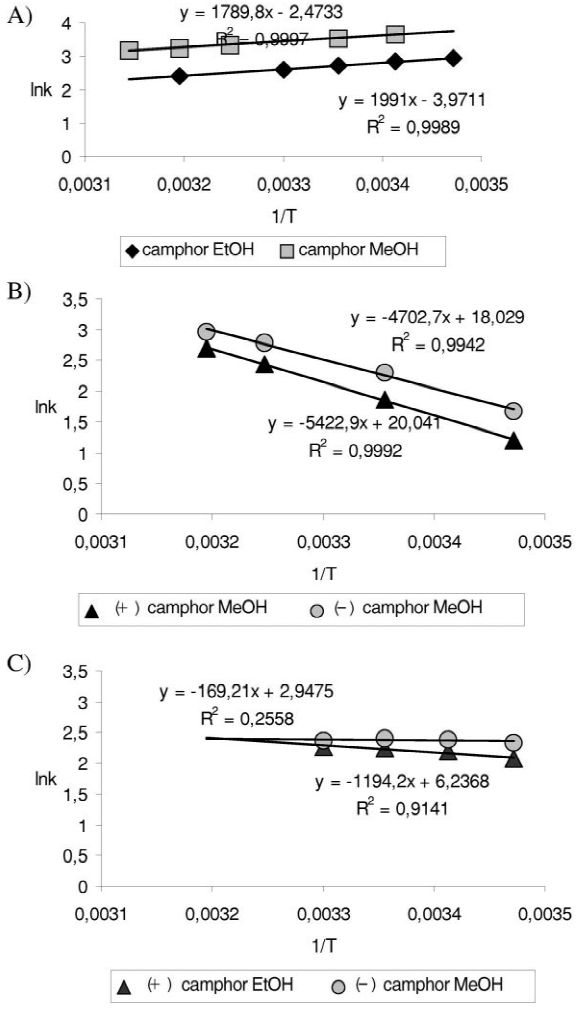

Fig. 3. Influence of mobile phase composition on the van't Hoff plots of camphor enantiomers; (A) 35% of alcohol, MeOH or EtOH without chiral selector, (B) 35% MeOH with 2×10^{-2} *M* α-CD, (C) 35% EtOH with 2×10^{-2} *M* α-CD; other chromatographic conditions as in Table 1.

ethanol than for methanol, which means that the difference in entropy change is responsible for the shorter elution of the solute when ethanol is used in the eluent.

4.4.2. System I

The enthalpy changes for the complexation of both camphor enantiomers were calculated from examination of the van't Hoff expression (Eq. (5)). In order to simplify this, it was assumed that the

$$\ln\varphi - \ln\left(\frac{1}{K_1K_2} + \frac{[CD]}{K_2} + [CD]^2\right)$$

expression does not change in the temperature range investigated (15–35°C), and that the slope of a plot of ln *k* against $1/T$ provides information about the difference in enthalpy change for each enantiomer. These assumptions are supported by the linear plots of ln *k* versus $1/T$ in this range of temperature as it is seen in Fig. 3 (one exception is (−)-camphor in 35% EtOH, Fig. 3C).

Differences in the change of free energy of complexation for each enantiomer at room temperature (20°C) were determined according to the equation:

$$\Delta G_{CD} = -RT \ln K_1K_2 \quad (11)$$

The entropy changes of complexation were determined from the relation:

$$\Delta G_{CD} = \Delta H_{CD} - T\Delta S_{CD} \quad (12)$$

The results are also collected in Table 5.

As we can see, the enthalpy of complexation of camphor by α-CD is much more negative than the enthalpy of transfer of the solute to the stationary phase, which indicates that complexation dominates over adsorption (short retention at low temperature). For both alcohols the negative free energy changes of complexation result from negative enthalpies of complexation, which means that complexation of camphor enantiomers by α-CD is enthalpy driven. The difference in free energy change for both alcohols is not large (more favourable complexation in methanol than in ethanol), but the enthalpy change is much more favourable for methanol. The large difference in enthalpy change for complex formation between both alcohols may suggest the active participation of the small methanol molecule in the complex formation.

Differences in free energy changes between both enantiomers were calculated as:

$$\Delta\Delta G_{CD} = \Delta G_{CDI} - \Delta G_{CDII} \quad (13)$$

where ΔG_{CD} for each enantiomer was calculated from Eq. (11) using apparent constant. The results are collected in Table 5. In all eluents, the difference

Table 5
Thermodynamic parameters of camphor enantiomers determined with various eluents[a]

System I						
Eluent	Enantiomer	$-\Delta H$ [kJ/mol]	$\Delta S/R + \ln \varphi$	$-\Delta G_{CD}$ [kJ/mol]	$-\Delta H_{CD}$ [kJ/mol]	$T\Delta S_{CD}$ [kJ/mol]
	(+)			21.5		
20% EtOH				$\Delta\Delta G = 1.5$		
	(−)			20.0		
	(+)			17.7	26.4	−8.7
35% EtOH		16.4±03	−3.9±0.3	$\Delta\Delta G = 1.6$		
	(−)			16.1	17.9	−1.8
	(+)			26.9		
20% MeOH				$\Delta\Delta G = 1.6$		
	(−)			25.3		
	(+)			22.6	60.0	−38.2
35% MeOH		14.9±0.6	−2.5±0.1	$\Delta\Delta G = 1.5$		
	(−)			21.1	54.0	−32.9

[a] $T = 293$ K; $-\Delta H$, ΔS are enthalpy and entropy changes of transfer of the solute between the mobile and stationary phase; $-\Delta H$, $\Delta S/R + \ln \varphi$ were calculated using Eq. (2); $-\Delta G_{CD}$, ΔH_{CD}, ΔS_{CD} are free energy, enthalpy and entropy changes of camphor–α-CD complexes formation; ΔH_{CD}, were calculated from Eq. (5); $-\Delta G_{CD}$ were calculated from Eq. (11) using apparent constants from Table 3; ΔS_{CD} were calculated from Eq. (12).

in free energy change ($\Delta\Delta G$) between both enantiomers of camphor is about 1.5 kJ/mol, which is in agreement with NMR data [16].

In summary, we can state that the observed data for retention and separation of camphor and α-pinene enantiomers using various different alcohols clearly demonstrate that in chromatographic systems the solvent may promote or inhibit the chiral recognition of the solute, depending on both solute and solvent nature. However, the details of this effect are still poorly understood.

By using a competition model for the analysis of camphor–α-CD chromatographic data in methanol- and ethanol-containing eluents the following information was obtained: (i) the quotient of the stability constants K_1K_2 for both enantiomers, denoted as absolute enantioselectivity E, always remains constant at a fixed value ($E \cong 1.9$), which may indicate that the complex composition does not change, (ii) the binding of camphor by the first molecule of CD is strongly influenced by the solvent and this process does not exhibit any enantioselectivity, (iii) it is the second complexation step that is responsible for the outstanding enantioselectivity ($E \cong 1.9$).

The enthalpy changes for formation of the camphor–α-CD complex are much more favourable than for transfer of camphor to the stationary phase, which means that complexation dominates over adsorption. Difference in free energy changes ($\Delta\Delta G$) between the enantiomers of camphor is about 1.5 kJ/mol at 20°C.

Unfortunately, some experimental results do not fit well with the competition model. The observed maximum value for the relation α versus methanol concentration (at 30% v/v) is difficult to explain. A decrease in the alcohol concentration should lead to an increase in the separation factor α, because it is accompanied by an increase in the equilibrium α-CD concentration. However, it is observed that at higher α-CD concentrations with lower methanol content the separation factor gets smaller. Other remarkable observations at 20% methanol are: (1) increased selectivity with raising temperature, and (2) discrepancies between experimental and calculated values for several chromatographic parameters. These unexpected results show that there is a range of temperatures (<35°C) and methanol concentrations (<30%) where the system behaves contrary to our earlier proposed assumptions. The question which phenomena are responsible for the observed dis-

crepancies currently remains unclear. Therefore, further chromatographic and NMR studies on this subject have to be performed.

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