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Interactions between native cyclodextrins and *n*-alcohols studied using thermostated thin-layer chromatography

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Abstract

Using thermostated thin-layer chromatography, the retention behavior of α-, β- and γ-cyclodextrin on a C_{18} stationary phase was studied. As mobile phases, a homologous series of *n*-alcohols, from ethanol to pentanol, and their mixtures with water were examined. Chromatographic experiments were performed either at a constant temperature (30°C) and over a wide range of binary mixtures (0–100%, v/v), for ethanol and propanol, as well as at fixed mobile phase composition and different temperatures from 5°C to 60°C. Using isoelution binary mobile phases, the effect of temperature on retention of cyclodextrins was examined. Results were compared with chromatographic retention data from previously reported work in which methanol–water binary phases were used. From linear Van 't Hoff plots thermodynamic parameters such as the change of enthalpy (ΔH^0) and the change of entropy (ΔS^0) were estimated. In each case the sign of the calculated parameters is negative. Nonlinearities of Van 't Hoff plots were observed when propanol or butanol was used as a component of binary mobile phase.

Keywords: Retention mechanisms; Thermostated thin-layer chromatography; Alcohols; Cyclodextrins

1. Introduction

It is well known, that cyclodextrins (CDs) and their derivatives have a wide range of applications including; modeling of enzymatic reactions, stereoselective membrane transport, enantiomers separations and many others. A great interest in three native cyclodextrins (α, β and γ), is caused mainly by their inclusion properties, high stability, simplicity in their purification, relatively low cost and low toxicity. Therefore, they have been often used as components of biotechnology, food, cosmetic or pharmaceutical products in order to improve the solubility, stability and also bioavailability of drugs [1].

A detailed knowledge of CD behavior in chromatographic systems is of great importance because it supports the theoretical basis of chiral separations and thus is in relation to other industrial applications of cyclodextrins. Cyclodextrins are commonly used as chiral selectors and for improving separation of other stereoisomers in gas chromatography (GC) [2], high-performance liquid chromatography (HPLC) [3], thin-layer chromatography (TLC) [4] and capillary electrophoresis (CE) [5].

On the other hand, a number of highly specific techniques for separation of CDs and their derivatives were recently developed and described. Using CE with dynamic fluorescence detection, native

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cyclodextrins were well separated as complexes with 2-anilinonaphthalene-6-sulfonic acid [6]. Zimmermann and Larsen [7] have applied aromatic anions as background electrolytes in CE for separation of α-, β-, γ- and δ-CDs. Moreover, CE was applied by Tait et al. to a measure substitution degree of the parent β-CD [8]. High-temperature high-resolution gas chromatography (HT-HRGC) has been used by Pereira et al. [9] for separation and analysis of β-CD derivatives. The retention properties of cyclodextrins have also been studied using HPLC [10–13]. Unfortunately, this system has several disadvantages for such purposes. Native cyclodextrins lack chromophors, hence they do not absorb UV light. Therefore, detectors of low sensitivity such as refractive index or polarimetric must be used instead of UV detection. This requires overload injections, which, because of an enormous adsorption effect on stationary phase, strongly influence retention measurements. Moreover, the mobile phase compositions which can be investigated is restricted, because under extreme conditions the retention time is too long or even the solute does not elute from the column.

Considering these disadvantages, TLC seems to be particularly well situated to the study of CD behavior in chromatographic systems. Recently, TLC separations of cyclodextrins were reported using normal-phase TLC [14], NH_2-bonded silica HPTLC [15], reversed-phase (RP) HPTLC [16] as well on polyamide plates [17]. In our previous work [18] it was demonstrated, that temperature is a very important factor, influencing applications of CDs for stereoselective separations. Generally, for these purposes CDs can be applied either as mobile or stationary phase additives. In the first case retardation factor should be close to unity. In the second case, when cyclodextrins are physically bonded to stationary phase, the R_F value should be as low as possible. Moreover a linear Van 't Hoff behavior of natural cyclodextrins and macrocyclic antibiotics has been observed, using wide range of temperatures and methanol in water binary mobile phases. This paper is a continuation of our earlier contribution concerning TLC behavior of native cyclodextrins using various mobile phases and a thermostated RP-HPTLC system.

2. Experimental

2.1. Reagents

α-CD was purchased from Reanal-Chinoin (Budapest, Hungary); β- and γ-CDs were a product of Merck (Darmstadt, Germany). Phenolphthalein (PP) and sodium carbonate were obtained from a commercial supplier and used as received. *n*-Alcohols (absolute ethanol 99.8%, propanol 99.9%, butanol 99.9% and pentanol 99.9%) were products of P.O.CH. (Gliwice, Poland) and were purified by double distillation (ethanol and propanol from anhydrous calcium oxide and calcium sulfate, respectively).

2.2. UV measurements

The absorption spectra were recorded using a Philips PU 8750 (UK) UV–Vis one-beam spectrophotometer. All measurements were carried out using standard 1 cm thick quartz cells, 1 nm sampling wavelength and 1000 nm min^{-1} scan speed. Temperature of the samples (30±0.2°C) was maintained using an internal heat exchanger and measured on-line by thermistor probe placed inside cell and connected to a Metex M-4650CR (Germany) digital multimeter.

An alkaline solution of PP at a concentration of 30 μ*M* was prepared in aqueous 0.02 *M* sodium carbonate (pH=10.5). The samples were modified by the addition of β-CD and alcohols at a concentration of 1 m*M* and 200 m*M*, respectively. Appropriate alkaline solutions without PP were used as references. All of the solutions were freshly prepared daily.

2.3. Chromatography

Chromatography was performed on 10 cm×3 cm RP-18W HPTLC plates (wettable with water, without fluorescent indicator) obtained from Merck (Darmstadt, Germany). The chromatographic chambers (110 mm×60 mm wide×15 mm deep) were saturated with the vapor of the mobile phase under 1 atm pressure (1 atm=101 325 Pa). Chromatographic experiments were performed at the temperatures 5, 10, 20, 30, 40, 50 and 60°C, controlled by circulating water from thermostat with an accuracy of ±0.5°C.

The plates were thermostated for 25 min before development in order to obtain proper temperature equilibrium. When the new temperature was started, the chromatographic device was thermostated for at least 30 min. The R_M values were calculated in the usual manner and are based on the average of at least five independent determinations of each solute.

Double distilled water was used for preparation of both CD stock solutions (1 mg ml^{-1}) and the alcohol–water binary mobile phases (%, v/v). The following binary mobile phases were prepared: (a) ethanol–water (from 10% to 40% step 5% and from 50% to 90% step 10%), (b) propanol–water (from 6% to 15% step 1% and from 20% to 90% step 10%), (c) butanol–water (0.5%; then from 1% to 8% step 1% and from 86% to 98% step 2%) and (d) pentanol–water (from 0.1% to 0.5% step 0.1%; then 1.5%; 2.0% and from 94.0% to 99.0% step 1.0%).

The mobile phases were filtered through a 1.5-μm membrane and degassed on ultrasonic bath UM-2 (Unitra-Unima, Olsztyn, Poland) prior to use.

The cyclodextrins were visualized by spraying the plates with a mixture of concentrated sulfuric acid–methanol (1:4, v/v) and heating at 140°C for 2–5 min. After this time the cyclodextrins were visualized as grey and black spots on the white background.

3. Results and discussion

As it can be seen from Figs. 1–3, and also was previously observed, using either RP-TLC [16,17] or HPLC [9] systems, native cyclodextrins do not elute when the concentration of water in binary organic–water mobile phase is close to 100%. This behavior contradicts the well-known concept that the retention of structurally similar solute compounds is determined by their solubility in the mobile phase, i.e., retention decreases when the solubility of analytes in mobile phase increases [19,20]. A good explanation for this unusual retention behavior of cyclodextrins, provides the equilibrium concept of Sybilska [3]. According to this idea, retention of cyclodextrins is determined by interactions of the ''empty'' CD and CD–cosolvent inclusion complexes with both stationary and mobile phases. This process depends strongly on the nature of cosolvent and the stationary phase. In this work, as cosolvents a homologous series of *n*-alcohols consisting of one to five carbon atoms has been chosen. In order to confirm that these alcohols enter the cyclodextrin cavity to form inclusion complexes, an experiment with PP was conducted. Results are shown in Fig. 4. It is well known [21] that alkaline solution of phenolphthalein absorbs visible light (curve A) and this absorption band is markedly lowered when cyclodextrin is added, due to formation of an inclusion complex (curve B). When an alcohol enters the CD cavity, in competition with phenolphthalein, the absorption band should be an intermediate between curve A and curve B. As can be seen in Fig 4, the addition of equal amounts of *n*-alcohols (from ethanol to pentanol) to the PP–CD system resulted in the systematic increase of phenolphthalein absorbance. Therefore, it can be concluded, that alcohols enter the cyclodextrins cavity and the strength of inclusion ability is related directly to the size and shape of the lipophilic part of the *n*-alcohol molecules. Similar results were reported previously, using azo dye–CD systems [22].

Figs. 1A–3A show the relationship between mobile phase composition and cyclodextrin R_F values for the systems investigated. Since for pentanol–water systems the R_F value is always zero, a corresponding figure is not given. Comparing the results obtained on methanol–water mobile phases [18] and for currently studied *n*-alcohols, it is apparent that transport of the cyclodextrins from the origin requires lower concentrations of alcohols from methanol to butanol. It is noteworthy, that to obtain the same elution effect of cyclodextrins, e.g., $R_F = 0.5$, the concentration of alcohol in mobile phase decreases approximately two-fold if the carbon chain of alcohol increases, e.g., for methanol 50%, ethanol 30%, propanol 14%, butanol 7%. For pentanol the estimated value is about 3.5%. However, due to differences in polarity of pentanol and water the homogeneous binary mixture in the concentration range from 2% to 94% does not exist. For studied ranges of pentanol–water mobile phases (0–2% and 94–100%) no elution of cyclodextrins was observed.

In order to study temperature effect on retention mechanism, the concentration of organic modifiers in mobile phases was prepared to give comparable

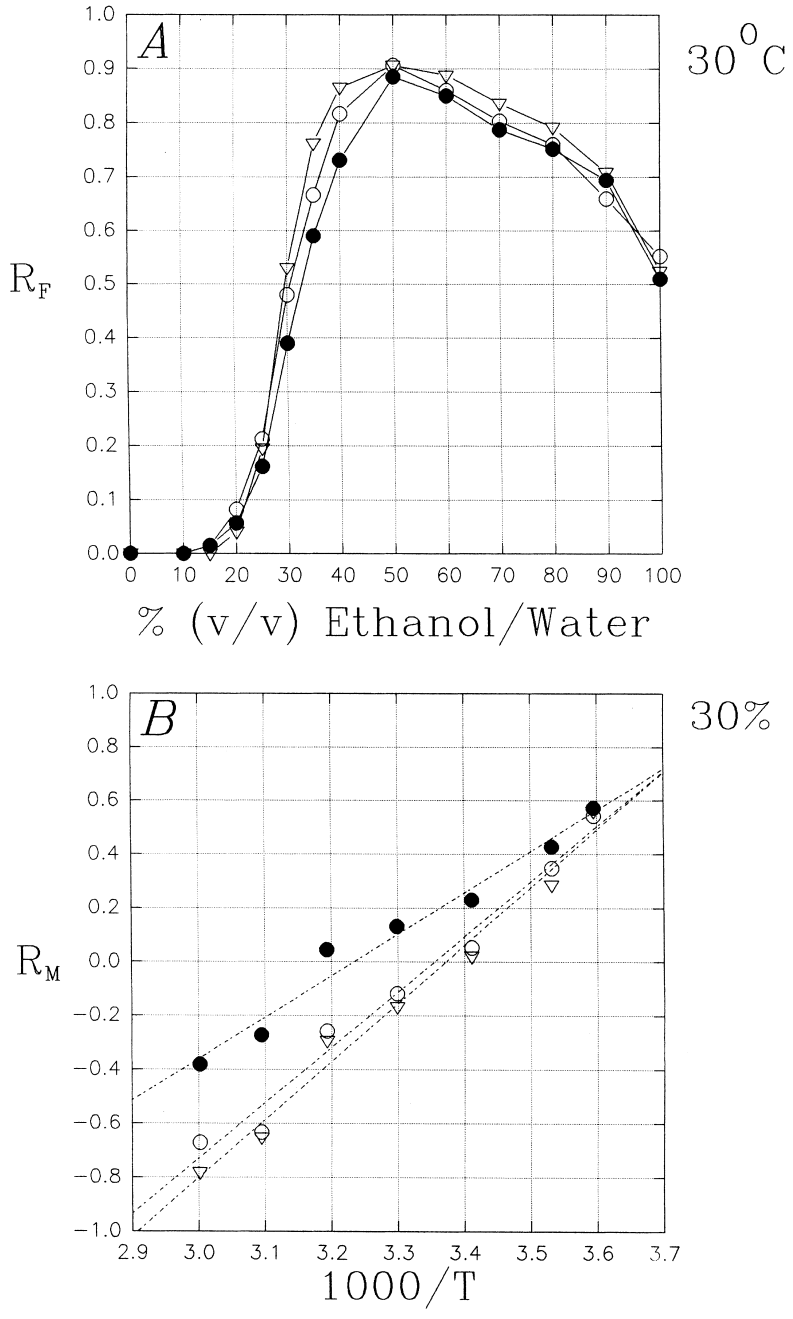

Fig. 1. Relationships between R_F (R_M) values of α-CD (○), β-CD (●) and γ-CD (▽) versus concentration of ethanol in water (graph A) and reciprocal of absolute temperature using 30% ethanol–water mixture as a mobile phase (graph B).

elution effect of cyclodextrin $R_F=0.5$ ($R_M=0$) according to data presented in Figs. 1A–3A. Therefore, the concentrations of ethanol, propanol and butanol were 30%, 14% and 7%, respectively. As can be seen from Figs. 1B–3B, temperature changes produce significant differences in the migration of

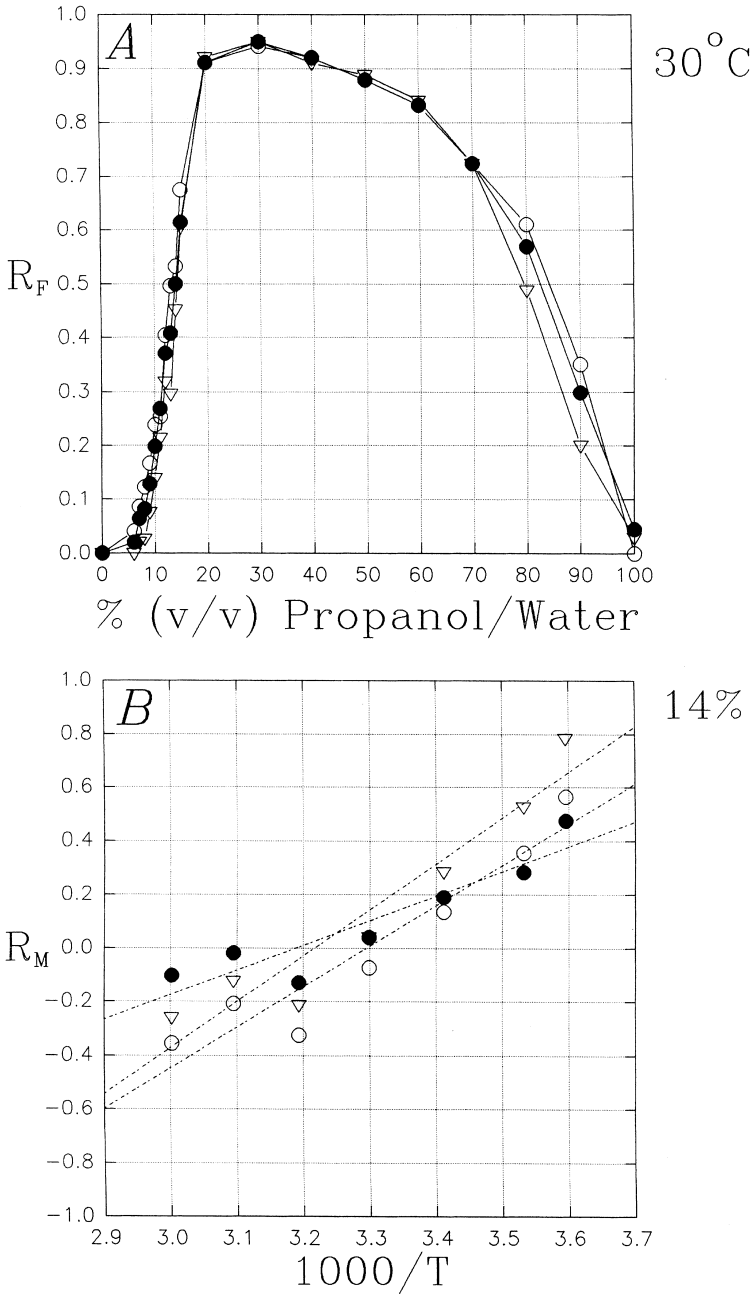

Fig. 2. Relationships between R_F (R_M) values of α-CD (○), β-CD (●) and γ-CD (▽) versus concentration of propanol in water (graph A) and reciprocal of absolute temperature using 14% propanol–water mixture as a mobile phase (graph B).

native cyclodextrins. Similarly to the previously discussed methanol–water system [18], in the ethanol–water system a typical Van 't Hoff behavior is observed at all temperatures studied. However, the selectivity of ethanol–water system is worse than using methanol–water mobile phases. In addition no changes in elution order of cyclodextrins is observed. In all the cases listed in Table 1 the values of

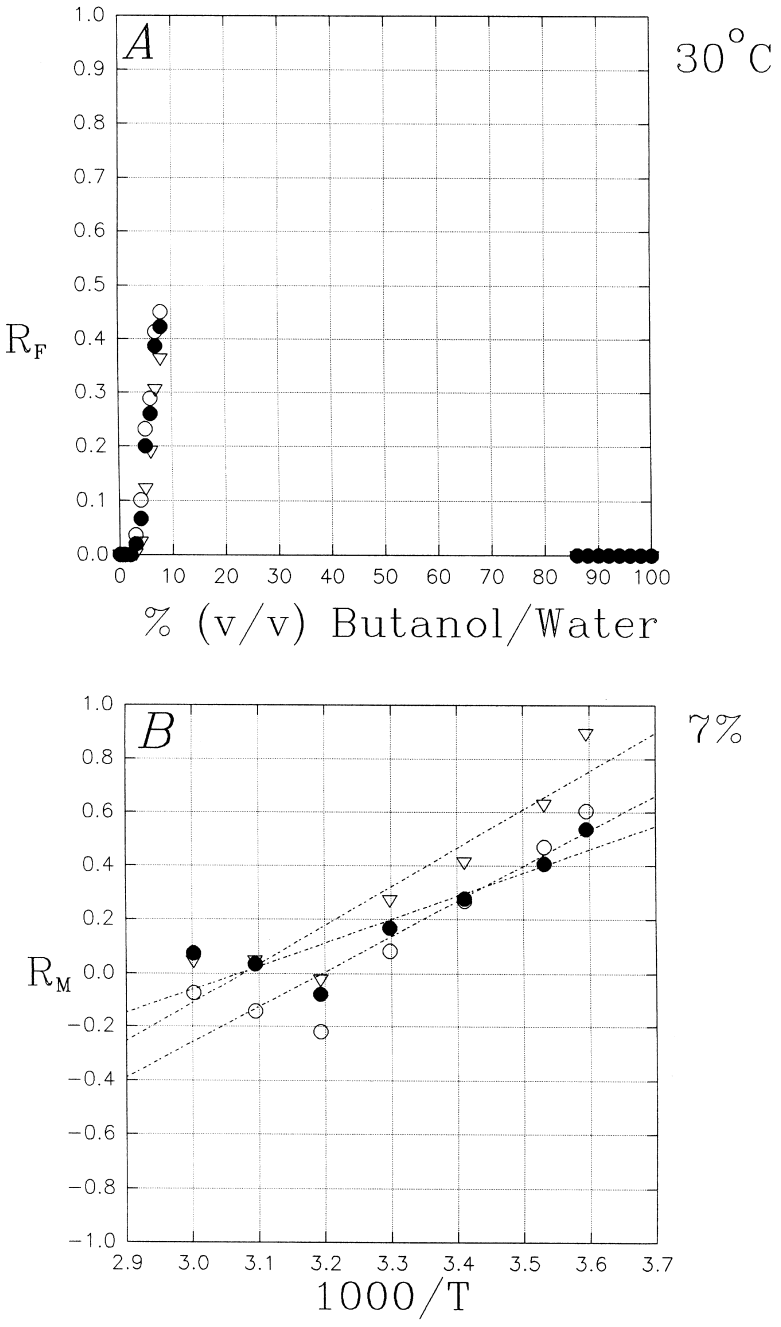

Fig. 3. Relationships between R_F (R_M) values of α-CD (○), β-CD (●) and γ-CD (▽) versus concentration of butanol in water (graph A) and reciprocal of absolute temperature using 7% butanol–water mixture as a mobile phase (graph B).

enthalpy and entropy are negative. Moreover, contrary to methanol–water, in the ethanol–water system very little differences in enthalpy changes for α- and γ-CD were observed. Using the propanol–water and butanol–water systems, deviations from the linear Van 't Hoff plots were observed. Therefore, for

Table 1
Regression coefficients (intercept, slope) and determination coefficient (r^2) of the regression equation R_M=intercept+slope×(1000/T) together with thermodynamic parameters (ΔH^0, ΔS^0) for α-, β- and γ-CD, measured by RP-HPTLC, using different mobile phase compositions (the values in parentheses indicate the standard error at 95% significance level; temperature range: 5–60°C; number of samples: 6)

Mobile phase (alcohol–water)	Solute	Intercept	Slope	r^2	ΔH^0 (kJ mol^{-1})	ΔS^0 (J mol^{-1} K^{-1})
30% Ethanol	α-CD	−6.9 (0.4)	2.1 (0.1)	0.9843	−40.21	−132.13
	β-CD	−5.0 (0.4)	1.5 (0.1)	0.9736	−28.72	−95.75
	γ-CD	−7.3 (0.4)	2.2 (0.1)	0.9819	−42.13	−139.79
14% Propanol	α-CD	−5.0 (0.7)	1.5 (0.2)	0.9109	−28.72	−95.75
	β-CD	−2.9 (0.6)	0.9 (0.2)	0.8560	–	–
	γ-CD	−5.5 (0.8)	1.7 (0.2)	0.9157	−32.55	−105.32
7% Butanol	α-CD	−4.2 (0.8)	1.3 (0.2)	0.8508	–	–
	β-CD	−2.7 (0.7)	0.9 (0.2)	0.7855	–	–
	γ-CD	−4.4 (0.8)	1.4 (0.2)	0.8714	–	–

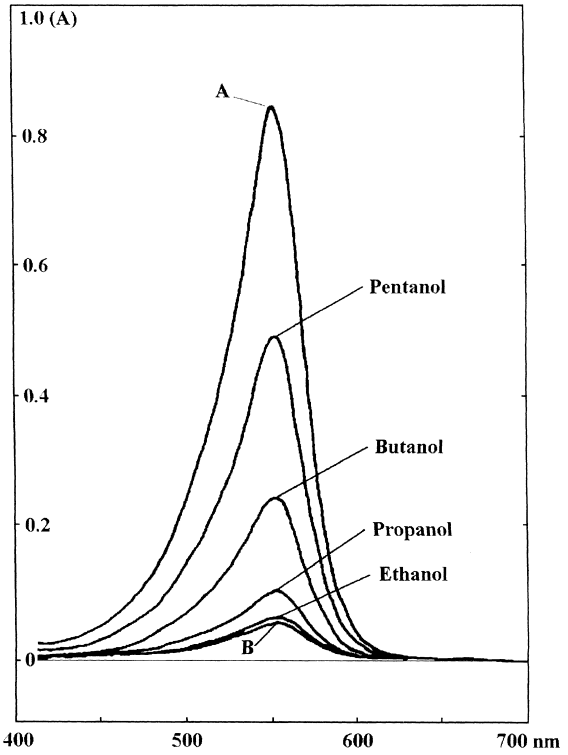

Fig. 4. Visible absorption spectra of PP (curve A) in the presence of β-CD (curve B) and after addition to PP–CD mixture *n*-alcohols in concentration of 200 m*M*; measured in alkaline solution (0.02 *M* Na_2CO_3; pH=10.5); PP and CD concentrations were 30 μ*M* and 1 m*M*, respectively; temperature 30°C.

these data points thermodynamic parameters were not calculated. It is well known, that non-linear Van 't Hoff behavior may be indicative of changes in the retention mechanism.

Conclusions

On the basis of both chromatographic and spectrophotometric experiments it was confirmed, that *n*-alcohols enter CD cavities influencing their retention properties.

Temperature changes of the chromatographic conditions produce significant differences in the migration of the investigated cyclodextrins. When methanol or ethanol is used as organic modifier a linear Van 't Hoff behavior of cyclodextrins is observed. In the case of propanol and butanol, a strong deviation from linear Van 't Hoff plots in the high temperature region indicate that the retention mechanism of cyclodextrin might be more complex.

References

[1] D. Duchene, F. Glomot, C. Vaution, in: D. Duchene (Ed.), Cyclodextrins and Their Industrial Uses, Editions de Sante, Paris, 1987, p. 211.
[2] E. Smolková-Keulemansová, S. Pokorna, L. Feltl, K. Tesarik, J. High Resolut. Chromatogr. Chromatogr. Commun. 11 (1988) 670.

[3] D. Sybilska, in: W.L. Hinze, D.W. Armstrong (Eds.), Ordered Media in Chemical Separation, ACS Symposium Series No. 342, American Chemical Society, Washington, DC, 1987, p. 219.
[4] W.L. Hinze, D.W. Armstrong, Anal. Lett. 13 (1980) 1093.
[5] J. Snopek, I. Jelínek, E. Smolková-Keulemansová, J. Chromatogr. 452 (1988) 571.
[6] S.G. Penn, R.W. Chiu, C.A. Monnig, J. Chromatogr. A 680 (1994) 233.
[7] K.L. Larsen, W. Zimmermann, J. Chromatogr. A 811 (1998) 193.
[8] R.J. Tait, D.J. Skanchy, D.P. Thompson, N.C. Chetwyn, D.A. Dunshee, R.A. Rajewski, V.J. Stella, J.F. Stobaugh, J. Pharm. Biomed. Anal. 10 (1992) 615.
[9] A.S. Pereira, E.F. Silva, F.R.A. Neto, J. Chromatogr. Sci. 36 (1998) 91.
[10] A.K. Chatjigakis, Ph.J.P. Cardot, A.W. Coleman, H. Parrot-Lopez, Chromatographia 36 (1993) 174.
[11] K. Koizumi, Y. Kubota, Y. Okada, T. Utamura, S. Hizukuri, J.-I. Abe, J. Chromatogr. 437 (1988) 47.
[12] T. Takeuchi, T. Miwa, Chromatographia 38 (1994) 453.
[13] H.L. Jin, A.M. Stalcup, D.W. Armstrong, J. Liq. Chromatogr. 11 (1988) 3295.
[14] J. Jindrich, J. Pitha, B. Lindberg, Carbohydr. Res. 275 (1995) 1.
[15] K. Koizumi, T. Utamura, T. Kuroyanagi, S. Hizukuri, J.-I. Abe, J. Chromatogr. 360 (1986) 397.
[16] P.K. Zarzycki, J. Nowakowska, A. Chmielewska, H. Lamparczyk, J. Planar Chromatogr. 8 (1995) 227.
[17] P.K. Zarzycki, J. Nowakowska, A. Chmielewska, H. Lamparczyk, J. Planar Chromatogr. 9 (1996) 260.
[18] P.K. Zarzycki, J. Nowakowska, A. Chmielewska, M. Wierzbowska, H. Lamparczyk, J. Chromatogr. A 787 (1997) 227.
[19] D.C. Locke, J. Chromatogr. Sci. 12 (1974) 433.
[20] T. Hanai, J. Chromatogr. 550 (1991) 313.
[21] P.K. Zarzycki, H. Lamparczyk, J. Chem. Educ. 73 (1996) 456.
[22] Y. Matsui, K. Mochida, Bull. Chem. Soc. Jpn. 52 (1979) 2808.