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Temperature-induced inversion of elution order in the enantioseparation of sotalol on a cellobiohydrolase I-based stationary phase

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Abstract

The effect of temperature on the resolution of (*RS*)-sotalol by immobilized cellobiohydrolase I (CBH I) was studied between 5 and 40°C and Van 't Hoff plots of ln k versus $1/T$ were acquired at different pH values of the aqueous mobile phase and in the presence of varying organic cosolvents. The elution order of the enantiomers reverses in the range between 17 and 28°C. Beyond this range, enantioseparations with comparatively high resolution factors are achieved either by decreasing or by increasing the temperature. The composition of the mobile phase influences the ''crossover'' temperature as well as the character of the global adsorption process of the (*R*)-(−)-enantiomer. Under certain conditions, (*R*)-(−)-sotalol exhibits an unusual endothermic adsorption behavior. Its retention time increases with increasing temperature. At room temperature (23°C) the enantiomeric elution order can also be regulated by the solvent additive.

Keywords: Enantiomer separation; Cellobiohydrolase I; Temperature effects; Mobile phase composition; Thermodynamic parameters; Sotatol

1. Introduction

Sotalol {4′-[1-hydroxy-2-(isopropylamino)ethyl] methanesulfonanilide} is a chiral β-adrenoceptor antagonist marketed as a racemate in the treatment of hypertension, angina pectoris and cardiac arrhythmias [1,2]. The β-receptor blocking activity is mainly attributed to the (*R*)-(−)-enantiomer (Fig. 1) [1], while both (*S*)-(+)- and (*R*)-(−)-sotalol are equipotent class III antiarrhythmic agents [1,2]. The stereodifferentiating pharmacological profile raised much interest in chiral separation methods for this compound.

In liquid chromatography, several chiral stationary phases (CSPs) have shown the ability to separate the enantiomers of a wide set of β-adrenoceptor antagonists, e.g., brush-type, protein and cellulose-derived CSPs [3–9]. However, the enantioseparation of sotalol proved to be troublesome and in many cases no or rather poor resolution was achieved [8–12]. The differing behavior observed may be causally related to the structural differences between sotalol and the classical β-blockers of the aryloxypropanolamine type (Fig. 1). Indeed, sotalol is

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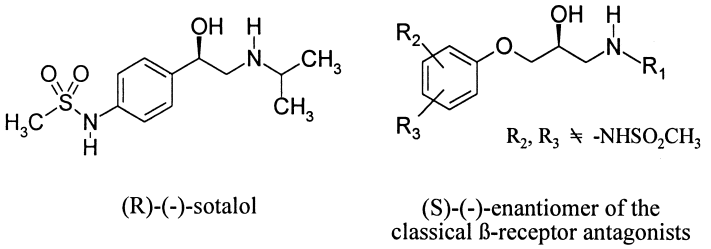

(R)-(-)-sotalol

(S)-(-)-enantiomer of the classical ß-receptor antagonists

Fig. 1. Structures of (*R*)-(−)-sotalol and the (*S*)-(−)-enantiomer of the classical β-receptor antagonists. According to the Cahn–Ingold–Prelog (CIP) rules the designation of the asymmetric carbon atom of the sotalol enantiomer is reversed in comparison to the corresponding enantiomer of the classical β-blockers of the aryloxypropanolamine type.

unique by its arylethanolamine moiety (pK_a=9.8) and the acidic sulfonamide function (pK_a=8.3) causing the zwitterionic properties of the molecule.

Among the protein-based CSPs immobilized cellobiohydrolase I (CBH I) was found to be the most suitable for chiral analyses of β-receptor antagonists. The CBH I column gives large resolution factors for almost all enantiomeric pairs of these compounds [6,13,14]. The major drawbacks of protein phases and CBH I columns in particular are strong peak tailings and low loading capacities even on analytical scale. Therefore, protein CSPs can only be restrictively applied to preparative purposes.

CBH I is an acidic glycoprotein with an isoelectric point of 3.9. As known for naturally occurring chiral polymers, the enzyme provides a variety of binding sites, including stereospecific and non-stereospecific ones, that may contribute to the retention of the analyte. Chiral recognition of basic compounds on CBH I is assumed to primarily depend on the formation of ion pair bindings with acidic amino acid residues located in the catalytic domain of the protein [6,15,16]. Hydrophobic interactions are involved in both achiral and chiral retention mechanisms [10,13,15].

Stereoselective separations on immobilised CBH I are generally optimized by the composition of the aqueous mobile phase [6,13–15]. Owing to the prime importance of ionic interactions, chiral resolution is mainly affected by the pH value of the buffer solution. The ionic strength of the buffer as well as the nature and concentration of organic modifiers are further parameters which were used to control the enantioseparation, whereas temperature effects were often disregarded. However, experiments on (*RS*)-propranolol have shown that the temperature also strongly influences the retention and enantioselectivity on CBH I [17,18]. In this particular case, unusual effects occur in the temperature range between 5 and 45°C. The retention of the more retained (*S*)-(−)-enantiomer increases with rising temperature, while the retention factor of the less retained (*R*)-(+)-enantiomer is reduced. The endothermic adsorption behavior of (*S*)-(−)-propranolol was only observed at higher pH values (pH 5.5). At lower pH (pH 4.7), the overall retention of each enantiomer is characterized by exothermic enthalpy changes. In both cases, at pH 5.5 and pH 4.7, an increase in temperature results in higher resolution factors.

Due to the comparatively low column temperatures, chiral separations by liquid chromatography are almost dominated by enthalpy control and thus enantioselectivity improves with decreasing temperature. Entropy-governed enantioseparations, as observed for the chiral resolution of (*RS*)-propranolol on CBH I, are rare. Cabrera and Lubda [19] reported an instance of an entropically driven enantioseparation on a β-cyclodextrin bonded phase in which the resolution factor of (*RS*)-prominal is diminished by decreasing temperature. It is evident that a progressive reduction of the column temperature will lower the enantioselectivity to a minimum resulting in a co-elution of the enantiomers at a certain temperature. Below this ‘‘crossover’’ temperature the enantiomers should elute in the opposite order.

Since the temperature range applicable for the optimization of gas chromatographic separations is broad, temperature dependent inversions of the enantiomeric elution order were first observed with this technique [20–24]. In high-performance liquid chromatography (HPLC) only few examples are noted.

Pirkle and Murray presented an interesting temperature study from a brush-type CSP [25]. A change in temperature causes a peak inversion between 10 and −25°C, whereas above 10°C the separation is enhanced as the temperature is increased. Balmér et al. described a reversal of the enantioselectivity on a cellulose-derived chiral column (Chiracel-OD) [26]. The inversion reported to take place only in the presence of a small water content in the nonaqueous mobile phase occurs between 35 and 45°C. A further example is given for a CSP based on α_1-glycoprotein (Chiral-AGP) where the enantiomeric elution order of mosapride and its metabolite was found to be temperature dependent and separation in the inverted order is achieved above 30°C and 20°C, respectively [27]. It should be mentioned that peak inversions by temperature changes were generally only observed for separations with marginal enantioselectivity.

Under special conditions (high pH, long column) the enantiomers of sotalol can also be separated on a CBH I column (Chiral-CBH, Chromtech, Hägersten, Sweden) [6], even though the resolution is rather poor when compared with those of the classical β-blockers. In our hands, this enantioseparation performed at ambient temperature was not reproducible day by day. This prompted us to study thoroughly the effect of the temperature on the chiral discrimination process of (*R*)-(−)- and (*S*)-(+)-sotalol by CBH I. We established the prime importance of this factor and could observe an inversion of the enantiomeric elution order within the investigated temperature range. Furthermore, (*R*)-(−)-sotalol was found to exhibit the same unusual endothermic adsorption behavior as previously reported for (*S*)-(−)-propranolol. In the present study we extensively investigate the influence of the mobile phase parameters (nature and concentration of organic modifiers, mobile phase pH) on the temperature dependence of the retention and enantioselectivity in the chiral separation of (*RS*)-sotalol.

2. Experimental

2.1. Chemicals

(*S*)-(+)-Sotalol hydrochloride and *rac*-sotalol hydrochloride were kindly provided by Bristol-Myers Squibb (Regensburg, Germany). All other chemicals were of HPLC- or analytical-grade.

2.2. Apparatus

The chromatographic system consisted of a Waters 501 HPLC pump (Waters, Eschborn, Germany), a Rheodyne Model 7725 injector equipped with a 20-μl loop and a FD-500 programmable fluorescence detector from Groton Technology (Concord, MA, USA). The temperature of the column was controlled by a Jetstream 2 peltier column thermostat (VDS/Optilab, Montabaur, Germany) providing an accuracy of ±0.5°C. Chromatographic data were recorded using ChromStar Light software from SCPA (Stuhr, Germany). The study was performed on a Chiral-CBH column (150×4.0 mm I.D., 5 μm) (Chromtech) coupled with a corresponding guard column (Chiral-CBH, 10×3.0 mm I.D., 5 μm).

2.3. Chromatographic conditions

The mobile phase was a 0.01 *M* sodium phosphate buffer (pH 6.0, 6.5, 7.0) containing 0%, 5%, 10%, 15% (v/v) 2-propanol (2-PrOH) or 0%, 5%, 10% (v/v) acetonitrile (MeCN) and 0.05 m*M* disodium EDTA. Prior to use the mobile phase was filtered through a 0.45 μm RC (regenerated cellulose) filter and degassed with a helium sparge. The flow-rate was adjusted to 0.9 ml/min. Fluorescence detection was carried out at 250 nm (excitation wavelength, λ_{ex})/315 nm (emission wavelength, λ_{em}). The hold-up time was determined with potassium bromide by UV detection at 230 nm (t_M=1.40 min at a flow-rate of 0.9 ml/min) and has been found not to change with the temperature. Sample solutions of *rac*-sotalol (30 μg/ml) were prepared with the mobile phase. To verify the enantiomeric elution order a (*S*)-(+)-sotalol enriched sample solution [(*S*)-(+)-sotalol HCl 15 μg/ml, (*R*)-(−)-sotalol HCl 7.5 μg/ml] was used. Chromatographic data were collected in four parallel runs. Most of the studies were performed on two different Chiral-CBH columns to confirm the validity of the observed effects.

2.4. Calculation

The retention factors of the (*S*)- and the (*R*)-

enantiomer k_S, k_R and the separation factor α are used to characterize the retention and the enantioselectivity of the chiral separations as follows: $k=(t-t_M)/t_M$; $\alpha=k_S/k_R$ for $k_S>k_R$ and $\alpha=k_R/k_S$ for $k_R>k_S$ where t is the retention time of one enantiomer, and t_M is the hold-up time.

The shape of the peaks is described by the asymmetry factor A_S, calculated from the widths of the descending slope of the peak (b) and the ascending slope of the peak (a) at 13.5% of the peak height as $A_S=b/a$ [28]. The resolution R_S was determined according to a modified resolution function for non-symmetrical peaks [28]. R_S is defined on the basis of the width of the trailing half of the first peak ($^1b_1$) and the width of the leading half of the second peak ($^2a_2$). Both $^1b_1$ and $^2a_2$ are measured at 13.5% of the height of each individual peak (h_1, h_2). The modified resolution function yields two resolution values for a pair of successive peaks, for the first peak

$$^1R_{S21}=\frac{t_2-t_1}{^1b_1+{}^2a_2\sqrt{1+0.5\ln h_2/h_1}}$$

and for the second peak

$$^2R_{S21}=\frac{t_2-t_1}{^1b_1\sqrt{1+0.5\ln h_1/h_2}+{}^2a_2}$$

3. Thermodynamics of retention and enantioselectivity

The molar Gibbs free energy of an adsorption process, ΔG^0, is expressed by:

$$\Delta G^0=\Delta H^0-T\Delta S^0=-RT\ln K \quad (1)$$

where R is the universal gas constant and T the absolute temperature (K). K represents the thermodynamic equilibrium constant. Assuming that the retention of an analyte on a stationary phase is limited to one single type of mechanism, the equilibrium constant is directly related to the retention factor k [18,29]:

$$k=K\phi \quad (2)$$

In Eq. (2), ϕ represents the phase ratio of the column, calculated by the volume of the stationary phase divided by the void volume of the column.

Hence, the Van 't Hoff equation can be used to describe the temperature dependence of the retention factor k [18]:

$$\ln k=-\frac{\Delta H^0}{R}\cdot\frac{1}{T}+\frac{\Delta S^0}{R}+\ln\phi \quad (3)$$

The plot of ln k versus $1/T$ permits the determination of the standard molar enthalpy of adsorption ΔH^0 from the slope of the line, while the intercept is related to the adsorption entropy ΔS^0. A linear relationship is obtained if ΔH^0 and ΔS^0 do not change within the relevant temperature range (which is usually the case). Non-linear Van 't Hoff plots are often due to a mixed retention mechanism and are generally to be expected if the surface of the stationary phase is heterogeneous [18].

In the separation of enantiomers by chromatographic methods chiral discrimination is determined by the difference between the free energy of adsorption of each enantiomer $\Delta_{S,R}\Delta G^0$ [30,31]:

$$\Delta_{S,R}\Delta G^0=\Delta_{S,R}\Delta H^0-T\Delta_{S,R}\Delta S^0=-RT\ln\frac{K_S}{K_R} \quad (4)$$

In Eq. (4), the subscript S corresponds arbitrarily to the more retained enantiomer, the subscript R to the less retained enantiomer. $\Delta_{S,R}\Delta G^0$ is composed of an enthalpic term, $\Delta_{S,R}\Delta H^0$, and an entropic term, $T\Delta_{S,R}\Delta S^0$, the latter of which is temperature dependent. At low temperatures enantioselectivity, expressed by $\Delta_{S,R}\Delta G^0$, is mainly influenced by $\Delta_{S,R}\Delta H^0$. With increasing temperature the enthalpic term will be more and more compensated by $T\Delta_{S,R}\Delta S^0$. At a certain temperature, the isoenantioselective temperature T_{iso}, $\Delta_{S,R}\Delta G^0$ equals zero and the enantiomers are not longer separated. Above T_{iso} the elution order of the enantiomers reverses. Enantioselectivity is now dominated by the entropic term.

The differences in adsorption enthalpy $\Delta_{S,R}\Delta H^0$ and adsorption entropy $\Delta_{S,R}\Delta S^0$ are conveniently obtained by plotting the natural logarithm of the separation factor α ($\alpha=K_S/K_R$) versus the reciprocal of the absolute temperature T [30,31]:

$$\ln\alpha=-\frac{\Delta_{S,R}\Delta H^0}{R}\cdot\frac{1}{T}+\frac{\Delta_{S,R}\Delta S^0}{R} \quad (5)$$

According to Eq. (4), the isoenantioselective temperature T_{iso} can be calculated as follows [31]:

$$T_{iso} = \frac{\Delta_{S,R}\Delta H^0}{\Delta_{S,R}\Delta S^0} \quad (6)$$

4. Results and discussion

CBH I-based chiral phases have been used successfully for the chiral analyses of almost all β-receptor antagonists. The (*S*)-enantiomer is reported to be always the more retained component [14]. Furthermore, the retention as well as the separation factor have been found to correlate with the hydrophobicity (log *P*) of the β-blockers [10]. The comparatively low enantioselectivity achieved in the enantioseparation of sotalol on this CSP may be causally related to both the structural differences and the high hydrophilicity of the compound.

To investigate the effect of temperature on the chiral resolution of sotalol by CBH I a study was carried out between 5 and 40°C. Since no details were given about the stability of Chiral-CBH at 35 and 40°C, high temperature exposure of the column was kept as short as possible. Thus, a consistent chromatographic performance of the protein CSP has been ascertained. Fig. 2 shows the chromatograms obtained at different temperatures when *rac*-sotalol is separated on Chiral-CBH using a mobile phase consisting of 5% 2-propanol in phosphate buffer, pH 7.0. The chromatographic data for the enantioseparations are given in Table 1. The retention time of the second eluted (*S*)-(+)-enantiomer rapidly decreases with increasing temperature, while the (*R*)-(−)-enantiomer exhibits an unusual endothermic adsorption behavior as previously observed for (*S*)-(−)-propranolol [17,18]. Hence, an increase in temperature results in slightly higher retention times for (*R*)-(−)-sotalol. As a consequence of the opposite behavior of both enantiomers, the enantioselectivity is reduced by raising the column temperature up to 25°C. Between 25 and 30°C the enantiomeric elution order reverses with the (*S*)-(+)-enantiomer being less retained at higher temperatures. The Van 't Hoff plots resulting from this temperature study are illustrated by Fig. 3. The negative slope of the plot of the (*R*)-(−)-enantiomer corresponds to an endothermic

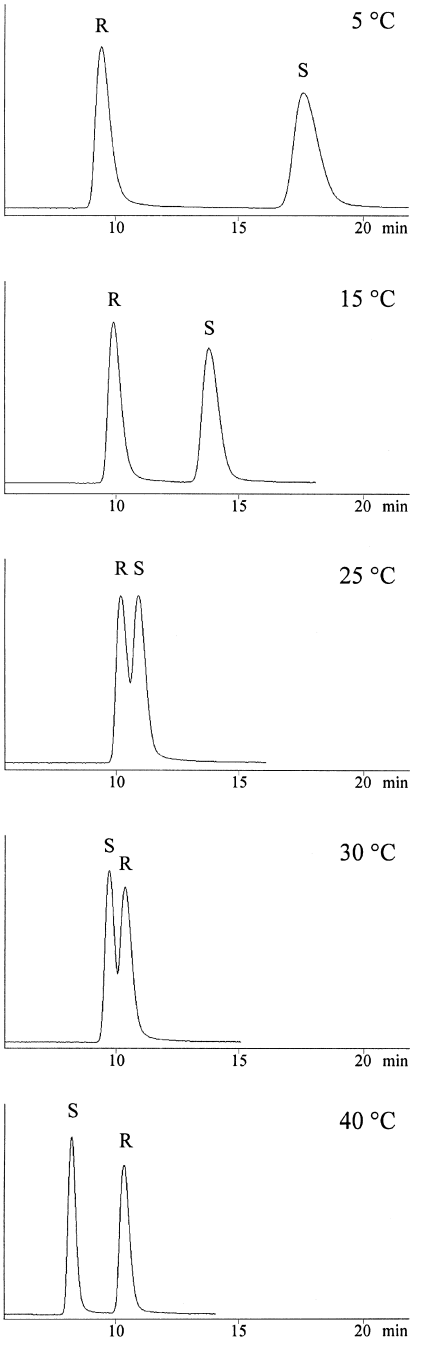

Fig. 2. Influence of temperature on the chiral separation of (*RS*)-sotalol on Chiral-CBH. Conditions: column, Chiral-CBH (5 μm), 150×4 mm I.D.; mobile phase, 5% 2-propanol in 0.01 *M* sodium phosphate buffer, pH 7.0; flow-rate, 0.9 ml/min; detection, fluorescence 250 nm (λ_{ex})/315 nm (λ_{em}); sample, 20 μl of a solution of (*RS*)-sotalol HCl (0.03 mg/ml) in the mobile phase.

Table 1
Effect of temperature on the chromatographic data for the chiral separation of (*RS*)-sotalol[a]

Temperature (°C)	k_S	k_R	α	$R_{S\,S}$	$R_{S\,R}$	$A_{S\,S}$	$A_{S\,R}$
5	11.58	5.79	2.00	5.36	5.90	1.53	1.61
10	10.11	5.93	1.71	4.43	4.75	1.56	1.80
15	8.79	6.06	1.45	3.35	3.51	1.49	1.71
20	7.76	6.17	1.26	2.14	2.19	1.49	1.75
25	6.78	6.26	1.08	–	–	–	–
30	5.92	6.39	1.08	–	–	–	–
35	5.29	6.42	1.21	2.36	2.26	1.50	1.65
40	4.87	6.36	1.31	3.37	3.22	1.39	1.54

[a] Column: Chiral-CBH (5 μm), 150×4 mm I.D. Mobile phase: 5% 2-propanol in 0.01 *M* sodium phosphate buffer, pH 7.0. Flow-rate: 0.9 ml min^{-1}.

adsorption enthalpy. The Van 't Hoff plots of the two enantiomers intersect at the isoenantioselective temperature (T_{iso}) which was calculated from the regression equations presented in Fig. 3 to be 27.8°C (300.8 K). Beyond T_{iso}, good separations with comparatively high resolution factors are obtained either by decreasing or by increasing the temperature. From 5 to 30°C the Van 't Hoff plots are nearly linear, whereas at higher temperatures non-linear behavior, especially for the descending plot, indicates a change in the retention mechanism. Chiral protein phases must be considered as heterogeneous surfaces which include various chiral and achiral binding sites contributing to the overall retention of the solute. Fornstedt et al. have illustrated the errors arising from the direct derivation of the thermodynamic parameters of adsorption from the Van 't Hoff plots of the retention factors [18]. Hence, in this paper we dispense with details about adsorption enthalpies and entropies.

Further experiments with varying mobile phases were created in order to extensively study the temperature dependence of the retention factors and the enantioselectivity in the chiral separation of (*RS*)-sotalol. The endothermic characteristic of the adsorption process of (*R*)-(−)-sotalol is not observed

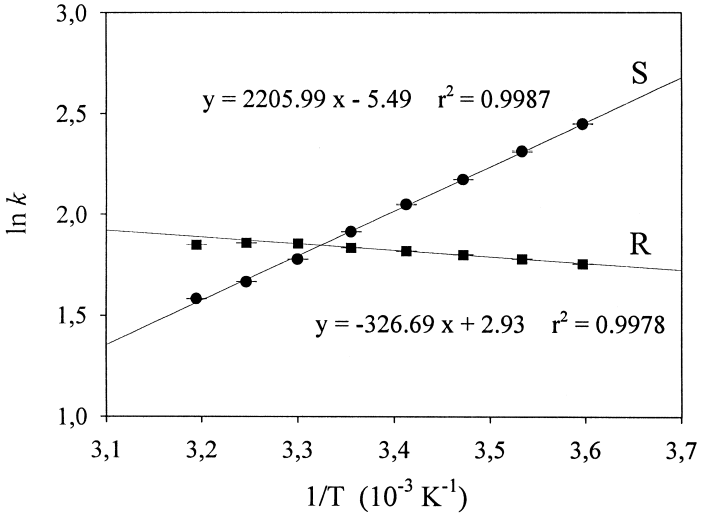

Fig. 3. Van 't Hoff plots for the separation of the enantiomers of sotalol on Chiral-CBH. Temperature range, 5–40°C; mobile phase, 5% 2-propanol in 0.01 *M* sodium phosphate buffer, pH 7.0; flow-rate, 0.9 ml/min; symbols, mean of four injections, (●) (*S*)-(+)-sotalol, (■) (*R*)-(−)-sotalol; error bars, standard deviation.

for any mobile phase used. In some cases, the retention time of the (*R*)-(−)-enantiomer remains almost constant over the entire temperature range. In other cases, both enantiomers exhibit an exothermic adsorption behavior. However, the temperature dependence of the retention factors of (*S*)-(+)- and (*R*)-(−)-sotalol always strongly differs so that an inversion in the elution order by changes in temperature is obtained in each series of experiments with the ''crossover'' temperature T_{iso} depending on the composition of the mobile phase. The reversal of the enantioselectivity generally occurs within the range of the ambient temperature, i.e., between 17 and 28°C.

The effect of the addition of an uncharged organic modifier to the buffer solution on the Van 't Hoff plots of (*S*)-(+)- and (*R*)-(−)-sotalol is presented in Fig. 4. An organic modifier influences the hydrophobic interactions of the solute with both the chiral and achiral binding sites [6,10,13,15]. As expected, the retention times of both enantiomers decrease by the addition of an organic solvent to the mobile phase. Owing to the increasing peak symmetry, the resolution R_S between the two peaks is improved. Furthermore, an organic solvent may induce conformational changes in the protein structure and thereby possibly affects the enantioselectivity [6,15]. Using a mobile phase without an organic modifier, the global adsorption processes of both enantiomers are exothermic. The addition of 5% 2-propanol or of 10% acetonitrile changes the character of the global adsorption of (*R*)-(−)-sotalol which now exhibits a slightly endothermic behavior. When the buffer solutions without an organic solvent or with 5% 2-propanol are used as the mobile phase, the peak coalescence occurs at 27.1°C and 27.8°C, respectively, and thus the (*R*)-(−)-enantiomer is first eluted at a temperature of 23°C. Using the mobile phase containing 10% acetonitrile, separation in the inverted order is already observed at temperatures above 18°C (T_{iso} = 17.8°C) and thereby the (*R*)-(−)-enantiomer is more retained at 23°C (Fig. 5). In the enthalpy-controlled separations below T_{iso} as well as in the entropically driven separations above T_{iso} an improvement of the enantioselectivity is achieved if 2-propanol is added to the buffer solution. Acetonitrile as the mobile phase additive has only a minor influence on the enantioselectivity and the higher resolution factors for the enantioseparations at 25°C and 30°C merely result from the comparatively low coalescence temperature T_{iso}.

Interesting effects on the thermodynamic functions

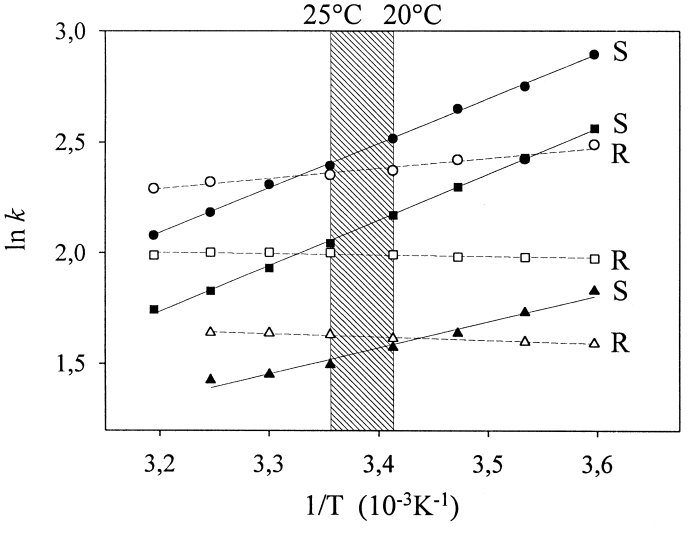

Fig. 4. Influence of the organic modifier on the Van 't Hoff plots of (*R*)-(−)- and (*S*)-(+)-sotalol. Temperature range, 5–40°C; mobile phase, 0.01 *M* sodium phosphate buffer, pH 7.0 ● ○ without an organic modifier (T_{iso} = 27.1°C), ■ □ with 5% 2-propanol added (T_{iso} = 27.5°C), ▲ △ with 10% acetonitrile added (T_{iso} = 17.8°C); symbols, mean of four injections, (*S*)-(+)-sotalol (filled symbols), (*R*)-(−)-sotalol (blank symbols).

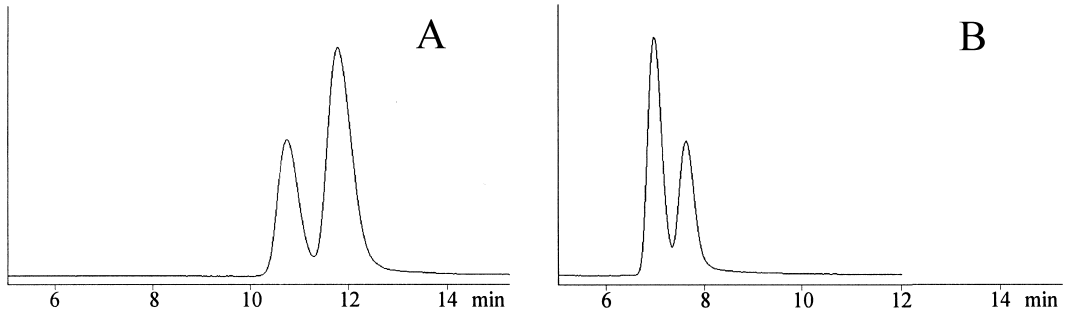

Fig. 5. Influence of the composition of the mobile phase on the elution order of the sotalol enantiomers on Chiral-CBH. Conditions: column, Chiral-CBH (5 μm), 150×4 mm I.D.; flow-rate, 0.9 ml/min; temperature, 23°C; detection, fluorescence 250 nm (λ_{ex})/315 nm (λ_{em}); sample, 20 μl of a solution of (*R*)-(−)- and (*S*)-(+)-sotalol HCl in the ratio 1:2; mobile phase, 0.01 *M* sodium phosphate buffer, pH 7.0 containing (A) 5% 2-propanol, (B) 10% acetonitrile.

of the global adsorption process are obtained by changes in the modifier concentrations. Figs. 6 and 7 show the Van 't Hoff plots of the (*S*)-(+)- and (*R*)-(−)-enantiomer at various concentrations of 2-propanol and acetonitrile, respectively. An increase in the 2-propanol concentration generally reduces the retention time of (*S*)-(+)-sotalol (Fig. 6). For the (*R*)-(−)-enantiomer the character of the adsorption behavior varies with the modifier concentration being endothermic with 5% 2-propanol and exothermic with 15% 2-propanol. Therefore, a higher percentage of 2-propanol causes a diminution of the retention time at temperatures between 10 and 40°C, but at 5°C the retention factor remains unchanged.

In chiral separations of classical β-blockers on CBH I a slight improvement of the enantioselectivity is usually obtained if the percentage of 2-propanol is enhanced [6,10,13]. In our studies on *rac*-sotalol such a positive effect is limited to the enantioseparations above T_{iso} which are governed by entropic factors, while for the enthalpy-controlled separations below T_{iso} an increase in the 2-propanol concentration results in smaller resolution factors (Table 2). Similar effects of the modifier concen-

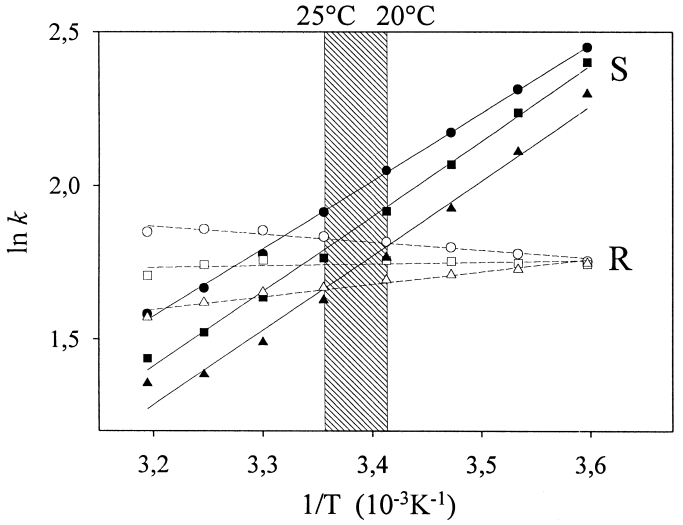

Fig. 6. Influence of the 2-propanol concentration on the Van 't Hoff plots of (*R*)-(−)- and (*S*)-(+)-sotalol. Temperature range, 5–40°C; mobile phase, 0.01 *M* phosphate buffer, pH 7.0 containing ● ○ 5% 2-PrOH (T_{iso}=27.6°C), ■ □ 10% 2-PrOH (T_{iso}=26.0°C), ▲ △ 15% 2-PrOH (T_{iso}=23.8°C); symbols, mean of four injections, (*S*)-(+)-sotalol (filled symbols), (*R*)-(−)-sotalol (blank symbols).

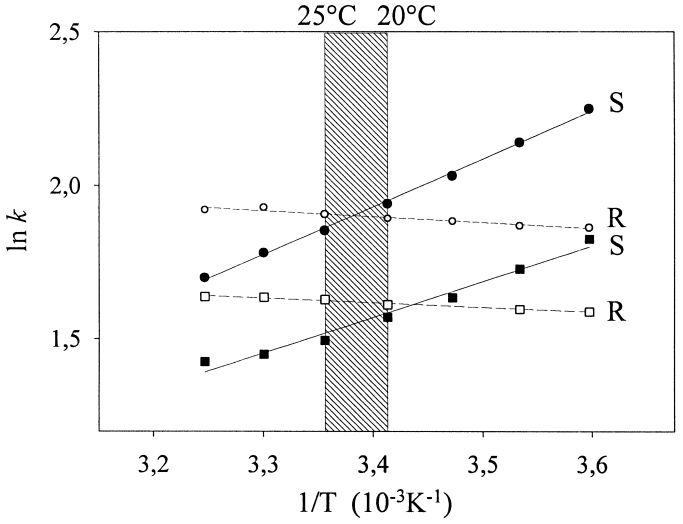

Fig. 7. Influence of the acetonitrile concentration on the Van 't Hoff plots of (*R*)-(−)- and (*S*)-(+)-sotalol. Temperature range, 5–35°C; mobile phase, 0.01 *M* phosphate buffer, pH 7.0 containing ● ○ 5% acetonitrile (T_{iso}=22.7°C), ■ □ 10% acetonitrile (T_{iso}=17.8°C); symbols, mean of four injections, (*S*)-(+)-sotalol (filled symbols), (*R*)-(−)-sotalol (blank symbols).

tration on the enantioselectivity were observed with acetonitrile as the mobile phase additive (Fig. 7). According to Fornstedt and co-workers [18,32], the stronger peak tailing generally obtained for (*R*)-(−)-sotalol (Tables 1 and 2) can be explained by the slower mass transfer kinetics of this enantiomer at the enantioselective adsorption sites.

The pH value of the buffer solution was found to be of prime importance for the chiral resolution of basic compounds on Chiral-CBH [6,10,13,15]. In the pH range used for the enantioseparations of β-blockers, i.e., pH 5 to 7, the net charge of the protein is negative, whereas the basic function of the solute is protonated. The Van 't Hoff plots of (*S*)-(+)- and (*R*)-(−)-sotalol chromatographed at different pH values are illustrated by Fig. 8. The retention times of both enantiomers extensively increase with increasing mobile phase pH. A reduction of pH influences the thermodynamic functions for the global adsorption process of (*R*)-(−)- and (*S*)-(+)-sotalol in an opposite way with the two Van 't Hoff plots approaching each other. As a result, the enantioselectivities of both the enthalpy- and entropy-controlled separations are rapidly lowered by a decrease of the mobile phase pH. Indeed, good separations are only obtained at pH 7.0. At lower pH

Table 2
Influence of 2-propanol concentration on the chiral separation of (*RS*)-sotalol at different temperatures[a]

Temperature (°C)	Organic modifier: 2-propanol (%)	k_S	k_R	α	$R_{S\,S}$	$R_{S\,R}$	$A_{S\,S}$	$A_{S\,R}$
10	5	10.11	5.93	1.71	4.43	4.75	1.56	1.80
	10	9.38	5.75	1.63	3.92	4.20	1.46	1.56
	15	8.26	5.64	1.46	2.90	3.07	1.30	1.46
35	5	5.29	6.42	1.21	2.36	2.26	1.50	1.65
	10	4.58	5.72	1.25	2.57	2.47	1.34	1.41
	15	3.99	5.05	1.27	2.49	2.38	1.42	1.37

[a] Column: Chiral-CBH (5 μm), 150×4 mm I.D. Mobile phase: 2-propanol in 0.01 *M* sodium phosphate buffer, pH 7.0. Flow-rate: 0.9 ml min^{-1}.

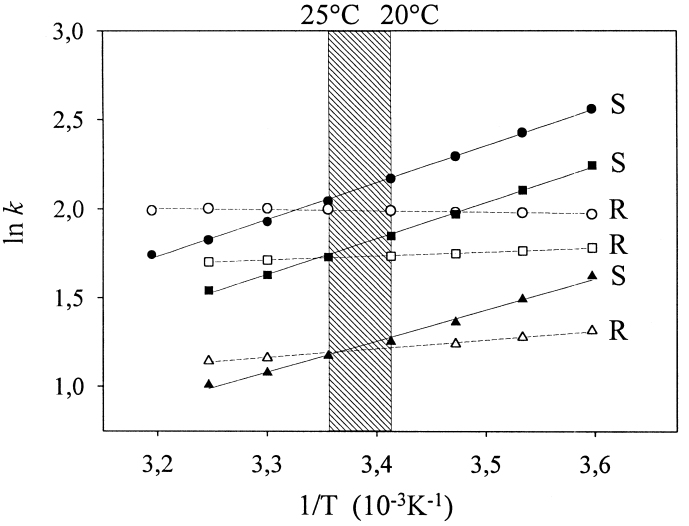

Fig. 8. Influence of the mobile phase pH on the Van 't Hoff plots of (*R*)-(−)- and (*S*)-(+)-sotalol. Temperature range, 5–35°C; mobile phase, 5% 2-propanol in 0.01 *M* phosphate buffer, ● ○ pH 7.0 (T_{iso}=27.5°C), ■ □ pH 6.5 (T_{iso}=26.0°C), ▲ △ pH 6.0 (T_{iso}=24.3°C); symbols, mean of four injections, (*S*)-(+)-sotalol (filled symbols), (*R*)-(−)-sotalol (blank symbols).

values (pH 6.5 and pH 6.0) the chromatographic resolution R_S is deteriorated by a strong peak tailing observed particularly for the (*R*)-(−)-enantiomer.

The influence of the ionic strength of the mobile phase on the enantioseparation of (*RS*)-sotalol by CBH I was studied at 23°C. Raising the buffer concentration from 10 to 20 m*M* strongly reduces the retention time of both sotalol enantiomers, while the resolution factor remains unchanged. Since no effect on the enantioselectivity was obtained, this mobile phase parameter was not further investigated.

5. Conclusion

The temperature is a determining factor for the chiral discrimination process of (*R*)-(−)- and (*S*)-(+)-sotalol by CBH I, as it influences not only the extent but also the sign of enantioselectivity. An inversion of the enantiomeric elution order was also induced by changes in the organic modifier added to the buffer solution.

The presented results emphasize the importance of a careful temperature control in order to achieve reproducible separations in chiral chromatography. Although reversals in enantioselectivity are rare, general predictions of the enantiomeric elution order on CSPs should be critically considered, especially if the extent of chiral recognition is marginal.

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References

[1] B.N. Singh, P. Deedwania, K. Nademanee, A. Ward, E.M. Sorkin, Drugs 34 (1987) 311.
[2] M.J. Antonaccio, A. Gomoll, Am. J. Cardiol. 65 (1990) 12A.
[3] Y. Okamoto, M. Kawashima, R. Aburatani, K. Hatada, T. Nishiyama, M. Masuda, Chem. Lett. (1986) 1237.
[4] W.H. Pirkle, J.A. Burke, J. Chromatogr. 557 (1991) 173.
[5] C. Vandenbosch, D.L. Massart, G. Egginger, W. Lindner, Trends Anal. Chem. 12 (1993) 168.
[6] J. Hermansson, A. Grahn, J. Chromatogr. A 687 (1994) 45.
[7] J. Hermansson, A. Grahn, J. Chromatogr. A 694 (1995) 57.
[8] J. Ekelund, A. van Arkens, K. Brønnum-Hansen, K. Fich, L. Olsen, P.V. Petersen, J. Chromatogr. A 708 (1995) 253.
[9] P.V. Petersen, J. Ekelund, L. Olsen, S.V. Ovesen, J. Chromatogr. A 757 (1997) 65.

[10] C. Vandenbosch, D.L. Massart, W. Lindner, Anal. Chim. Acta 270 (1992) 1.
[11] C. Vandenbosch, T. Hamoir, D.L. Massart, W. Lindner, Chromatographia 33 (1992) 454.
[12] A. Ceccato, P. Hubert, J. Crommen, J. Chromatogr. A 760 (1997) 193.
[13] I. Marle, P. Erlandsson, L. Hansson, R. Isaksson, C. Pettersson, G. Pettersson, J. Chromatogr. 586 (1991) 233.
[14] R. Isaksson, C. Pettersson, G. Pettersson, S. Jönsson, J. Ståhlberg, J. Hermansson, I. Marle, Trends Anal. Chem. 13 (1994) 431.
[15] I. Marle, S. Jönsson, R. Isaksson, C. Petterson, J. Chromatogr. 648 (1993) 333.
[16] C. Divne, J. Ståhlberg, T. Reinikainen, L. Ruohonen, G. Petterson, J.K.C. Knowles, T.T. Teeri, T.A. Jones, Science 265 (1994) 524.
[17] S. Jönsson, A. Schön, R. Isaksson, C. Pettersson, G. Pettersson, Chirality 4 (1992) 505.
[18] T. Fornstedt, P. Sajonz, G. Guiochon, J. Am. Chem. Soc. 119 (1997) 1254.
[19] K. Cabrera, D. Lubda, J. Chromatogr. A 666 (1994) 433.
[20] K. Watabe, R. Charles, E. Gil-Av, Angew. Chem., Int. Ed. Engl. 28 (1989) 192.
[21] V. Schurig, J. Ossig, R. Link, Angew. Chem., Int. Ed. Engl. 28 (1989) 194.
[22] B. Koppenhoefer, B. Lin, J. Chromatogr. 481 (1989) 17.
[23] W.A. König, D. Icheln, T. Runge, B. Pfaffenberger, P. Ludwig, H. Hühnerfuss, J. High Resolut. Chromatogr. 14 (1991) 530.
[24] V. Schurig, F. Betschinger, Chem. Rev. 92 (1992) 873.
[25] W.H. Pirkle, P.G. Murray, J. High Resolut. Chromatogr. 16 (1993) 285.
[26] K. Balmér, P.-O. Lagerström, B.-A. Persson, G. Schill, J. Chromatogr. 592 (1992) 331.
[27] A. Karlsson, A. Aspegren, Chromatographia 47 (1998) 189.
[28] P.J. Schoenmakers, J.K. Strasters, Á. Bartha, J. Chromatogr. 458 (1988) 355.
[29] J. Yang, D.S. Hage, J. Chromatogr. A 725 (1996) 273.
[30] B. Koppenhoefer, E. Bayer, Chromatographia 19 (1984) 123.
[31] V. Schurig, J. Chromatogr. A 666 (1994) 111.
[32] T. Fornstedt, G. Zhong, Z. Bensetiti, G. Guiochon, Anal. Chem. 68 (1996) 2370.