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Adsorption isotherms and retention behavior of 1,1′-bis(2-naphthol) on CHIRIS AD1 and CHIRIS AD2 columns

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Abstract

The separation of the atropoisomers of 1,1′-bis(2-naphthol) was studied on CHIRIS AD1 and CHIRIS AD2, two Pirkle-type chiral stationary phases. Satisfactory selectivity was found only on CHIRIS AD2. The ternary mobile phases comprised hexane, dichloromethane and methanol. The effects of their composition and of the temperature on the retention under analytical conditions and on the single-component and competitive isotherms were investigated. The retention of the *R*- and *S*-isomers on CHIRIS AD1 and CHIRIS AD2 is controlled by the enthalpic contribution to adsorption, but the effect of the mobile phase on the retention should be attributed mainly to the entropic contribution. The adsorption of the less retained *R*-isomer is controlled by the achiral interactions, which are the same as for the *S*-isomer. The single-component and competitive isotherms of the *R*- and *S*-isomers are adequately described by the sum of a Langmuir term for the achiral contribution to adsorption and a linear-term characterising the selective or chiral adsorption of the *S*-isomer in the concentration range experimentally available, i.e. within the solubility limit of 1,1′-bis(2-naphthol).

Keywords: Chiral stationary phases, LC; Adsorption isotherms; Atropoisomer separation; 1,1′-bis(2-Naphthol)

1. Introduction

Owing to ever increasing demand, the development of methods for obtaining pure enantiomers has become very important in pharmaceutical industry. The direct separation by preparative HPLC is now widely used to prepare optically pure drugs. Various types of chiral stationary phases (CSP) are available for analytical and preparative chiral separations. Unfortunately, there is no universal CSP generally suitable for all types of chiral separations and the column and separation conditions should be carefully selected and optimised for

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each specific problem. An excellent review of the recent advances in the development of chiral stationary phases was published by Lammerhofer and Lindner [1].

So-called Pirkle stationary phases represent an important type of CSP. They have been widely used for almost twenty years. Their chiral selectivity is based on the π-electron donor–acceptor interactions. Usually, a π-electron acceptor group such as 3,5-dinitrophenyl glycine (DNPG) or a π-electron donor group, such as 2-naphthylamine, are chemically bonded to a silica gel support material. Proper selection of a suitable π-electron donor or acceptor allows the adjustment of the separation selectivity of a Pirkle-CSP. The elution order of the enantiomers can even be affected. Pirkle-CSPs are usually employed in normal-phase systems, with mobile phases containing 2-propanol or acetonitrile in hexane.

A new type of Pirkle-CSP was introduced lately, containing 2,4,5,6-tetrachloro-1,3-dicyanobenzene as an intermediate group between a chiral selector and the silica gel surface to which it is covalently bonded via a propyl- or a butyl- "spacer" group [2–4]. The 2,4,5,6-tetrachloro-1,3-dicyanobenzene group can be substituted by chiral selector π-electron-donor or -acceptor groups, enabling the synthesis of a broad spectrum of new, highly stable and efficient CSPs [5,6]. This type of CSPs is available commercially from the IRIS company. CHIRIS columns contain one or two relatively small chiral selectors with one or more chiral adsorption centres. The cyanobenzene group provides additional π–π-electron interactions with optical isomers. The columns are generally suitable for the chiral separations of strong π-electron acceptors, such as 3,5-dinitrobenzoyl derivatives of amino acids, and also of π-electron donors, for example, substituted phenols. The columns show sufficient stability even for the separations of strong acids and bases [7,8].

In this work, we tested the possibility of using a CHIRIS AD1 column with a phenyl group on the chiral centre and a CHIRIS AD2 column with an aliphatic branched chain on the chiral centre (see structures in Fig. 1) for the separation of the *R*- and *S*-isomers of 1,1′-bis(2-naphthol). This molecule contains no asymmetrical carbon atom, but the interaction between the two hydroxyl groups in *ortho*-positions to the covalent single bond connecting the two naphthalene rings causes steric hindrance and prevents free rotation around this connecting bond. So, 1,1′-bis(2-naphthol) has two atropoisomers, *R*- and *S*-, with the naphthalene rings swivelled out of plane by an average angles of $+10^\circ$ and -10°, respectively.

In preparative HPLC, the production costs should be kept as low as possible, which requires the selection of a stationary phase with a high selectivity and a high loading capacity as a pre-requisite for the achievement of a high production rate. Hence, it is usually necessary to overload the column, i.e. to operate with non-linear adsorption isotherms. The equation of the isotherm describing the experimental distribution data should be determined to allow the optimization of the separation conditions. Several models have been suggested to fit non-linear isotherms to single-component adsorption data describing the distribution of a component between the stationary and the mobile phases. The most common and simplest isotherm is the two-parameter Langmuir isotherm [9]:

$$Q = \frac{ac}{1 + bc} \tag{1}$$

Here, Q is the concentration of the sample compound in the stationary phase, c that in the mobile phase, a (dimensionless) and b (l/mol) are the coefficients of the isotherm ($a = k_0/\phi$, where k_0 is the retention factor of the sample compound at infinite dilution, i.e. in analytical chromatography; $\phi = V_S/V_M$, the ratio of the volumes of the stationary, V_S; and the mobile, V_M, phases in the column and $b = a/q_s$, where q_s (mol/l) is the column saturation capacity).

Often the experimental data fit poorly to the Langmuir model and more complex isotherm models should be used. If a compound can be adsorbed on two different adsorption centres (1 and 2), the distribution can often be described by a bi-Langmuir model, with different coefficients a_1, b_1 and a_2, b_2 characterising the adsorption energies and saturation capacities of the two adsorption centres 1 and 2, $q_{s1} = a_1/b_1$ and $q_{s2} = a_2/b_2$, respectively [10]:

$$Q = \frac{a_1 c}{1 + b_1 c} + \frac{a_2 c}{1 + b_2 c} \tag{2}$$

The bi-Langmuir isotherm Eq. (2) is often useful to describe the distribution of two enantiomers if enantioselective and non-selective interactions can be ascribed

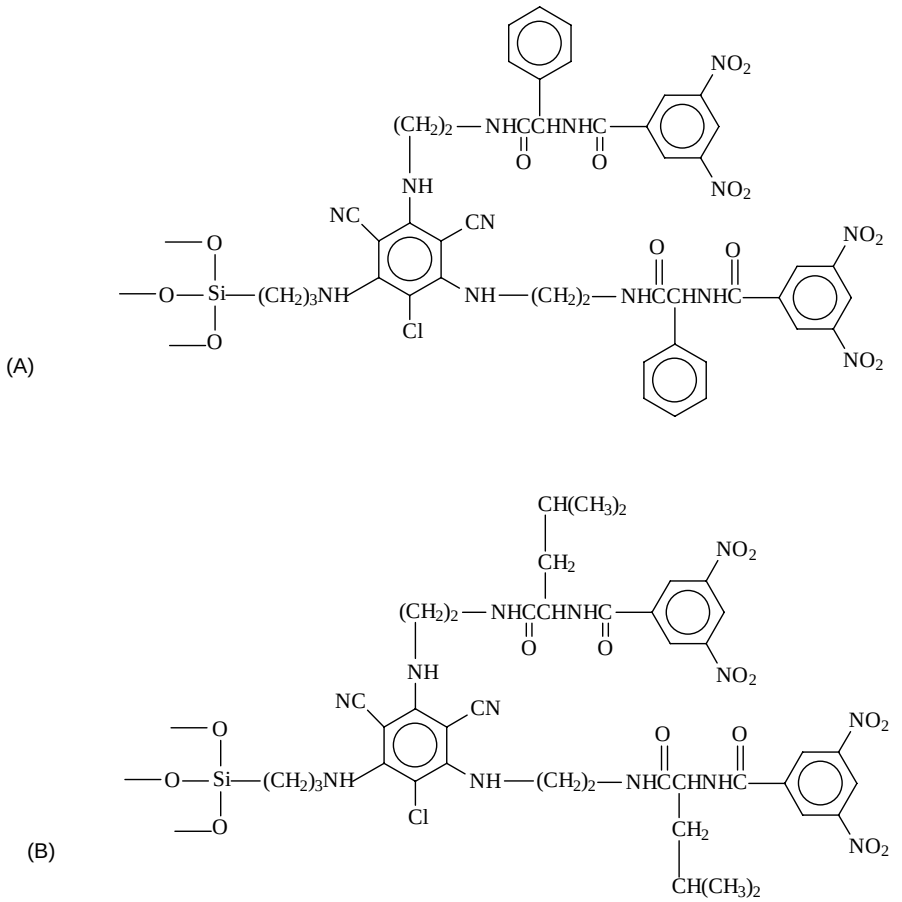

Fig. 1. Structures of the: (A) CHIRIS AD1 and (B) CHIRIS AD2 chiral stationary phases.

to different types of adsorption sites. Both enantiomers interact equally with the non-selective sites 1 and the selectivity arises from the difference between the enantioselective interactions with the chiral adsorption centres 2 [11].

The coefficients of the isotherms are affected by the properties of the stationary phase and by the composition of the mobile phase used for the separation. The main factors affecting the distribution isotherm are the temperature, the mobile phase composition, and for ionizable compounds, the pH and the ionic strength.

In the present work, we investigated the effects of the mobile phase composition and of the temperature on the retention time and the chiral selectivity of the atropoisomers of 1,1′-bis(2-naphthol) on both CHIRIS AD1 and CHIRIS AD2.

2. Experimental

2.1. Sample test compounds

R,*S*-1,1′-bis(2-naphthol) (racemate), *R*-1,1′-bis(2-naphthol), *S*-1,1′-bis(2-naphthol), reagent grade, TCI, Tokyo, Japan.

2.2. Columns

CHIRIS AD1, 5 μm, 150 mm × 4.6 mm (V_m = 1.78 ml), and CHIRIS AD1, 5 μm, 150 mm × 4.6 mm

($V_m = 1.81$ ml), both from IRIS Technologies L.L.C., Lawrence, KS, USA (Fig. 1).

2.3. Instrumentation

To acquire the data necessary for the determination of the equilibrium isotherms, an HP 1090 M liquid chromatograph (Hewlett-Packard, Palo Alto, CA, USA) was used, equipped with a 3-DR solvent delivery system and solvent reservoirs continuously stripped with helium to degas the mobile phase and the sample solution, an automatic sample injector, a column switching valve, a temperature-controlled column compartment, a diode-array UV detector and a data workstation.

2.4. Mobile phases

Methanol, dichloromethane and *n*-hexane, all HPLC grade (Lichrosolv), were obtained from E.M. Merck, Darmstadt, Germany. The solvents were kept dry by storing their flasks over dried molecular sieves Dusimo S 5A. They were filtered on Millipore 0.45 μm filters before the use. The mobile phases were prepared by mixing the components in the required ratios and were degassed by ultrasonication before use. The sample solutions used for the chromatographic measurements and for the determination of the distribution data were prepared by weighing the required amounts of sample solutes and dissolving them in the mobile phase.

2.5. Measurement of the retention data and determination of the distribution isotherms of enantiomers by frontal analysis

The retention data were measured at the required temperature by injecting 20 μl volumes of the racemate and of the pure isomers at a mobile phase flow rate of 1 ml/min, using UV detection at 355 nm. The overloaded injection profiles were measured for sample volumes of 250, 500 and 750 μl. All experiments were performed at least in duplicate.

The equilibrium isotherms were measured using the frontal analysis method [12]. The mobile phase was stored in one of the solvent flasks of the solvent delivery system of the liquid chromatograph and the solution of sample in a solvent of the same composition in another such flask. The gradient-delivery system was used to pump and mix the two solutions in the ratio needed for the frontal analysis experiments.

The ratio of the flow-rates of the two solutions controls the concentration of the solute delivered continuously to the column. It was adjusted from 0 to 100% in successive 5 or 10% steps. Time was allowed for the stabilization of the detector signal after each concentration change. The flow-rate (1 ml/min) and the column temperature (25, 35 or 45 °C) were kept constant during all the experiments.

In each experiment, the solute concentration at equilibrium in the stationary phase was determined from the integral mass balance equation, using the experimental retention volume (inflection point of the break-through curve), corrected for the volume of the tubing between the mixing point of the liquids pumped in each channel and the column top (0.88 ml) [13]. The solutions of the individual atropoisomers and of the racemate of 1,1′-bis(2-naphthol) in the appropriate mobile phase were used for measuring the isotherms. The elution volumes of the breakthrough curves and the concentrations of the individual sample compounds corresponding to the intermediate plateaus on the detector response were measured for 10–20 subsequent steps of frontal analysis. The data obtained were used in the appropriate mass-balance equation (Eq. (3)) [13] using a spread-sheet program run on a Pentium personal computer. The ADSTAT software (Trilobyte, Prague, Czech Republic) was used to fit the isotherm data by non-linear regression analysis:

$$Q_{i+1} = Q_i + \frac{(c_{i+1} - c_i)(V_{i+1} - V_m)}{V_s} \quad (3)$$

Here, c_i and c_{i+1} are the concentrations of the compound in the mobile phase in equilibrium with the stationary phase in the column in the steps i and $(i+1)$, respectively, Q_i and Q_{i+1} the concentrations of the adsorbed compound in the steps i and $(i+1)$, respectively, V_{i+1} is the retention volume at the inflection point of the $(i+1)$th step on the frontal analysis curve, V_m the column hold-up volume, i.e. the volume of the mobile phase in the column determined as the elution volume of a non-retained marker, here, tri-*tert*-butyl benzene which was shown not to be retained in ternary mobile phases containing the relatively high concentrations of polar solvents used in this work, and V_S the volume of stationary phase in the column,

determined as the difference between the geometrical volume of the empty column and the hold-up volume.

3. Results and discussion

3.1. Retention behaviour of 1,1′-bis(2-naphthol) under linear chromatography conditions

The normal-phase separation of the atropoisomers of 1,1′-bis(2-naphthol) on both CHIRIS AD1 and CHIRIS AD2 was attempted in various binary mobile phases made of mixtures of: hexane–2-propanol,–dichloromethane and –1,4-dioxane. All attempts were unsuccessful. The selectivity was too low or negligible in all the binary mobile phases tested. By contrast, the two isomers could be resolved in ternary mobile phases consisting of hexane, dichloromethane, and methanol. The maximum concentration of methanol allowed by its limited solubility in the ternary mobile phase used was 5% (v/v).

To investigate the effects of the working conditions on the separation, the retention volumes, V_R, were measured for small amounts of the racemate and the pure atropoisomers injected in various mobile phases, containing 1–5% methanol and 39–69% dichloromethane in hexane, at seven different temperatures between 20 and 50 °C. As we had no possibilities to stabilize and control the column temperature at sub-ambient values, we could not perform measurements in a broader temperature range, which could have provided additional information. In all mobile phases and at all temperatures, the *R*-isomer was eluted before the *S*-isomer. As expected, the retention factors, $k = (V_R/V_m - 1)$ of the two isomers decreased with increasing temperature and increasing concentrations of methanol or of dichloromethane in the mobile phase. Fig. 2 illustrates the linear decrease of $\log k$ with increasing logarithm of the volume fraction of the polar solvent, φ, in the mobile phase. This linear relationship is often observed in normal-phase systems [14,15]. The same linear character was observed for the log–log plots of the retention of both atropoisomers versus the concentration of either dichloromethane or methanol, at all temperatures. However, because of the greater polarity of methanol, a 1% increase of the methanol concentration causes approximately the same decrease of the retention as a 10% increase of

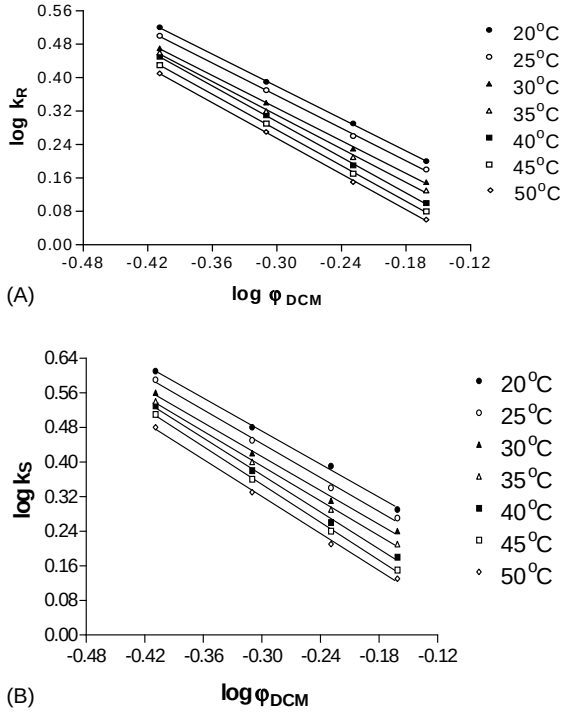

Fig. 2. Plots of the retention factors of the *R*-isomer (k_R, A) and *S*-isomer (k_S, B) of 1,1′-bis(2-naphthol) vs. the volume fraction of dichloromethane in mobile phases containing 2% (v/v) methanol at temperatures between 20 and 50 °C. Column CHIRIS AD2, 5 μm, 150 mm × 4.6 mm.

the dichloromethane concentration. The slopes of the straight lines in Fig. 2 increase only slightly with increasing temperature. The selectivity ($\alpha = k_S/k_R$) is practically independent of the composition of the mobile phase and, as shown in Fig. 3, is better on CHIRIS AD2 ($\alpha = 1.23$) than on CHIRIS AD1 ($\alpha = 1.13$), even though the isomers are more strongly retained on CHIRIS AD1.

The effect of the column temperature on the retention can be described by the well-known van't Hoff equation relating $\log k$ and the reciprocal of the thermodynamic temperature, $1/T$ (T (K)), Eq. (4) [16–18]:

$$\ln k = \ln K_D + \ln \Phi = -\frac{\Delta G^0}{RT} + \ln \Phi$$
$$= -\frac{\Delta H^0}{RT} + \frac{\Delta S^0}{R} + \ln \Phi \quad (4)$$

Here, K_D is the distribution constant of a solute, R the ideal gas constant, ΔG^0, ΔH^0 and ΔS^0 are the

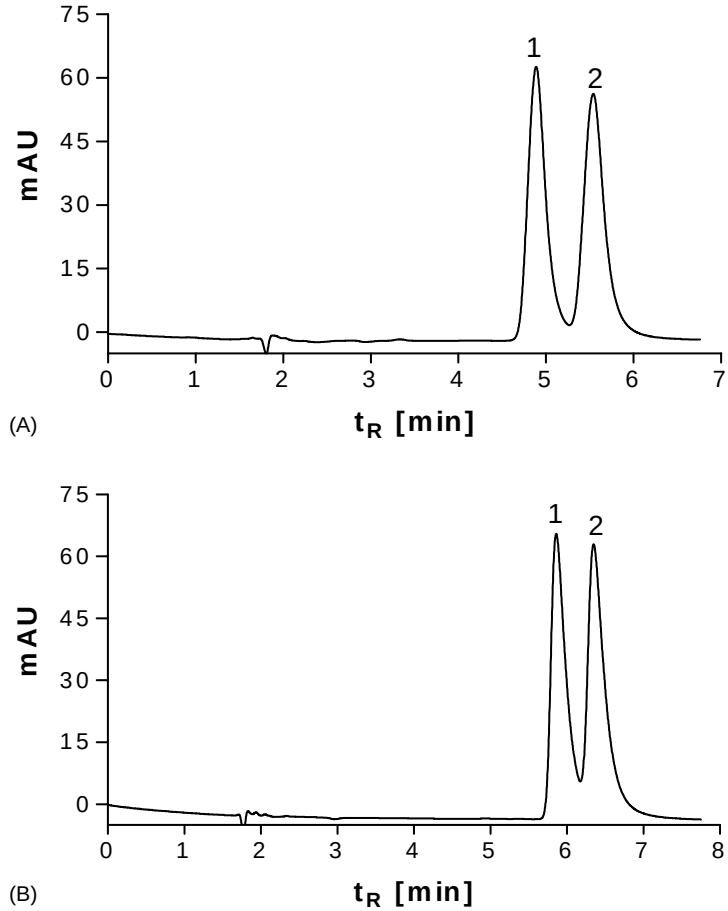

Fig. 3. Separation of 5 μl of 1,1′-bis(2-naphthol) racemate on: (A) CHIRIS AD2 and (B) CHIRIS AD1 columns (both 5 μm, 150 mm × 4.6 mm) in ternary mobile phase of hexane–dichloromethane–methanol, 39:59:2, at 30 °C. UV detection at 355 nm: (1) *R*-isomer; (2) *S*-isomer.

standard molar Gibbs energy, molar enthalpy and molar entropy of adsorption, respectively, and Φ the phase ratio of the column, i.e. the ratio of the volumes of stationary and mobile phases, $\Phi = V_S/V_m$.

The experimental van't Hoff plots for the two isomers on both the CHIRIS AD1 and CHIRIS AD2 columns in all mobile phases are linear with correlation coefficients better than 0.9997. The temperature affects only moderately the separation selectivity of the two isomers: α decreases by approximately 0.04 for a temperature increase of 5 °C. From the slopes and intercepts of the van't Hoff plots (Eq. (4)), the standard enthalpy and the standard entropy of adsorption of the 1,1-bis(2-naphthol) atropoisomers were determined. For this purpose, like for the determination of the adsorption isotherms (see below), the volume of the stationary phase was determined as the difference between the geometrical volume of the empty column and the volume of mobile phase in the column, determined as the elution volume of tri-*tert*-butyl benzene, used as non-retained marker.

The plots of the adsorption enthalpies, ΔH°, and entropies, ΔS°, versus the concentration of methanol or dichloromethane in the mobile phase for both CHIRIS AD1 and CHIRIS AD2 have similar profiles, illustrated in Fig. 4 for CHIRIS AD2. A negative value of $-\Delta H^\circ$ means a positive contribution to the adsorption energy. Its larger negative value explains the stronger retention of the *S*-isomer with respect to the *R*-isomer. For both enantiomers, $-\Delta H^\circ$ is approximately

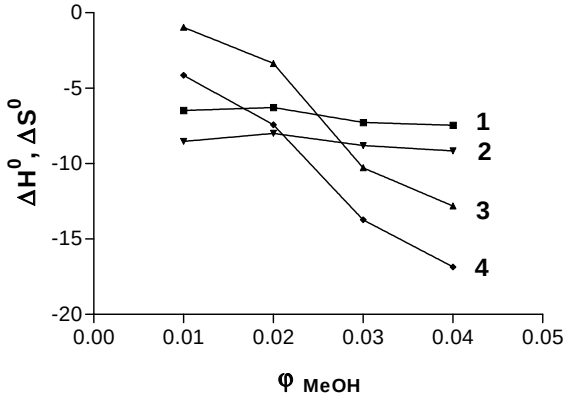

Fig. 4. Influence of the concentration of methanol, φ (vol.%) $\times 10^{-2}$, in ternary mobile phases with 39% dichloromethane, on the standard enthalpy, ΔH° [kJ mol^{-1}] (plots 1 and 2) and entropy, ΔS° [J mol^{-1} K^{-1}] (plots 3 and 4) of adsorption of the atropoisomers of 1,1′-bis(2-naphthol) on a CHIRIS AD2 column. *R*-isomer: plots 1 and 3; *S*-isomer: plots 2 and 4.

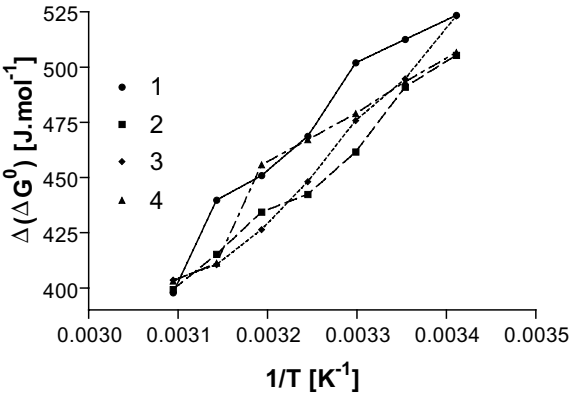

Fig. 5. Effect of temperature (T (K)) on the differences of Gibb's free energy of adsorption, $\Delta(\Delta G^\circ)$, between the *S*- and *R*-isomers of 1,1′-bis(2-naphthol). Column: CHIRIS AD2, 150 mm × 4.6 mm, 5 μm, ternary mobile phases of HX–DCM–MeOH: (1) 59:39:2; (2) 49:49:2; (3) 39:59:2; HX, hexane; DCM, dichloromethane; MeOH, methanol.

independent of the concentration of methanol or dichloromethane in the mobile phase. The parallel evolutions of both ΔH° and ΔS° when the methanol concentration increases from 10 to 40% is obviously consistent with the constant separation factor, independent of the methanol concentration. The value of $-\Delta H^\circ$ is approximately three–four times higher than that of the entropic contribution (with $\Delta S^\circ < 0$), which decreases the adsorption free energy, which can probably be attributed to a better organized structure of the adsorbed molecules (note the 1000-fold difference in the scales for the two functions). The (negative) entropic contribution to adsorption increases in more polar mobile phases, i.e. phases containing a higher concentration of methanol or dichloromethane, probably because of a better solvation of the polar 1,1′-bis(2-naphthol) molecules. The plots also show a lower adsorption entropy for the more strongly retained *S*-atropoisomer. Similar plots as with CHIRIS AD2 but with lower differences between the isomers were observed also with CHIRIS AD1.

These results show that even though the retention on the CHIRIS CSPs is caused mainly by the enthalpy of adsorption, the effect of the mobile phase on the retention should be attributed mainly to entropic contributions. As the rate of decrease in the entropy of adsorption per unit concentration of polar solvent in the mobile phase is practically the same for the two 1,1′-bis(2-naphthol) isomers, the difference in the Gibbs free energy of adsorption of the two isomers, $\Delta\Delta G^\circ$, is independent of the mobile phase composition and so is the separation selectivity. On the other hand, $\Delta\Delta G^\circ$ decreases at elevated temperatures, as illustrates Fig. 5.

3.2. Single-component adsorption isotherms of 1,1′-bis(2-naphthol) isomers on the CHIRIS AD1 and CHIRIS AD2 columns

The single-component adsorption isotherms of the pure 1,1′-bis(2-naphthol) isomers were measured by frontal analysis in the concentration range of 0.002–0.021 mol/l, in three ternary mobile phases on CHIRIS AD2 and in one ternary mobile phase on CHIRIS AD1. Low solubility precluded the determination of the isotherms in a wider concentration range. The frontal analysis breakthrough curves were measured using gradually increasing concentrations of 1,1′-bis(2-naphthol) in 10 successive steps. From each breakthrough curve, the adsorbed amount and the concentration of the solute in the stationary phase were evaluated as described in Section 2. After a plateau on the breakthrough curve was reached, the column was washed with the pure mobile phase until elution of the whole amount adsorbed and another breakthrough curve was measured with a higher solute concentration in the next step.

Table 1
Parameters of the single-component isotherms (Eqs. (5) and (6)) of the *R*- and *S*-atropoisomers of 1,1′-bis(2-naphthol)

HX–DCM–MeOH	Temperature (°C)	a_{RL}	b_{RL}	a_{SL}	b_{SL}	a_{SBL}	D^2
CHIRIS AD2 column							
49:49:2	25	6.1	13.2	6.2	12.6	0.8	0.9999
49:49:2	35	4.8	16.5	5.8	18.8	0.6	0.9998
58:39:3	35	4.9	15	5.3	8.7	0.7	0.9999
CHIRIS AD1 column							
58:39:3	35	6.7	20.8	7.5	23.8	0.8	0.9998

D^2—coefficient of regression; columns CHIRIS AD2 and CHIRIS AD1, both 150 mm × 4.6 mm, 5 μm. Ternary mobile phases: HX, hexane; DCM, dichloromethane; MeOH, methanol.

The experimental isotherm data sets were analysed using non-linear regression to fit the data to an isotherm model. The results are shown in Table 1. The distribution of the less retained *R*-isomer is adequately described by the Langmuir model (Eq. (1)). To test the assumption that the difference in retention between the *R*- and *S*-isomers should be attributed to the adsorption of the latter on another type of adsorption sites, the experimental distribution data of the *S*-isomer were fitted to the bi-Langmuir model, Eq. (2). However, the regression yielded negative isotherm coefficients, which lack physical meaning, until the value of the parameter b_2 in Eq. (2) was set to zero during the fitting procedure. The bi-Langmuir model with forced $b_{SBL} = 0$ provided very good fit to the experimental distribution data, with correlation coefficients of 0.9998–0.9999. Hence, the following equations were employed to describe the distribution of the 1,1-bis(2-naphthol) isomers:

$$Q_R = \frac{a_{RL} c_R}{1 + b_{RL} c_R} \quad (5)$$

$$Q_S = \frac{a_{SL} c_S}{1 + b_{SL} c_S} + a_{SBL} c_S \quad (6)$$

The parameters a_{SL}, b_{SL} describing the adsorption of the *S*-isomer on the first type of adsorption sites are in reasonable agreement with the parameters a_{RL}, b_{RL} determined by fitting the Langmuir model to the distribution data of the *R*-isomer in independent experiments (Table 1). Further, the adsorption parameters on the selective type of sites, a_{SBL}, are approximately one order of magnitude lower than the parameters a_{RL} and a_{SL} for non-selective adsorption. This behavior is in reasonable agreement with a model assuming the contribution of similar non-specific adsorption to both atropoisomers, while additional, selective chiral centers contribute only to the adsorption of the *S*-isomer. The negligibly low values of the parameter b_{SBL} suggest that the enantioselectivity of the CHIRIS columns can probably be attributed to a low-energy adsorption on the enantioselective sites that are present at a relatively high concentration on the CHIRIS adsorbent surface. It is probable that, at higher *S*-isomer concentrations, a finite value could be obtained for the parameter b_{SBL}, but the low solubility of the analytes limits the concentration range within which the adsorption isotherm data of the *S*-isomer could be measured.

The equilibrium adsorption data of the *S*-isomer fit well to the simple Langmuir isotherm model but the comparison of the Langmuir isotherm parameters *a* and *b* does not allow a straightforward comparison of the adsorption behavior of the two isomers. Finally, as will be shown later (Section 3.3), the model based on the Eqs. (5) and (6) requires only three parameters to describe the competitive distribution of the two atropoisomers, whereas at least four parameters would be necessary to describe the distribution assuming Langmuir models with different column saturation capacity for each isomer. In agreement with the decrease of the retention at higher temperatures under linear conditions, the adsorption isotherm parameters a_{RL}, a_{SL} and a_{SBL} decrease with increasing temperature (Table 1).

Fig. 6 compares the single-component adsorption isotherms of *R*- and *S*-1,1-bis(2-naphthol) on CHIRIS AD1 and CHIRIS AD2 in a ternary solvent mixture of hexane–dichloromethane–methanol, 58:39:3. The isotherms on CHIRIS AD1 are more curved (higher parameters b_{RL}, b_{SL} in Table 1, lower saturation capacity) and show much less significant differences between the adsorption of the *R*- and *S*-isomers than on CHIRIS AD2, which appears to be the more suitable for overloaded preparative separations as well as for analytical ones.

3.3. Competitive adsorption isotherms of 1,1′-bis(2-naphthol) isomers on the CHIRIS AD2 columns

Because of the lower selectivity of CHIRIS AD1 for the 1,1′-bis(2-naphthol) isomers which would

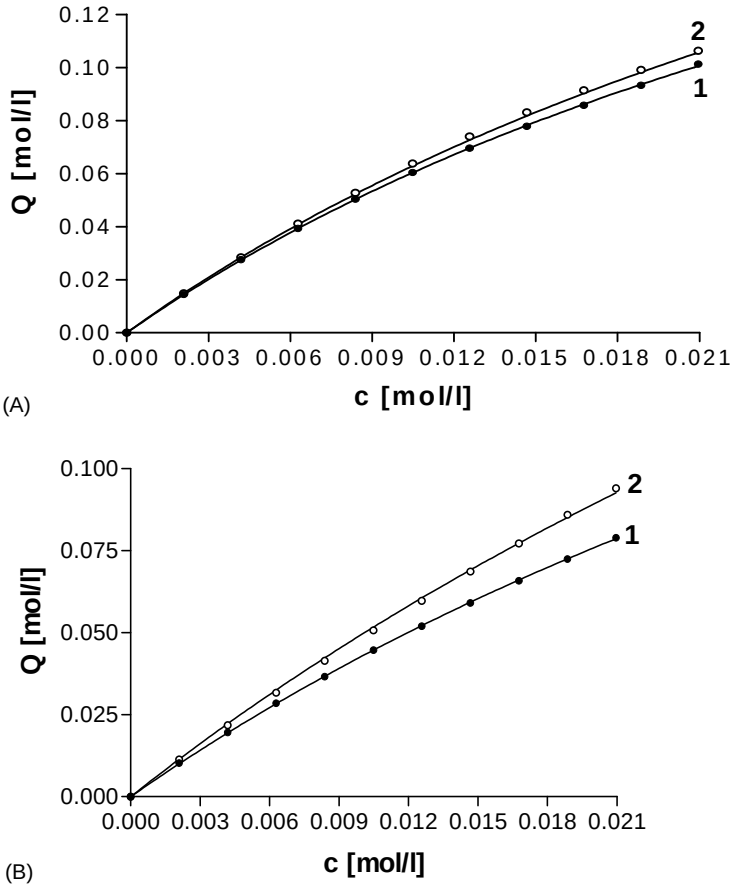

Fig. 6. Single-component isotherms of *R*- and *S*-1,1′-bis(2-naphthol) in a ternary mobile phase hexane–dichloromethane–methanol 58:39:3 (v/v): (A) on a CHIRIS AD1, 150 mm × 4.6 mm, 5 μm and (B) CHIRIS AD2, 150 mm × 4.6 mm, 5 μm, columns at 35 °C. (1) *R*-isomer and (2) *S*-isomer.

provide poor binary breakthrough curve data, the competitive adsorption isotherms were determined only on CHIRIS AD2. For the determination of the competitive isotherm parameters, the racemate solution, containing equimolar solutions of the two isomers at concentrations up to 0.0175 mol/l were used for frontal analysis measurements, as shown in Section 2. An advantage of applying this technique to optical isomers is that these show equal response factors in UV detection, i.e. equal molar absorption coefficients at any wavelength. As the column was equilibrated with the mobile phase after the end of each breakthrough step, the analysis of the breakthrough curve was simplified and the concentrations of racemate at the final plateaus could be used for the detector calibration, to determine the concentration of the *R*-isomer at the mid-plateau of the breakthrough curve. The composition of the ternary mobile phases and the temperatures used for the determination of the competitive isotherms are given in Table 2.

As the single-component isotherms were adequately described by Eqs. (5) and (6), we adapted the model for competitive distribution, assuming that the adsorption of both the *R*- and *S*-isomers on the non-selective sites can be described by a simple Langmuir isotherm with the same parameters a_R and b_R (equal retention and saturation capacities) and that an additional linear term with a parameter a_S accounts for the selective adsorption of the *S*-isomer on the chiral adsorption sites. Then, the competitive isotherm of the *R*-isomer

is described by the Langmuir Eq. (7), with the sum of the concentrations of the two isomers in the denominator; and Eq. (8) with an additional linear term can be used to describe the distribution of the *S*-isomer:

$$Q_R = \frac{a_R c_R}{1 + b_R(c_R + c_S)} \quad (7)$$

$$Q_S = \frac{a_R c_S}{1 + b_R(c_R + c_S)} + a_S c_S \quad (8)$$

The parameters a_R, b_R and a_S listed in Table 2 were determined by using a single matrix including the distribution data for both the *R*- and the *S*-isomers, subject to a common non-linear regression analysis. High values of the coefficients of regression (0.9996–0.9999) demonstrate that only three isotherm parameters are necessary to describe adequately the competitive distribution of the two isomers, supporting further the assumption of independent contributions of the non- and enantio-selective adsorption sites in the chiral separation. The data in Table 2 and the isotherm profiles in Fig. 7 demonstrate a decrease of the isotherm parameters a_R and a_S with increasing temperature and with increasing concentration of polar solvents—either methanol or dichloromethane—in the mobile phase. The values of the competitive isotherm parameters in Table 2 are in reasonable agreement with the values of the single-component isotherm parameters in Table 1, if we take into account that—because of

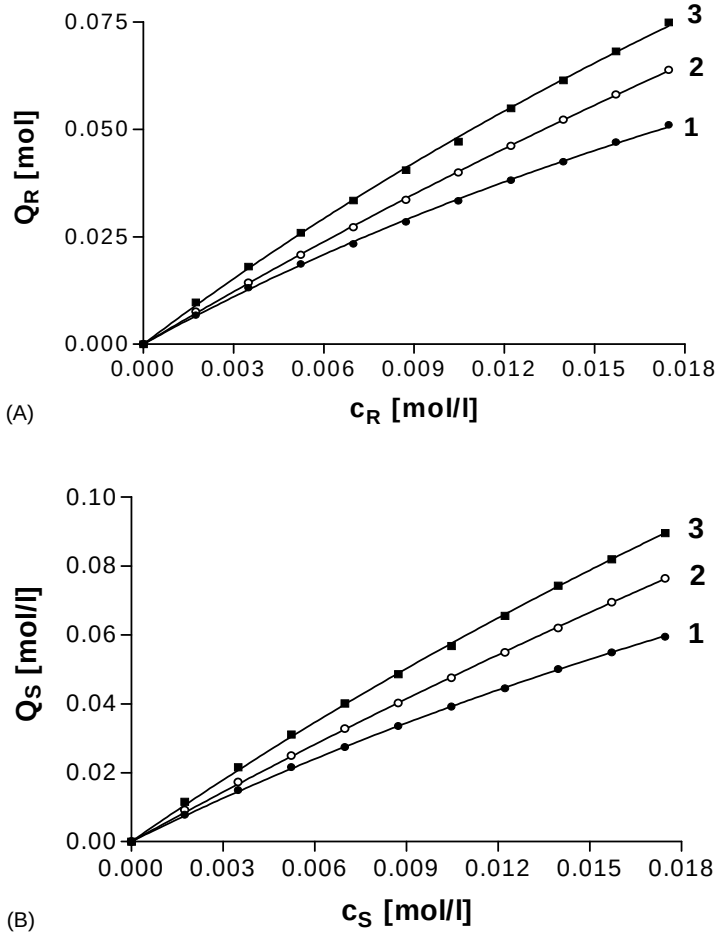

Fig. 7. Competitive isotherms of the *R*,*S*-1,1′-bis(2-naphthol) racemate in ternary mobile phases of hexane–dichloromethane–methanol: (1) 29:69:2, (2) 39:59:2, and (3) 49:49:2, at 25 °C. CHIRIS AD2 column, 5 μm, 150 mm × 4.6 mm; temperature, 25 °C. (A) *R*-isomer and (B) *S*-isomer. The experimental data were fitted to Eqs. (7) and (8).

Table 2
Parameters (a_R, a_S and b_R) of the competitive isotherms (Eqs. (7) and (8)) of the *R*,*S*-1,1′-bis(2-naphthol) racemate

HX–DCM–MeOH	Temperature (°C)	a_R	b_R (l/mol)	a_S	RSC	D^2
29:69:2	25	3.88	9.73	0.52	1.01×10^{-7}	0.9997
	35	3.16	8.81	0.47	2.89×10^{-8}	0.9999
	45	2.79	6.37	0.37	6.16×10^{-8}	0.9997
39:59:2	25	4.17	4.13	0.72	1.07×10^{-7}	0.9998
	35	3.87	4.06	0.61	1.46×10^{-7}	0.9997
	45	3.46	4.09	0.48	1.10×10^{-7}	0.9997
49:49:2	25	5.28	7.06	0.89	3.49×10^{-7}	0.9996
	35	4.97	6.44	0.65	3.16×10^{-7}	0.9996
	45	4.76	5.59	0.58	1.85×10^{-7}	0.9997
56:39:5	25	3.5	4.48	0.55	3.60×10^{-8}	0.9999
	35	3.27	3.39	0.43	2.01×10^{-8}	0.9999
	45	2.94	3.14	0.32	7.54×10^{-8}	0.9997
57:39:4	25	4.18	5.08	0.72	1.72×10^{-7}	0.9997
	35	3.88	5.03	0.57	4.77×10^{-8}	0.9999
	45	3.42	5	0.51	7.28×10^{-8}	0.9998
58:39:3	25	5.18	8.33	0.83	2.07×10^{-7}	0.9997
	35	4.71	6.34	0.76	1.26×10^{-7}	0.9998
	45	4.23	5.93	0.7	1.84×10^{-7}	0.9997

RSC, residual sum of quadrates; D^2, coefficient of regression. Column CHIRIS AD2, 150 mm × 4.6 mm, 5 μm. Ternary mobile phases: HX, hexane; DCM, dichloromethane; MeOH, methanol.

different form of the denominators of the Langmuir terms in Eqs. (5)–(8), the competitive parameters b_R are expected to be half the values of the corresponding single-component parameters, which was indeed approximately the case. The effect of the mobile phase on b_R is less straightforward and cannot be generalized on the basis of the present data.

Using the competitive isotherm parameters, the overloaded band profiles for injections of mixtures of the *R*- and *S*-isomers of 1,1-bis(2-naphthol) on CHIRIS AD2 were calculated using the Rouchon algorithm [10]. Fig. 8 shows the good agreement between the calculated and experimental chromatograms for the injection of a 500 μl sample containing 0.021 mol/l of the *R*-isomer and 0.01 mol/l of the *S*-isomer and provides an additional proof for the validity of the isotherm model described by Eqs. (7) and (8). Under the conditions shown in Fig. 8, the preparative separation of the racemate of 1,1′-bis(2-naphthol) could be carried out with production rates of 2 and 1 mg min^{-1} cm^{-2} and recovery yields of 37 and 17% for the *R*- and *S*-isomers, respectively. These recovery yield are the fractions of the atropoisomer feed that could be collected and recovered pure (99%). The

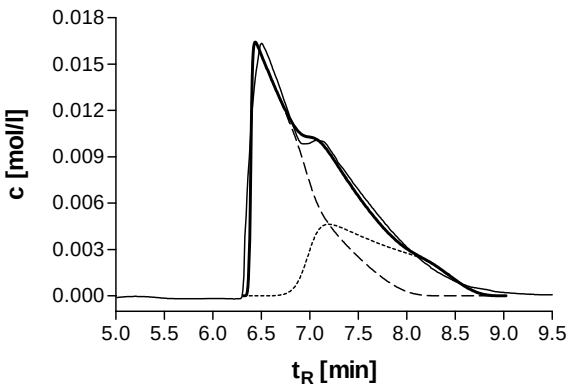

Fig. 8. Comparison of the (full line, thin) experimental and calculated band profiles for a 500 μl sample of a mixture of: (dashed line) *R*-1,1′-bis(2-naphthol) and (dotted line) *S*-1,1′-bis(2-naphthol), with concentrations of: 0.021 mol/l (*R*-isomer) and 0.0105 mol/l (*S*-isomer). Ternary mobile phases of hexane–dichloromethane–methanol, 58:39:3, at 35 °C, column CHIRIS AD2, 150 mm × 4.6 mm, 5 μm; thick, full line represent calculated total band profile for the *R*- and *S*-isomers.

intermediate, mixed fraction corresponding to the overlapping bands of the two isomers can be recycled to avoid product loss.

4. Conclusions

The retention of the *R*- and *S*-atropoisomers of 1,1′-bis(2-naphthol) on CHIRIS AD1 and CHIRIS AD2 in ternary mobile phases made of hexane, dichloromethane and methanol is caused by the enthalpic contribution to adsorption, but the effect of the mobile phase on the retention should be attributed mainly to its effect on the entropic contribution. As the rate of decrease of the adsorption entropy per unit concentration of polar solvent in the mobile phase is practically equal for the two isomers, the difference in Gibbs free energy of adsorption that controls the selectivity of the chiral separation of the two isomers, $\Delta\Delta G^{\circ}$, is practically independent of the mobile phase composition, but decreases with increasing temperature. The chiral selectivity of CHIRIS AD1 is too low to achieve adequate chiral separation of the *R*- and *S*-isomers of 1,1′-bis(2-naphthol). CHIRIS AD2 can be used for this purpose, but a ternary mobile phase is required to achieve a satisfactory separation.

The adsorption of the less retained *R*-isomer is controlled essentially by the non-selective interactions, which are the same for the two isomers. The single-component and the competitive isotherms of the *R*- and *S*-isomers can be adequately described by a model combining a Langmuir term for the non-selective contribution to adsorption and a linear term for the enantioselective adsorption of the *S*-isomer. The simplicity of this model is due to the fact that the poor solubility of 1,1′-bis(2-naphthol) limits the concentration range that is experimentally available.

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