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Measurements of association constants between enantiomers and chiral selectors by capillary gas chromatography. Theoretical and practical considerations

Pablo J. Lebed, Sonia Keunchkarian, Jaiver Osorio Grisales, Cecilia B. Castells*

Laboratorio de Investigación y Desarrollo de Métodos Analíticos (LIDMA), División Química Analítica, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, 47 y 115 (1900) La Plata, Argentina

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ABSTRACT

The association constants of several volatile enantiomers with octakis(3-O-butanoyl-2,6-di-O-pentyl)-γ-cyclodextrin at temperatures between 50 and 100 °C were measured by gas–liquid chromatography using capillary columns coated with different amounts of chiral selector dissolved in polysiloxane OV-1701 and prepared with a precisely determined phase ratio. Simple expressions were deduced to estimate the apparent distribution constants from accurate hold-up and retention times along with that known phase ratio at each temperature. The enantiomer-chiral selector association constants were then calculated from the linear regression of the apparent constants as a function of the chiral selector concentration. One aim of this study consisted in discussing all the experimental uncertainties inherent in the determination of enantiomer/selector association constants with chiral analytes, and how these fundamental measurements can be performed precisely without resorting to the use of reference solutes.

1. Introduction

Derivatives of cyclodextrins (CDs) are widely used as stationary phases for enantioseparations of volatile compounds in chiral gas chromatography (GC) [1,2]. These cyclic molecules have five stereogenic centers in every glucose moiety. This large number of stereogenic centers in addition to the conical structure of the CD molecule which can induce solute inclusion into the cavity explains their broad chiral recognition capability. Octakis(3-*O*-butanoyl-2,6-di-*O*-pentyl)-γ-cyclodextrin is composed of eight glucose units whose hydroxyl groups were converted in either ether or ester groups. This chiral selector (CS) is well soluble in polysiloxanes of medium polarities, and the columns based on this CD display chiral enantiorecognition toward a very broad group of chiral molecules [3,4].

GC using packed columns constitutes a well-accepted method for determining gas–liquid partitioning as well as complex-formation constants between a given selector and specific volatile compounds. This approach is highly precise provided that adsorption onto the support is negligible. The technique has been extensively used also in chiral chromatography [5]. Since, however, most CSs usually exhibit selectivity factors rarely exceeding 1.3 units, capillary columns containing the CS in question constitute a potentially better alternative. The use of capillary columns results in enough efficiency to compensate for the usually only moderate enantiorecognition ability of CSs. Moreover, the absence of a solid support makes the system much simpler and more accurate for the measurement of any gas–liquid equilibrium property [6].

Capillary columns have been widely used to estimate association constants by resorting to the retention increment model [7,8]. In that approach, a reference solute, usually an *n*-alkane is chromatographed along with the chiral solute. This reference molecule is chosen so as to have no interactions with the CS, and this very practical approach allows a distinction between retention contributions from both the nonchiral and the chiral components of the stationary phase and circumvents the necessity for knowledge of the phase ratio of the different columns.

The aim of this work was: (i) to discuss the direct measurement of association constants between a chiral selector and an enantiomer using capillary columns of exactly measured phase ratios; (ii) to apply simple fundamental equations to obtain the association constants of several enantiomers with the CS octakis(3-*O*-butanoyl-2,6-di-*O*-pentyl)-γ-cyclodextrin dissolved in OV1701; (iii) to critically evaluate the uncertainties associated to each experimental measurement and how these uncertainties would affect the association constants; (iv) to evaluate the contribution of the achiral matrix to the apparent enantioselectivity factors, and (v) to critically compare the constants obtained here with the values that

* Corresponding author. Tel.: +54 221 4228328; fax: +54 221 4271537.
E-mail address: castells@isis.unlp.edu.ar (C.B. Castells).

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would be estimated from the retention increment model under the same conditions.

2. Theoretical

When an enantiomer X is chromatographed in a column containing a pure solvent S (*e.g.* polysiloxane), the solute infinite dilution gas–liquid partition coefficient, $K_L{}^\circ$, and retention factor are related as follows: [9]

$X(g) \rightleftharpoons X(l)$

$$K_L{}^\circ = k^\circ \beta^\circ = \frac{RT}{\gamma_s^\infty \; p^\circ \nu_s} \tag{1}$$

where k° denotes the solute retention factor in a column having a phase ratio β° (*i.e.* the ratio between dead volume and the stationary phase volume). R is the gas constant and T the absolute temperature, p° and γ_s^∞ are, respectively, the solute's vapor pressure and infinite dilution activity coefficient in solvent S at temperature T; and ν_s denotes the solvent molar volume at the same temperature.

When the enantiomer X is injected in a column containing different concentrations of the selector A dissolved in the achiral solvent S, the equilibrium for the XA-complex formation is:

$X(1) + A \rightleftharpoons XA$

$$K_{XA}{}^\circ = \frac{a_{XA}}{a_X \, a_A} \cong \frac{m_{XA}}{m_X \, a_A} \tag{2}$$

where $K_{XA}{}^\circ$ represents the thermodynamic association constant between enantiomer and CS, a_i represents the activity for species i with the convention that a_i approaches to concentration at infinite dilution. Under analytical chromatographic conditions the activities of the solute and the complex can be replaced by molal concentrations (m_i) since those species are infinitely diluted. For practical reasons, the equilibrium would also be expressed by the stoichiometric association constant K_{XA} ($= K_{XA}{}^\circ \gamma_A$), where γ_A denotes the CS activity coefficient.

Enantiomer retention in these columns is related to an apparent association constant, K_{app}, as follows:

$$K_{app} = k \times \beta = \frac{RT}{\gamma_{s+A}^\infty \; p^\circ \nu_{A+s}} (1 + K_{XA} \; m_A) \tag{3}$$

where k represents the retention factor for the enantiomer in the column containing the CS at concentration m_A and phase ratio equal to β. The solute activity coefficient in the solvent mixture $S+A$ is denoted as γ_{s+A}^∞. Similarly, the molar volume of the mixture, ν_{S+A}, would be different from that of the pure solvent. Eq. (4) results from combining Eqs. (1) and (3):

$$K_{app} = K_L{}^\circ \frac{\gamma_S^\infty \; \nu_S}{\gamma_{s+A}^\infty \; \nu_{A+s}} (1 + K_{XA} \; m_A) = K_L{}^{\circ,*} (1 + K_{XA} \; m_A) \tag{4}$$

In Eq. (4), the constant $K_L{}^{\circ,*}$ is equal to $K_L{}^\circ$ if the ratio $\gamma_S^\infty \; \nu_S / \gamma_{s+A}^\infty \; \nu_{A+s}$ equals 1. Similarly, a plot of K_{app} as a function of m_A is a straight line only if γ_{s+A}^∞ and ν_{S+A} do not depend on m_A, but rather bear a linear relationship to CS molality. An assumption made here is that there are no synergistic effects between the achiral polysiloxane and the chiral selector.

Now, for any enantiomeric pair, the thermodynamic enantioselectivity, $\alpha_{D/L}$, is defined by the ratio $K_{DA}{}^\circ / K_{LA}{}^\circ$, which value is equivalent to the ratio between stoichiometric complex-formation constants K_{DA}/K_{LA}, where arbitrarily a stronger interaction between the D-enantiomer and the CS has been considered. The estimation of $\alpha_{D/L}$ is possible only if these complex-formation constants can be measured. The usual and more accessible ratio between retention factors for both enantiomers k_D/k_L will lead to an apparent enantioselectivity, $\alpha_{D/L,\,app}$:

$$\alpha_{D/L,app} = \frac{(1 + K_{DA} \; m_A)}{(1 + K_{LA} \; m_A)} \tag{5}$$

which value is always smaller than the true enantioselectivity, and the consequent apparent enantioselectivity factor would approach the true enantioselectivity only if ($1 \ll K_{XA} m_A$).

The above treatment was independent of reference solutes; the apparent constants can be obtained from the measurements of retention factors of symmetric peaks in chiral columns of known phase ratios at each temperature.

3. Experimental

3.1. Instrumentation and materials

Chromatographic measurements were performed in an HP6890 (Agilent, Palo Alto, CA, USA) gas chromatograph equipped with flame ionization detection and manual injection. Split injection was used and the split ratio was kept at 1:100. The solute retention times were acquired with the software Clarity (DataApex, Czech Republic).

Fused silica tubing capillary of 250 μm i.d. was provided by MicroQuartz (München, Germany), OV-1701 (5% cyanopropyl, 7% phenyl) was purchased to Supelco (Bellefonte, PA, USA), and octakis-(3-*O*-butanoyl-2,6-di-*O*-pentyl-)-γ-cyclodextrin (LIPODEX-E) was obtained from Cyclolab Ltd. (Budapest, Hungry). For comparison, we used a commercial Lipodex E column, 25 m × 250 μm i.d., bought from Macherey-Nagel (Macherey-Nagel GmbH & Co. KG, Düren, Germany).

The racemic solutes were obtained from either, Aldrich–Sigma (St. Louis, MO, USA) or Fluka (Ronkonkoma, NJ, USA). Ethyl chloroformiate (97%) and trifluoroacetic anhydride (>99%) were purchased from Aldrich and Fluka, respectively.

Chemical waste was delivered for a proper residual treatment.

3.2. Density measurements

A 2-mL Guy-Lussac pycnometer was employed to measure stationary phase densities. The pycnometer was initially calibrated within the range between 30 and 130 °C by using high purity *n*-tetradecane whose densities were taken from literature [10]. Next, the densities of pure OV-1701 and of a mixture containing 10% (w/w) CS at temperatures between 30 and 100 °C were measured. The obtained density values were then fitted to the following two polynomial equations:

$$\rho_{OV1701}(\text{g mL}^{-1}) = 1.29_7(\pm 0.032) - 0.0010(\pm 0.0002)\,T + 2.7(\pm 1.7) \times 10^{-7}\,T^2, \quad R^2 = 0.9998, \quad \text{s.e.} = 0.0004 \tag{6}$$

$$\rho_{10\%\,CS/OV1701}(\text{g mL}^{-1}) = 1.1_9(\pm 0.13) - 0.00020(\pm 0.00008)\,T - 1.1(\pm 1.0) \times 10^{-6}\,T^2, \quad R^2 = 0.9991, \quad \text{s.e.} = 0.0007 \tag{7}$$

where T represents the temperature in degrees Kelvin. The data showed that the density of the mixture is slightly larger than that of pure polysiloxane at all temperatures.

The densities of the two mixtures with the higher CD contents were estimated by extrapolation.

3.3. Column coating

Capillaries of 20 m in length without any surface pretreatment were prepared by a static coating procedure detailed elsewhere

Table 1
Retention times of methane and hold-up times calculated from Eq. (9) in the four columns.

Temperature (°C)	Column 1		Column 2		Column 3		Column 4	
	$t_{R(CH_4)}$	t_M	$t_{R(CH_4)}$	t_M	$t_{R(CH_4)}$	t_M	$t_{R(CH_4)}$	t_M
100	0.863	0.862 ± 0.002	0.958	0.955 ± 0.002	0.853	0.845 ± 0.009	0.850	0.849 ± 0.003
90	0.845	0.841 ± 0.003	0.938	0.936 ± 0.003	0.838	0.828 ± 0.003	0.833	0.826 ± 0.001
80	0.828	0.823 ± 0.002	0.922	0.916 ± 0.002	0.820	0.809 ± 0.007	0.817	0.810 ± 0.004
70	0.810	0.808 ± 0.004	0.902	0.898 ± 0.002	0.803	0.792 ± 0.010	0.798	0.791 ± 0.004
60	0.793	0.788 ± 0.005	0.882	0.876 ± 0.003	0.788	0.777 ± 0.015	0.780	0.773 ± 0.003
50	0.773	0.766 ± 0.008	0.862	0.854 ± 0.001	0.768	0.759 ± 0.009	0.762	0.757 ± 0.011

Table 2
Physicochemical properties of the capillary columns.

	Column 1	Column 2	Column 3	Column 4
% (w/w) Selector in OV-1701	0	10.03	16.82	26.26
Cyclodextrin concentration (molal)	–	0.0383	0.0695	0.1223
Solution concentration[a] (mg/mL)	4.084	4.065	4.874	5.000
Column phase ratio[b], β (±0.2)				
50 °C	245.9	251.1	211.3	209.2
60 °C	244.0	248.8	209.2	206.8
70 °C	242.0	246.6	207.2	204.7
80 °C	240.1	244.4	205.3	202.7
90 °C	238.2	242.1	203.2	200.3
100 °C	236.3	239.6	200.8	197.4
Film thickness[c] (μm) (±0.004)				
50 °C	0.253	0.248	0.298	0.304
100 °C	0.264	0.260	0.312	0.318

[a] Concentration of the solution (OV1701 + cyclodextrin dissolved in dichloromethane) used to fill the capillary.
[b] Calculated from Eq. (8). Absolute uncertainty estimated by error propagation rules.
[c] Estimated from $d_f = \left(\frac{d_c}{2}\right)\left[\frac{1-\beta}{(\beta+1)^{0.5}}\right]$, where d_c represents the column diameter.

[6,11,12]. In this study, stationary phase solutions were prepared in dichloromethane. After coating, the capillaries were conditioned with nitrogen flow at 120 °C overnight. The column efficiencies were measured by injecting *n*-dodecane at 100 °C. Column phase ratio was accurately determined at each column temperature from [13,14]:

$$\beta = \frac{\rho_s(T)}{C_0}\exp[\alpha_{Si}(T - T_0)] - 1 \quad (8)$$

where $\rho_s(T)$ represents the stationary phase density at temperature T, C_0 denotes the concentration of the solution (expressed as (w/v)) used to fill the capillary at the temperature T_0, and α_{Si} denotes the thermal expansion coefficient of the silica capillary wall.

3.4. Chromatographic measurements

All separations were performed under isothermal conditions. We first selected solutes that were very well resolved in the commercial Lipodex-E column in order to get enantioseparation even in the column containing 10% (w/w) of CD. Retention times were measured at the peak maxima at a precision of 0.001 min and not fewer than three times at each temperature.

The estimation of hold-up time was performed by non-linear regression of the retention times of *n*-alkanes from *n*-pentane to *n*-decane as a function of carbon number to the equation [15]:

$$t_R(n) = t_M + e^{((A+B(n-1)+\ln\ (1-C-n^2))} \quad (9)$$

where $t_R(n)$ is the retention time of an *n*-alkane of *n* carbon atoms, and A, B, C and t_M are the nonlinear fitting parameters of the multiparametric equation. Table 1 lists the values for t_M and $t_{R(CH_4)}$. The differences, amounting up to 0.008 min, are significant in the calculation of *k*-values for solutes scarcely retained whereas become negligible for those well retained.

The methane retention times along with the β values have been used to estimate the partition coefficient for methane in every column and temperature through the equation:

$$K_{L(CH_4)} = \left(\frac{t_{R(CH_4)}}{t_M - 1}\right) \times \beta \quad (10)$$

These $K_{L(CH_4)}$ data were then used to calculate the t_M in each chromatographic run when methane was co-injected with the solute at any pressure inlet condition and column temperature.

4. Results and discussion

4.1. Capillary column properties

Table 2 summarizes the physical properties of the columns. The β-values as well as the film thickness at two temperatures are included. We estimated that the probable absolute error in the tabulated β is ±0.2 units (about 0.1%), at least for columns 1 and 2. The high precision associated with those β-values (four significant figures) was possible since a large amount of coating solution was prepared and the densities could be determined at high precision. The absolute errors in the β values for columns 3 and 4 are expected to be somewhat larger, because the densities of mixtures with 16 and 26% CD were estimated by linear extrapolation with the CS amount.

The performance characteristics of our columns were compared with those of a similar column from a commercial company in Fig. 1. The chromatograms recorded from the elution of *n*-dodecane at 90 °C from the Column 4 and from the commercial one are shown in plots A and B. Fig. 1C summarizes the efficiencies (theoretical plates/m) measured with *n*-dodecane at 100 °C for all the home-fabricated capillaries and for the commercial column. When the asymmetry factors for the *n*-dodecane peaks were calculated, the results ranged between 1.00 and 1.18 in all columns and at all temperatures, whereas the *n*-dodecane profile in the

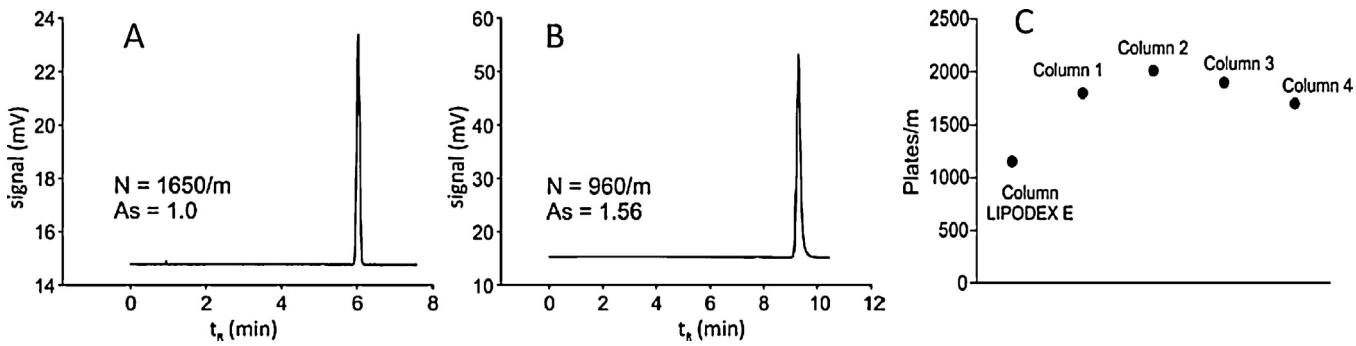

Fig. 1. Chromatograms of *n*-dodecane eluted from Column 4 (A) and from the Lipodex E column (B) at 90 °C. Plot (C): Efficiencies achieved with the columns prepared in this work and that measured with the Lipodex E column, with all columns operated at the same carrier linear velocities. Solute: *n*-dodecane. Column temperature: 100 °C.

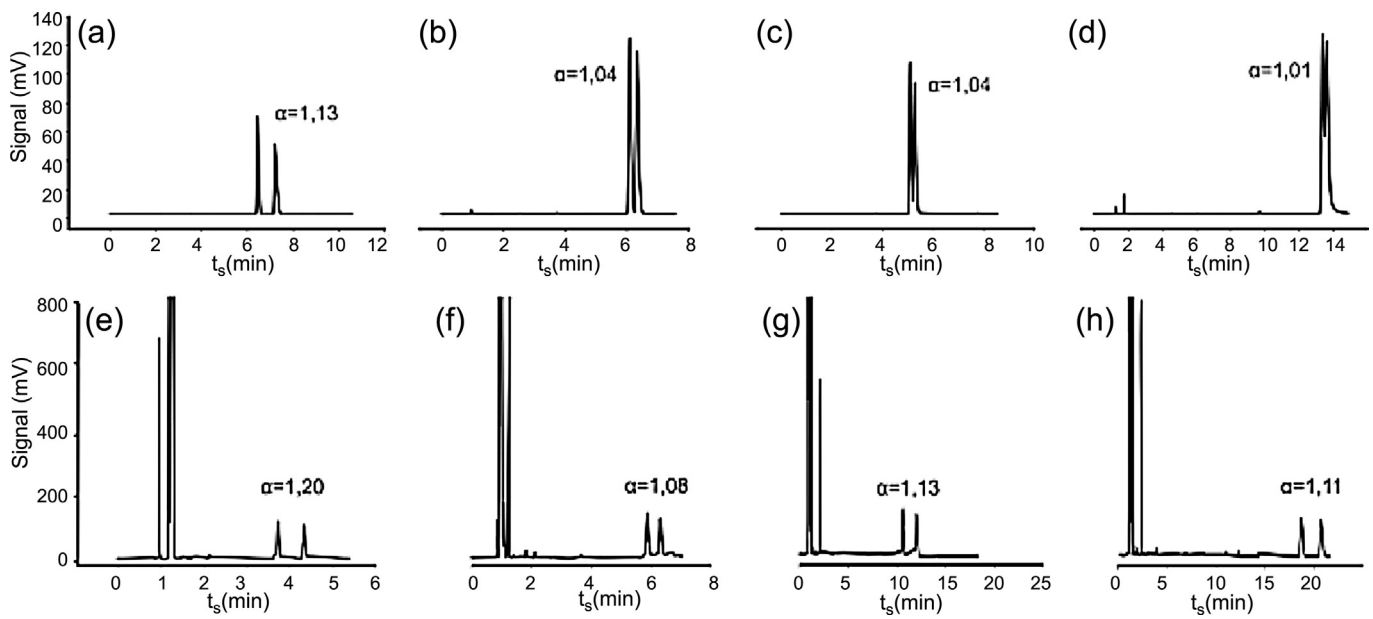

Fig. 2. Chromatograms of the chiral separation with the column 4 of (a) 3,3,5-trimethylcyclohexanone at 90 °C, (b) 2,5-dimethoxytetrahydrofuran at 50 °C, (c) 4-methyl-2-pentanol at 50 °C, (d) 1-phenyl-1-ethanol at 100 °C, (e) 2-chloropropanoic acid-ECF at 70 °C, (f) 2-bromopropanoic acid-ECF at 70 °C, (g) 2-bromobutanoic acid-ECF at 70 °C, (h) 2-bromovaleric acid-ECF at 70 °C. EFC denotes the derivatization with ethyl chloroformate.

commercial Lipodex E showed asymmetry factors between 1.30 and 1.56. Unfortunately, the information about the properties of the commercial column (*e.g.* film thickness, the achiral polymeric matrix and selector concentration) was not provided, therefore, a thoroughly equitable comparison between that column and the others was not possible. Fig. 2 shows chromatograms of some racemates eluted from Column 4.

4.2. Determination of enantiomer-chiral selector association constants

A simple algebraic arrangement between Eq. (10) and the definition of retention factor leads to the expression used to calculate K_{app} from solute retention time, t_R, and β-values at each temperature:

$$K_{app} = \left(\frac{t_R}{t_{R(CH_4)}}\right)(\beta + K_{L(CH_4)}) - \beta \quad (11)$$

Since β can be known with relative standard deviations below 0.1% (*cf.* Table 2), the random errors associated with the estimation of apparent constants K_{app} are determined by the measurement of retention times of both the enantiomer and methane.

These apparent association constants are thermodynamically meaningless, since they depend on the chiral selector concentration (*cf.* Eq. (3)). The experimental K_{app} values were plotted against the molal concentration of the CD-selector. Fig. 3 illustrates the results for four representative solutes at a constant column temperature. A similar behavior was observed for other solutes and temperatures. With the exception of a few solutes, most of the data points fell into straight lines. Table 3 lists the coefficients of linear regressions along with their corresponding standard deviations for all solutes at a single temperature. These results clearly indicated that the K_{app} values fitted very well to the linear model in most instances: satisfactory correlation coefficients were obtained and, as a whole, the standard errors associated with the linear models were relatively low. The largest discrepancies corresponded to a few solutes (amino acids) for which a curvature was detected in their K_{app} *vs.* selector molality. The following conclusions can be drawn from the observed linearity for most of the enantiomers:

i) The CS activity and its molal concentration are proportional quantities within the concentration range used in this study and within the experimental error, *i.e.*, the stoichiometric association constant is not dependent on the selector molality as expressed in Eq. (4).

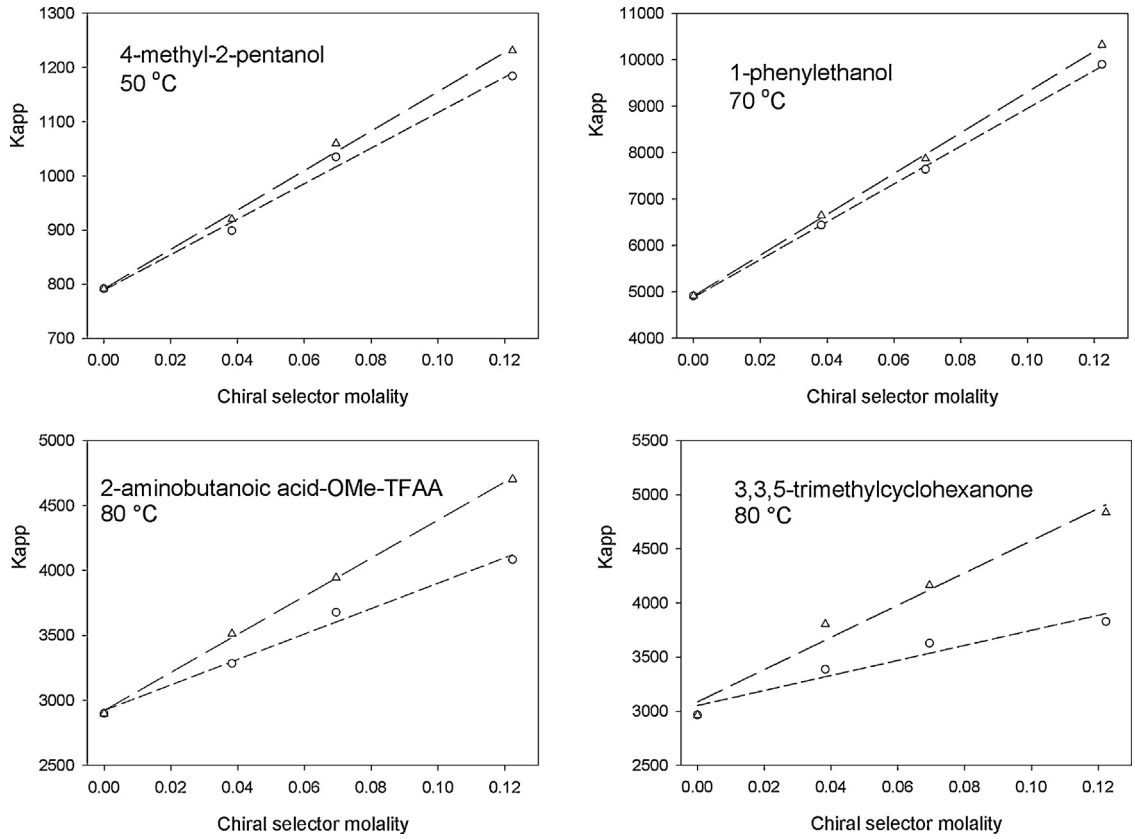

Fig. 3. Plots of apparent association constants, K_{app}, as a function of chiral selector molality. The solutes and temperatures are indicated in each plot.

Table 3
Results of least-square regressions between enantiomer K_{app} and selector molality and the estimated enantiomeric association constants.

Solute	Temp. (°C)	K°	First eluted enantiomer				Second eluted enantiomer			
			Intercept ($\times 10^{-2}$)	Slope ($\times 10^{-3}$)	R^2	K_i	Intercept ($\times 10^{-2}$)	Slope ($\times 10^{-3}$)	R^2	K_i
2M4PeOH	50	791	7.9 ± 0.1	3.3 ± 0.2	0.99	4.2 ± 0.3	7.9 ± 0.1	3.6 ± 0.2	0.99	4.6 ± 0.2
2-Br-C5	60	389	3.89 ± 0.008	0.59 ± 0.02	0.99	1.52 ± 0.05	4.9 ± 0.1	1.8 ± 0.2	0.98	1.82 ± 0.05
335-TMC	70	2965	29.8 ± 0.4	9.6 ± 0.9	0.99	3.2 ± 0.3	30.2 ± 1.4	17 ± 3	0.97	6 ± 1
1-PhEtOH	70	4913	48.8 ± 0.5	40.7 ± 0.7	0.99	8.3 ± 0.2	49.1 ± 0.7	43.9 ± 0.9	0.99	9.0 ± 0.2
ClC3ECF	60	1033	10.5 ± 0.4	3.3 ± 0.9	0.93	3.1 ± 0.8	10.8 ± 1.3	6 ± 3	0.84	6 ± 3
BrC3ECF	60	1824	18.5 ± 0.6	5.9 ± 1.4	0.94	3.2 ± 0.7	18.7 ± 1.3	8 ± 2	0.89	4 ± 2
BrC4ECF	70	2170	22.0 ± 0.8	6.2 ± 1.6	0.93	2.8 ± 0.8	22.3 ± 1.6	8 ± 3	0.86	4 ± 2
BrC5ECF	70	4185	42.1 ± 0.6	9.4 ± 1.4	0.97	2.2 ± 0.3	42.7 ± 2.0	14 ± 4	0.91	3 ± 1
ClC3OMe	50	927	9.9 ± 0.7	3.4 ± 0.9	0.87	3.4 ± 0.9	11.1 ± 1.8	8.9 ± 2.5	0.86	8 ± 3
BrC3OMe	50	1719	18.3 ± 1.1	6.9 ± 1.5	0.91	3.8 ± 0.9	19.1 ± 1.9	12 ± 3	0.92	6 ± 1
BrC4OMe	60	2122	22.5 ± 1.3	7.5 ± 1.8	0.90	3.3 ± 0.8	23.7 ± 2.4	17 ± 3	0.93	7 ± 2
BrC5OMe	50	7484	79.6 ± 4.9	29.5 ± 6.8	0.91	3.7 ± 0.9	87 ± 12	70 ± 16	0.90	8 ± 2
BrC6OMe	70	5393	53.7 ± 0.6	12.2 ± 1.3	0.99	2.3 ± 0.2	54.1 ± 0.4	18.4 ± 0.8	0.99	3.4 ± 0.2
3OHC4OMeTFA	60	1525	16.4 ± 2.9	10.9 ± 6.3	0.75	7 ± 4	16.4 ± 2.9	19 ± 6	0.90	11 ± 4
AbuOMeTFA	60	9213	92.8 ± 0.9	60.5 ± 1.3	0.99	6.5 ± 0.2	93.9 ± 3.7	96 ± 5	0.99	10.2 ± 0.7
Leu	80	8063	79.7 ± 2.5	17.8 ± 5.4	0.92	2.2 ± 0.7	79.9 ± 1.9	22 ± 4	0.97	2.8 ± 0.5
Norleu	80	9968	98.5 ± 2.9	21.5 ± 6.4	0.92	2.1 ± 0.7	98.8 ± 2.3	27 ± 5	0.97	2.7 ± 0.5

Abbreviations: 2M4PeOH – 2-methyl-4pentanol; 2-Br-C5 – 2-bromopentane; 335TMC – 3,3,5-trimethylcyclohexanone; 1-PhEtOH – 1-phenylethanol; ClC3ECF – 2-chloropropionic acid ethylchloroformate (ECF); BrC3ECF – 2-bromopropionic ECF; BrC4ECF – 2-bromobutyric ECF; BrC5ECF – 2-bromovaleric ECF; ClC3OMe – 2-chloropropionic methyl ester (OMe); BrC3OMe – 2-bromopropionic OMe; BrC4OMe – 2-bromobutyric OMe; BrC5OMe – 2-bromovaleric OMe; BrC6OMe – 2-bromohexanoic OMe; 3OHC4OMeTFA – 3-hydroxybutyric methyl ester trifluoroacetic anhydride (OMeTFA); AbuOMeTFA – 2-aminobutyric acid OMeTFA; leu – leucine; norleu – norleucine.

ii) The solute activity coefficient and mixture molar volume, γ^{∞}_{S+A} and ν_{S+A}, are either, totally independent or, otherwise, their values are linearly dependent on the selector molal concentration.
iii) Within the limitations given by these fittings, the linearity observed from these plots allow us to infer that the 1:1 stoichiometric ratio for the association enantiomer/CS would be correct.
iv) Since a few systems displayed nonlinear curves for the K_{app} *vs.* CS molality (*i.e.*, derivatized amino acids), we concluded that the solute activity coefficients in pure OV1701 were different from those in the (CD + OV1701) mixtures.

The validity of these measurements can be corroborated indirectly from the results shown in Table 3. For most solutes, the calculated intercepts are statistically equal for both enantiomers and, furthermore, they are also not statistically different from the corresponding K_L°. Two possible criticisms of the se estimations would be raised. The first, related to the

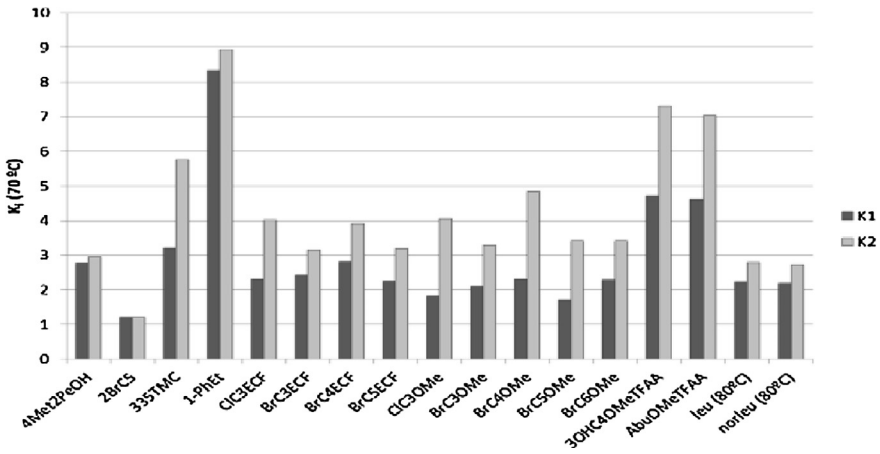

Fig. 4. Comparison of stoichiometric association constants between enantiomers and octakis(3-*O*-butanoyl-2,6-di-*O*-pentyl)-γ-cyclodextrin at 70 °C.

interpretation of the regression results, is that the figures could be affected by the weight given to the K_{app} values obtained for $m = 0$; for that reason, these experimental values were removed and the regressions were repeated with the remaining data. The results indicated similar intercepts and slopes that differed by less than 4% from those calculated from the first regression. The second criticism is related to the nonequivalence of ν_S and ν_{S+A} as determined from the density measurements. Nevertheless, the decrease in the molar volume for the 10%CD in OV1701 relative to the pure polysiloxane at the same temperature (amounting to about 1.5%) is virtually insignificant for these measurements. In summary, from the previous considerations we conclude that the approximation $K_L^{\circ} \approx K_L^{\circ,*}$ would be valid within the limits imposed by the errors in our measurements.

According to Eq. (4), the stoichiometric association constants between the enantiomer and the derivatized γ-CD can be calculated from the ratio between slope and intercept. The results of these estimations were also presented in Table 3. The standard deviations associated with these thermodynamic quantities were estimated from the rules of classical error propagation.

4.3. Enantioselective and nonenantioselective interactions

Strong nonchiral interactions, as reflected in large K_L° values, impair the separation process and would prevent baseline enantioresolutions. A second detrimental analytical consequence of strong nonstereoselective interactions is the increase in retention times (and decrease in peak height) proportional to the values of K_L°. Several authors have discussed the influence of the achiral polymer (*i.e.*, the solvent) used to dissolve the CD [11,16,17]. They revealed that, for constant CS concentrations, the use of solvent polymers of low or null polarity led to systems with higher enantiorecognition ability. Fig. 4 compares the K_i-values for all the solutes at 70 °C. The presence of an aromatic group (1-phenylethanol) practically doubles the association constants for both enantiomers as compared to the values for most other solutes. Schurig (7) has extensively discussed the implications of large K_i-values on selectivity: the enantioselective interactions become predominant over the nonenantioselective ones, that is, $K_{XA}\, m_A > 1$ and, consequently, the apparent enantioseparation factor becomes less dependent on CS concentration at much lower molalities.

The main observation is that the association constants are large between *both* enantiomers of 1-phenylethanol with the CS. This additional stability of these two complexes, attributed to the aromatic moiety, leads to an increase in both retention factors but, as a whole disfavors enantioresolution, and thus should be considered as a nonenantioselective interaction. From the analytical point of view, solutes with small *differences* between their interactions with the CS need relatively higher amounts of the latter to achieve a reasonable enantioresolution.

Very different conclusions can be drawn from a comparison of the K_i-values for carboxylic esters: the methyl esters of 2-bromocarboxylic acids are systematically smaller than the values for the corresponding ethyl esters (the ethyl chloroformate derivatives), *i.e.*, the additional methylene group confers more stability on the complex formed by the first enantiomer of these ethyl esters, but the K_2-values are slightly higher for the methyl esters. These results indicate that these specific interactions (resulting from the $-OCH_3$ and $-OC_2H_5$ groups) are truly enantioselective so that, consequently, the methyl esters have significantly larger enantioseparation factors than the corresponding ethyl esters. Venema et al. observed a similar behavior for the complexing of 2-bromo and 2-chlorocarboxylic acids with perpentyl-β-CD [18] as well as with permethyl-β-CD [19].

A similar comparison can be made with the stability constants corresponding to the esters of 2-chloro- to those of 2-bromopropanoic acids. The K_1-values of both the 2-bromopropanoic methyl and ethyl esters are slightly larger than the analogous 2-chloropropanoic esters, but the stability of the second enantiomer for each pair is greater for molecules containing a chloro substituent in C2 than for the bromo derivatives. These propionates constitute another example of enantioselective interaction between a halogen atom linked to C2 and the CS. Enantioselectivity measurements with perpentyl and permethyl-β-CD, however, had given larger enantioselective values for esters of 2-bromopropanoic acid than for the 2-chloropropanoic derivatives [18,19]. Similar conclusions can be drawn from the analysis of the association constants at the other temperatures.

4.4. Enantioselectivity factors

The use of K_{app} constants to estimate the apparent enantioselectivities and the associated apparent thermodynamic functions has been reported many times. As was postulated by Nowotny et al. [20], enantioselectivity is strongly influenced by the CS concentration in the achiral solvent so that the use of these apparent enantioselectivity results hinders both the assignment of meaningful association constants and the determination of correct bulk-solution thermodynamic quantities. Several authors have determined the difference between the contributions due to the enantiorecognition process and those arising from nonenantioselective interactions between the enantiomers with the achiral polymer. Schurig denominated chemical and physical contributions to these two components, respectively [21]. In Fig. 5, we

Table 4
Values of the retention increments, R'_1, and enantioselectivity factors estimated using as reference solutes the *n*-alkanes from heptane to decane measured at 70 °C.

Solute	C7	C8	C9	C10	C (=R_2/R_1)[a]	α (=R_2/R_1)[b]	$\alpha_{D/L}$[c]	α_{app}[d]
2-Me-4PeOH	0.71	0.68	0.65	0.63	1.04	1.044	1.07	1.02
2-Br-C5	0.21	0.19	0.17	0.16	1.17	1.20	1.20	1.03
335-TMC	0.66	0.63	0.61	0.58	1.68	1.71	1.79	1.26
1-PhEtOH	1.59	1.55	1.51	1.47	1.07	1.07	1.07	1.04
ClC3ECF	0.49	0.46	0.44	0.42	1.63	1.67	1.74	1.20
BrC3ECF	0.49	0.46	0.44	0.42	1.26	1.28	1.30	1.08
BrC4ECF	0.55	0.52	0.50	0.48	1.38	1.41	1.40	1.13
BrC5ECF	0.44	0.41	0.39	0.37	1.36	1.39	1.43	1.11
ClC3OMe	0.59	0.56	0.54	0.52	1.71	1.75	2.21	1.27
BrC3OMe	0.63	0.60	0.58	0.56	1.34	1.36	1.56	1.13
BrC4OMe	0.67	0.64	0.62	0.60	1.70	1.74	2.10	1.28
BrC5OMe	0.56	0.53	0.51	0.49	1.56	1.60	2.00	1.20
BrC6OMe	0.41	0.38	0.36	0.35	1.42	1.46	1.49	1.12
3OHC4OMeTFA	0.70	0.66	0.64	0.62	1.60	1.63	1.58	1.22
AbuOMeTFA	1.02	1.02	1.02	1.02	1.40	1.41	1.53	1.20
leu (80 °C)	0.47	0.45	0.43	0.41	1.20	1.21	1.25	1.06
norleu (80 °C)	0.46	0.44	0.42	0.40	1.19	1.20	1.25	1.06

[a] Reference analyte: *n*-octane.
[b] Reference analyte: *n*-decane.
[c] $\alpha_{D/L} = K_2/K_1$.
[d] Apparent enantioselectivities in column 4.

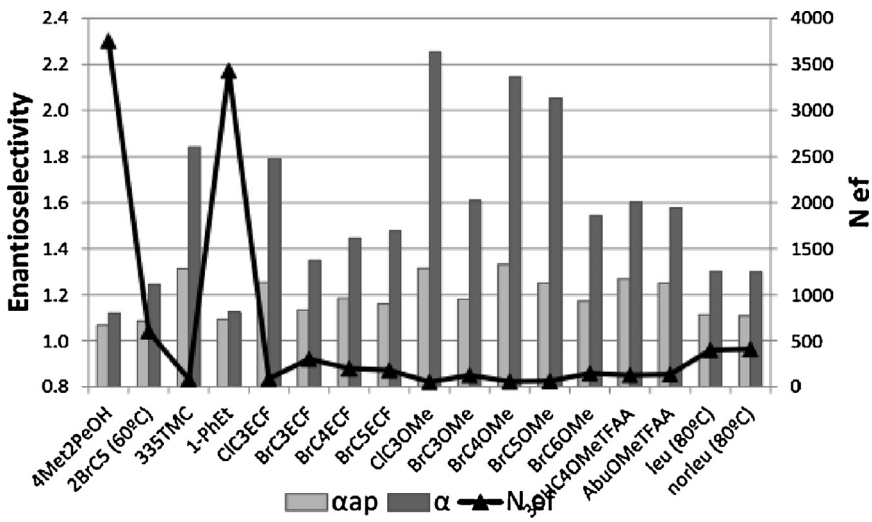

Fig. 5. True and apparent enantioselectivity factors measured with Column 4 (see Table 1) and at 70 °C (left ordinate) and effective plate number necessary to separate with resolutions equal to unity (right ordinate).

compared the α_{app}-values measured in Column 4 with the true enantioselectivity factors at 70 °C. The plot clearly indicates the large differences between both quantities for certain solutes. On the right axis of the figure, we plotted the effective theoretical plates that would be required to get resolution factors equal to unity if nonenantioselective interactions would be eliminated. In all the instances fewer than 1000 effective plates, that is, columns of a few meters length, would be more than sufficient for acceptable enantioseparation.

Jung et al. [22] first proposed the concept of retention increment, R', in enantioselective GC. This concept was adapted from previous studies involving olefins in argentation chromatography [23–26]. Initially, the authors used this R' concept with CS based on metallic complexes, but in 1991 they applied the same concept to cyclodextrin complexes [22]. Retention increment, Rí ($R' = K_{XA}m_A$) stands for the increment in solute retention attributable to the presence of the CS in the polysiloxane. The value is experimentally accessible from the determination of the relative retentions between the enantiomer *X*, and a reference substance in the chiral column, $r_X(=k_X/k_{ref})$, as well as in a column containing only the achiral polymer, $r^\circ(=k_x{}^\circ/k_{ref}{}^\circ)$, as follows: $R' = (r_X - r^\circ)/r^\circ$. In this approach, the chosen reference compound should not interact with the CS. With the assumption that this reference molecule is retained only through a partitioning in the achiral polymer, a direct measurement of the column phase ratio is not necessary and can be estimated indirectly from the reference and solute retention factors. The retention increment model has been used extensively to obtain enantioselectivity factors from the ratio between R'_2/R'_1 (where the 1 and 2 refer to the first and the second eluted enantiomer) for several systems [22,27–29]. The retention-increments R' have proved to be very precise to provide thermodynamic data using only three columns, and independently from the reference standard chosen, for systems which exhibit high enenatioselectiviy factors [30,31].

We calculated R' values using as a reference the *n*-alkanes from *n*-C7 to *n*-C10 at all the temperatures. Table 4 gives the results at 70 °C. Clearly, the values depend on the substance chosen as a reference. The formation of inclusion complexes between *n*-alkanes and permethyl-β-cyclodextrin was reported by Casu et al. [32] who isolated the formed complexes as solids and studied their thermal behavior. Other authors admitted that even *n*-alkanes would form associations with certain CDs [22,28]. The R' measurements made by those authors depended on the *n*-alkane; they reported differences of up to 10%.

Table 5
Thermodynamic properties for the complex formation between enantiomer and the chiral selector octakis(3-*O*-butanoyl-2,6-di-*O*-pentyl)-γ-cyclodextrin.

Solute	$-\Delta H^{\circ}{}_1$ [a] (±s)[b] (kJ mol^{-1})	$-\Delta H^{\circ}{}_2$ [a] (±s)[b] (kJ mol^{-1})	$-T(\Delta S^{\circ}{}_1)$ [c] (kJ mol^{-1})	$-T(\Delta S^{\circ}{}_2)$ [c] (kJ mol^{-1})	$\Delta(\Delta G^{\circ})$ [d] (kJ mol^{-1})	$\Delta(\Delta H^{\circ})$ [e] (±s) (kJ mol^{-1})	$-T\Delta(\Delta S^{\circ})$ (kJ mol^{-1})
2M4PeOH	−23(±1)	−26(±1)	−20.2	−22.6	−0.19	−2.6(±0.5)	2.4
2-Br-C5	−23(±2)	−28(±1)	−22.1	−27.0	0.00	−5(±1)	4.9
335TMC	−24(±4)	−32(±2)	−20.2	−27.2	−1.66	−9(±2)	7.0
1-PhEtOH	−22.7(±0.7)	−23.7(±0.6)	−16.6	−17.4	−0.20	−1.0(±0.1)	0.8
ClC3ECF	−25(±1)	−33.3(±0.7)	−23.0	−29.4	−1.58	−7.2(±0.6)	5.6
BrC3ECF	−23(±1)	−28(±1)	−20.5	−24.9	−0.75	−6.7(±0.9)	5.9
BrC4ECF	−27(±2)	−34(±2)	−24.3	−30.0	−0.95	−7.0(±0.5)	6.1
BrC5ECF	−20(±12)	−31(±1)	−17.7	−27.7	−1.01	−11(±1)	9.9
ClC3OMe	−29(±5)	−37(±2)	−27.7	−32.6	−2.26	−7(±3)	4.9
BrC3OMe	−27(±0.5)	−30.6(±0.4)	−24.5	−27.2	−1.26	−4.3(±0.2)	3.0
BrC4OMe	−38(±2)	−43(±2)	−35.5	−38.2	−2.11	−6(±2)	3.4
BrC5OMe	−47(±3)	−48(±2)	−46.0	−44.2	−1.98	−1(±1)	−1.3
BrC6OMe	−18(±1)	−26(±1)	−15.5	−22.4	−1.14	−8.0(±0.2)	6.9
3OHC4OMeTFA	−37(±1)	−44.7(±0.8)	−32.4	−39.0	−1.26	−7.9(±0.4)	6.6
AbuOMeTFA	−40(±3)	−41(±2)	−35.8	−35.2	−1.21	−1.6(±0.5)	0.4
leu (80 °C)	−28(±2)	−31(±2)	−25.9	−28.3	−0.65	−3.3(±0.6)	2.7
norleu (80 °C)	−24(±1)	−29(±2)	−22.0	−25.7	−0.64	−4.6(±0.4)	4.0

[a] From the linear regression of ln K_{assoc} vs. (1/*T*). 1 and 2 refers to the first and second eluted enantiomer.
[b] Standard deviations in parenthesis.
[c] Calculated from the differences ($\Delta G^{\circ} - \Delta H^{\circ}$) at 70 °C.
[d] Calculated from $-RT \ln \alpha$ (70 °C).
[e] From linear regression of ln α vs. (1/*T*).

These, or even larger, differences were also obtained in the present study. In the last two columns of Table 4, we included the enantioselectivities estimated as the ratio between R'_2/R'_1 using C8 and C10, respectively, as references. The differences in α-values are not significant, but these values were systematically smaller than the true α-values calculated from the K_i, which result constitutes clear evidence of the existence of interactions between these alkanes and the γ-CD in OV1701.

In our opinion, the approach of using the R' concept should be reserved for the situation in which the density of the stationary phase would be difficult to measure, for instance, when the chiral selector has been bonded with the polymer *via* a covalent linkage or after a polymer crosslinking. For systems formed by the selector dissolved in the achiral matrix, however, estimations of the K_{assoc} permit a precise determination of the true enantioselectivity factors.

4.5. Influence of temperature

Temperature is fundamental in chiral GC. Usually, both the retention factors and enantioselectivity decrease as the temperature is increased. That behavior was observed for all these systems. The thermodynamic properties for the complex formation between the enantiomers and the CS have been calculated from least squares regressions of the association constants and the enantioselective factors as a function of the reciprocal of temperature, which approach constitutes an acceptable treatment provided that the thermodynamic functions are independent of temperature.

The differences in the change in free energy change ($\Delta\Delta G^{\circ}$), enthalpy ($\Delta\Delta H^{\circ}$) and entropy ($\Delta\Delta S^{\circ}$) for the association of each enantiomer with the CS estimated are true (not apparent) thermodynamic quantities. The non-enantioselective interactions that contribute to solute retention in the CS + OV1701 systems have been differentially determined, and the association constants calculated are independent on CS concentration. Table 5 summarizes the results from these regressions. Enantioseparations are clearly dominated by the enthalpic differences in the association. As discussed above, the esters of 2-chloropropionic acids are better separated than the corresponding to 2-bromopropionic acids, and the differences can be attributed to enthalpic differences. The contrasting feature between the two kind of molecules are the size and the larger dipolar moment of the 2-chloropropionates. Similarly, the higher polarity of the methyl as compared to the ethyl esters of the same carboxylic acid would be the cause for the greater enantioselectivities of those methyl derivatives. The association constant between methyl 2-chloropropionate and octakis(3-*O*-butanoyl-2,6-di-*O*-pentyl)-γ-cyclodextrin has been reported in the literature. The informed $\Delta\Delta G^{\circ}$ value cited (560 cal/mol) is quite similar to that measured in this work, but the $\Delta\Delta H^{\circ}$ value was about 90% larger than the value estimated by us [33].

From a practical perspective, in the present work, improvements in resolution were achieved at relatively low temperatures but at the expense of excessively long analysis times. For any optimization strategy one must resolve a given enantiomeric pair within the shortest analysis time. Therefore, a thorough understanding of the equilibria between each enantiomer with the CS becomes fundamental.

5. Conclusions

The main conclusions drawn from the results are as follows:

- We demonstrated that efficient columns can be constructed by a rapid static method, one that had been used successfully in the past for coating chiral and non-chiral liquid phases on capillary columns. We were able to fabricate columns with very precise

phase ratios if a few experimental precautions were considered in preparing the solutions used to fill the capillaries and if careful measurements of the stationary phase densities were obtained.

- We proposed a reliable method for determining enantiomeric association constants using capillary columns. According to the basic equations of chromatography, and without resorting to any *ad-hoc* hypothesis, the enantiomeric association constants could be estimated with high precision. These results together with the method used to construct the capillary columns, conferred on capillary gas chromatography not only its well-known and established maturity as an analytical technique but also the feasibility of using these capillary columns to determine system physicochemical properties such as enantiomeric association constants.
- The results of true enantioselectivity were compared with values of enantioselectivity estimated from the model of the retention increments. We verified that the retention increment values depended on the *n*-alkane used as reference and practically all the values obtained were systematically smaller than those arising from the use of association constants. We suggest that straightforward chromatographic retention concepts are sufficient to obtain reliable association constants provided that column phase ratios could be accurately known.

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