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Approach to the thermodynamics of enantiomer separation by gas chromatography
Enantioselectivity between the chiral inhalation anesthetics enflurane, isoflurane and desflurane and a diluted γ-cyclodextrin derivative

V. Schurig*, M. Juza

Institut für Organische Chemie, Universität Tübingen, Auf der Morgenstelle 18, 72076 Tübingen, Germany

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Abstract

The thermodynamics of enantioselectivity, $-\Delta_{D,L}(\Delta G)$, $-\Delta_{D,L}(\Delta H)$, $\Delta_{D,L}(\Delta S)$ and T_{iso}, have been determined by gas chromatography employing the concept of the retention increment R' for the inhalation anesthetics enflurane (**1**), isoflurane (**2**) and desflurane (**3**) and the selector octakis(3-*O*-butanoyl-2,6-di-*O*-*n*-pentyl)-γ-cyclodextrin (**4**) in the polysiloxane SE-54. It is shown that the separation factor α is concentration-dependent. Therefore, the separation factor α should not be employed as a criterion for enantioselectivity in diluted systems. The $-\Delta_{D,L}(\Delta G)$ data for **1** and **4** are corroborated by ^{1}H NMR spectroscopic measurements.

Keywords: Enantiomer separation; Thermodynamic parameters; Enflurane; Isoflurane; Desflurane

1. Introduction

Diethyl ether was introduced into medicine as a human inhalation anesthetic by Crawford W. Long from Jefferson, Georgia in 1842 (published in 1849) and by William Thomas Green Morton in Boston, 1846 [1]. By substituting hydrogen for halogen, improved narcotic gases were developed with reduced toxicity and flammability and increased volatility and potency. At present, the haloethers enflurane (**1**) and isoflurane (**2**) (cf. Fig. 1) are the most frequently administered inhalation anesthetics while the highly volatile narcotic gas desflurane (**3**) is in its clinical test phase.

The consequences of the (unintended) introduction of a chiral centre into the haloethers and its impact on enantioselective biological action [2–5] have been overlooked in the past and chiral inhalation anesthetics are still produced and clinically administered as racemic mixtures. As this situation may be subject to reconsideration in the future, the analytical and preparative enantiomer separation of chiral inhalation anesthetics is of great current interest. With the advent of cyclodextrin-containing stationary phases in gas chromatography [6,7], racemic halogenated hydrocarbons and haloethers can successfully be resolved.

* Corresponding author.

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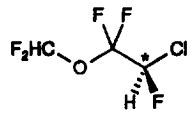
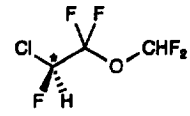
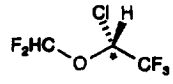
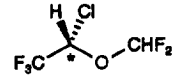
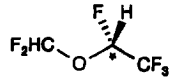
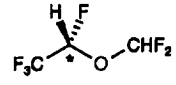
	(*R*)-configuration	(*S*)-configuration
Enflurane 1		
Isoflurane 2		
Desflurane 3		

Fig. 1. Chiral haloethers employed as human inhalation anesthetics.

Thus, Meinwald et al. reported the first enantiomer separation of **1**, **2** and halothane [C*HClBr(CF_3)] on 25 m fused-silica capillary columns coated with hexakis(2,3,6-tri-*O*-*n*-pentyl)-α-cyclodextrin and with octakis(6-*O*-methyl-2,3-di-*O*-*n*-pentyl)-γ-cyclodextrin, respectively, at 30°C [8]. We found that octakis(3-*O*-butanoyl-2,6-di-*O*-*n*-pentyl)-γ-cyclodextrin **4** (cf. Fig. 2) [9], when diluted in polysiloxanes such as OV-1701 or SE-54 [10], resolves **1–3** and halothane [11–13].

The large chiral separation factors $\alpha > 2$ for **1** and $\alpha > 1.3$ for **2** and **3** observed on **4** (cf. Fig. 3) permitted their preparative gas chromatographic enantiomer separation in high chemical and enantiomeric purity [11–13].

In parallel investigations, Vigh et al. separated the enantiomers of **1** and **2** on undiluted pertrifluoroacetylated γ-cyclodextrin, a commercially available synthetic mixture of isomers and homologues [14–16].

The resolved haloethers **1–3** represent small chiral linear molecules while the resolving agent **4** contains a large cavity (cf. Figs. 1 and 2). Therefore, the occurrence of large chiral separation factors α is surprising and the determination of thermodynamic

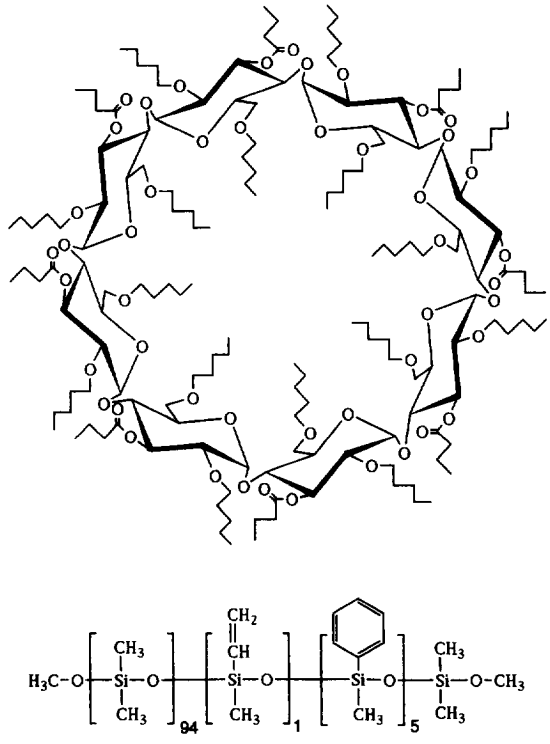


Fig. 2. The chiral selector octakis (3-*O*-butanoyl-2,6-di-*O*-*n*-pentyl)-γ-cyclodextrin **4** [9] and the polysiloxane solvent SE-54.

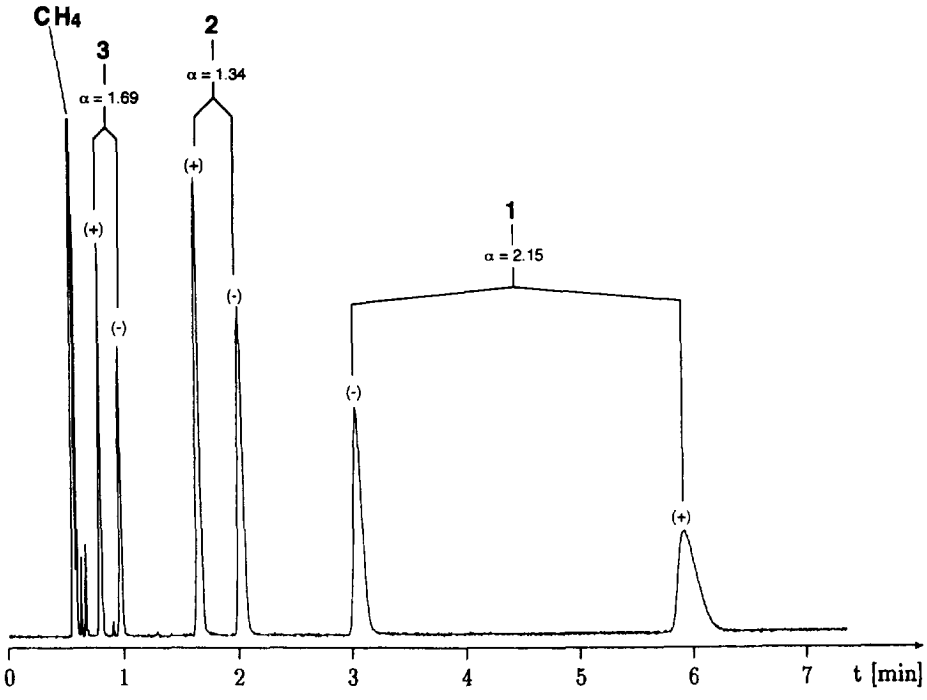


Fig. 3. Simultaneous analytical gas-chromatographic enantiomer separation of the inhalation anesthetics **1–3**. Fused-silica capillary column (25 m×0.25 mm I.D.) coated with **4** dissolved in SE-54 (column II), film thickness 0.5 μm, at 26°C. Carrier gas: 1.1 bar helium.

data of enantiomer discrimination is of considerable interest. Preliminary data on the thermodynamics of enantiomer discrimination between **1–3** and **4** in the polysiloxane SE-54 have been reported previously [13].

Here, we describe in great detail a general approach suitable for the determination of thermodynamic parameters of enantioselectivity obtained from gas-chromatographic retention data. Most importantly, in the present treatment the enantioselectivity of the diluted cyclodextrin derivative is distinguished from nonenantioselective contributions to retention arising from the achiral polysiloxane solvent. As previously advanced in complexation gas chromatography [17–21] and inclusion gas chromatography [22], the present methodology is based on the concept of the retention increment (or chemical capacity factor) R' accessible from relative retention data. The approach described here is of general applicability to chiral stationary phases consisting of diluted systems.

2. Experimental

2.1. Instrumentation

A 5300 Mega gas chromatograph (Fisons, Mainz, Germany) was used for the thermodynamic measurements. Temperatures were controlled by the oven display and checked by an additional digital temperature sensor (±0.1K). Retention times (in tenth of s) were recorded with a Shimadzu C-R6A integrator.

2.2. Materials

Enflurane (**1**) and isoflurane (**2**) were obtained from a local hospital. Desflurane (**3**) was kindly provided by Pharmacia, Erlangen, Germany. γ-Cyclodextrin was a courtesy of the Consortium für Elektrochemische Industrie, Munich, Germany.

The selector (**4**) was synthesized according to the published procedure of König et al. [9], except that the acylation was accomplished in triethylamine at

50°C. The product was carefully purified by repeated column chromatography over silica gel with ethyl acetate–*n*-heptane (from 1:10 to 1:3, v/v). The high chemical purity of **4** was proved by electro spray mass spectrometry [23] and by NMR spectroscopy establishing the absence of under- and over-pentylated and -acylated congeners.

2.3. Columns

Reference column: a fused-silica capillary column (50 m×0.25 mm I.D.) was heated at 200°C in a slow stream of hydrogen for 5 h and, without further deactivation, coated by the static method with the reasonably apolar polysiloxane SE-54 (dimethyl polysiloxane with 5% phenyl, 1% vinyl, CP polarity index 8). The film thickness was 0.5 μm. Helium was used as carrier gas at an inlet pressure of 2.1 bar.

Reactor columns: fused-silica capillary columns (25 m×0.25 mm I.D.) were heated at 200°C in a slow stream of hydrogen for 5 h, and, without further deactivation, coated by the static method with 5.23% (w/w) **4** dissolved in the polysiloxane SE-54 affording reactor column I (~0.02 *m*) and with 10.135% (w/w) **4** in SE-54 affording reactor column II (~0.04 *m*), respectively. The film thickness was 0.5 μm each. Helium was used as carrier gas at an inlet pressure of 1.1 bar.

2.4. Thermodynamic measurements

Thermodynamic measurements were performed as previously described [17,22]. Thus, the two reactor columns I and II (see above) were co-installed in the injector and operated in parallel, thus ensuring exactly the same column temperature. For column temperatures below 30°C, the columns were placed in a water bath, whose temperature could easily be checked by a digital (±0.1K) and by a conventional thermometer. The gas holdup time t_M was determined by co-injecting methane. The Kováts plots log t' vs. n (n=number of carbon atoms) for a homologous series of *n*-alkanes (*n*-butane to *n*-heptane) were linear on the reference column and on the two reactor columns I and II [regression lines were $y=-2.151+0.445x$ (reference column, $r=0.999$); $y=-2.417+0.445x$ (reactor column I, $r=0.999$); $y=-2.338+0.446x$ (reactor column II, $r=0.999$)]. All adjusted retention times t'_R of **1–3** (i.e., for the unresolved racemates on the reference column and for the separated enantiomers on the reactor columns) were related to t'_R* of each one of four reference compounds, i.e., *n*-pentane, *n*-hexane, *n*-heptane and diethyl ether which were co-injected as mixture, except when peak-overlapping occurred, to give relative retentions r° and r, respectively ($r=t'_R/t'_R$*). As the relative retentions on the reference column, r°, are low, a 50 m long column was used and special attention was paid to the correct determination. Thus, values for r° were measured at 0, 15, and 30 to 100°C, in steps of 5°C, and ln r° was plotted against $1/T$. These data were used for a linear regression, which allowed the precise interpolation of the r° values needed for a desired temperature (cf. Fig. 4).

Thermodynamic data were calculated according to Eq. (16), Eqs. (19,20). The regression analysis was performed according to the least square method to calculate the slope ($-\Delta_{D,L}(\Delta H)$), intercept ($\Delta_{D,L}(\Delta S)$), standard deviations and coefficients of the determinations using the program LOTUS 1-2-3 for Windows, version 1.1 (Lotus Development) on a personal computer. This resulted in slightly different values (with lower standard deviations) to the previously published data in Ref. [13] which were obtained with a pocket calculator. All $R \ln R'_D/R'_L$ vs. $1/T$ plots were linear with regression coefficients greater than 0.996.

3. Theory

Consider a volatile solute B (selectand) migrating through a gas-chromatographic reactor column containing a dilute solution of the nonvolatile additive A (selector) in the nonvolatile inert solvent S (cf. Fig. 5, right).

When a 1:1 associate is formed rapidly and reversibly between A and B, two *separate* equilibria are distinguished

$$B_{(g)} \rightleftharpoons B_{(l)} \qquad K^\circ_L \qquad (1)$$

and

$$A + B_{(l)} \rightleftharpoons AB \qquad K \qquad (2)$$

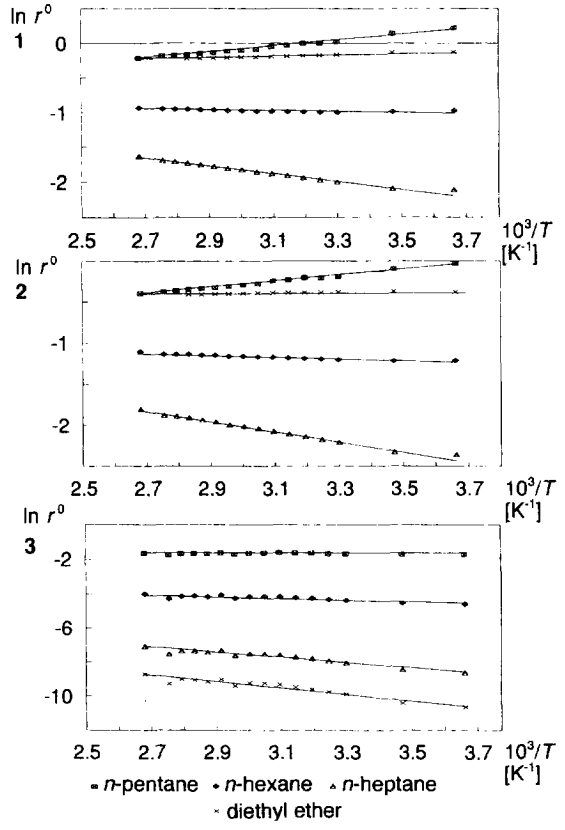


Fig. 4. Linear interpolation of r° of **1–3** by plotting ln r° vs. $1/T$ obtained on the reference column containing pure SE-54 for the four reference compounds B* *n*-pentane, *n*-hexane, *n*-heptane and diethyl ether between 0 and 100°C. Enflurane **1** (top), isoflurane **2** (middle) and desflurane **3** (bottom). For conditions see Section 2.

or

$$K_L^\circ = \frac{c_{B(l)}}{c_{B(g)}} \tag{3}$$

and

$$K = \frac{a_{AB}}{a_A a_{B(l)}} = \frac{c_{AB}}{c_A c_{B(l)}} \frac{\gamma_{AB}}{\gamma_A \gamma_{B(l)}} \tag{4}$$

where the subscripts g and l refer to the gas and the liquid phase, respectively. K_L° is the distribution constant (partition coefficient) of B between the gas and the pure liquid phase S (=physical contribution to retention, neglecting the presence of A in S), K is the thermodynamic association constant of A and B in S (=chemical contribution to retention) and a_i is the activity of the species i, with the convention that

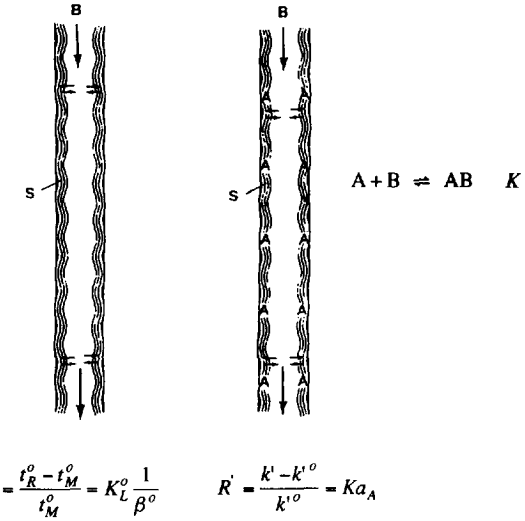


Fig. 5. The principle of association gas chromatography. Reference column (left) and reactor column (right).

$a_i \rightarrow c_i$ when $c \rightarrow 0$. As both AB and $B_{(l)}$ usually are highly diluted in the gas-chromatographic experiment, Eq. (4) can be rewritten

$$K a_A = \frac{c_{AB}}{c_{B(l)}} \tag{5}$$

The total concentration of the selectand B in the liquid phase S is $c_{B(l)} + c_{AB}$. Hence, the apparent distribution constant K_L, assuming no volume change on dissolution of B, is [24]

$$K_L = \frac{c_{B(l)} + c_{AB}}{c_{B(g)}} \tag{6}$$

With Eqs. (3,5) this can be rewritten

$$K_L = \frac{c_{B(l)}}{c_{B(g)}} + \frac{c_{B(l)}}{c_{B(g)}} \frac{c_{AB}}{c_{B(l)}} = K_L^\circ + K_L^\circ K a_A \tag{7}$$

or

$$K a_A = \frac{K_L}{K_L^\circ} - 1 \tag{8}$$

Thus, the thermodynamic association constant K can be obtained from the plot of K_L vs. a_A (Eq. (7)) [24–26].

From Eq. (8), a very useful relationship between K and chromatographic retention data can be developed when a reference column, containing the

pure solvent S (cf. Fig. 5, left, symbols referring to the reference column are marked with a circle, e.g., K_L°, k'°) and an inert reference compound B* not interacting with A and coinjected with B, is employed [17,27–29] (symbols referring to B* are marked with an asterisk, e.g., k'^*).

The distribution constant of the selectand B between the gas and the pure liquid phase S, K_L°, is defined by

$$K_L^\circ = k'^\circ \beta^\circ \tag{9}$$

and the apparent distribution constant of the selectand B between the gas and the liquid phase S containing the selector A, K_L, is defined by

$$K_L = k' \beta \tag{10}$$

where k'° is the retention factor of B in the pure solvent S, k' is the retention factor of B in the solution of A in S, β° is the phase ratio of the reference column and β is the phase ratio of the reactor column. Eq. (8) can now be rewritten as

$$K a_A = \frac{k'}{k'^\circ} \frac{\beta}{\beta^\circ} - 1 \tag{11}$$

For the ideal reference compound B* not interacting with A it is required that $K^*=0$. From Eq. (11), it follows for B* that

$$\frac{k'^*}{k'^{\circ *}} \frac{\beta}{\beta^\circ} = 1 \tag{12}$$

The ratio β/β° is equal for B and co-injected B* and Eqs. (11,12) can be combined to give

$$K a_A = \frac{k'}{k'^\circ} \frac{k'^{\circ *}}{k'^*} - 1 = R' \tag{13}$$

With

$$\frac{k'^\circ}{k'^{\circ *}} = \frac{t_R'^\circ}{t_R'^{\circ *}} = r^\circ \tag{14}$$

and

$$\frac{k'}{k'^*} = \frac{t_R'}{t_R'^*} = r \tag{15}$$

a relationship referring to relative retention data r° and r is obtained [17]

$$K a_A = \frac{r}{r^\circ} - 1 = \frac{r - r^\circ}{r^\circ} = R' \tag{16}$$

where r is the relative retention of the selectand B with respect to the inert reference compound B* on the reactor column containing the selector A with the activity a_A in the inert solvent S (cf. Fig. 5, right), and r° is the relative retention of the selectand B with respect to the same reference compound on a reference column containing the pure solvent S, devoid of the additive A (cf. Fig. 5, left). Whereas it is experimentally difficult, if not impossible, to determine adjusted retention times $t_R'^\circ$ and t_R' of B with strictly identical parameters for the reference and reactor column, the relative retention data r° and r in Eq. (16) are independent of the type of the columns (packed vs. capillary) and from all operating conditions except the temperature. Thus, in the absence of interfacial adsorption effects [30], gas-chromatographic parameters such as the phase ratio (determined by the volume of the mobile and stationary phase or film thickness and internal diameter, respectively), flow-rate of the mobile phase, column pressure drop and column length can be ignored. However, since r refers to retention factors k' or adjusted retention times t_R', respectively, the gas holdup time t_M of the columns has to be determined either as the time of the air peak, if present, or, as an approximation, of the peak of coinjected methane. The validity of Eq. (16) has previously been scrutinized by careful and extensive experiments [17,19,31].

According to Eq. (16), the retention increment (or chemical capacity factor) R' is linearly related with a_A at a given temperature when a 1:1 molecular complex is formed between A and B (as can be expected when minute amounts of B are injected into the column). While the retention increment R' can accurately be measured, an error in the *absolute* value of the thermodynamic association constant K according to Eq. (16) may arise due to the incertitude of the activity a_A of the additive A in the solvent S. In very dilute solutions the activity a_A may be related to the molarity M_A or, preferably, to the molality m_A concentration scale (the unit molality is independent of the temperature, and, for practical reasons, it is advantageous to add A to the weighed amount of S) [17]. In the binary separation system of enantiomers it suffices to consider relative thermodynamic association constants. Since the enantiomers compete for the same selector A in S, the

ratio K_D/K_L is directly related to the ratio of their retention increments R'_D/R'_L, accessible from relative retention data r_D, r_L and r°, and, significantly, this ratio is independent of the activity of A in S, a_A (the subscripts D, dextro, right, and L, levo, left, denote enantiomers irrespective of their absolute configuration; D is arbitrarily eluted after L from the column), i.e.,

$$\frac{K_D}{K_L} = \frac{R'_D}{R'_L} = \frac{r_D - r^\circ}{r_L - r^\circ} \tag{17}$$

Moreover, as enantiomers can not be separated on the reference column containing an achiral solvent S, r° is identical for the enantiomers D and L. Hence, in principle, r° need not be determined separately but can be extrapolated from two sets of data of the relative retention, r, for the enantiomers (D and L) at two arbitrary (unknown) activities (or concentrations) of A in S of the reactor columns I and II.

$$r^\circ = \frac{r_D^{(I)} r_L^{(II)} - r_L^{(I)} r_D^{(II)}}{(r_D^{(I)} + r_L^{(II)}) - (r_L^{(I)} + r_D^{(II)})} \tag{18}$$

Eq. (18) can be used to assess the nonenantioselective contributions to retention, r°. Sometimes, r° is difficult to measure directly, e.g., when selectors are chemically bonded to polysiloxanes (Chirasil-Val [32] and Chirasil-Dex [33]). The validity of Eq. (18) which is based on that of Eq. (16) has previously been verified [19] and will be confirmed in the present work.

Enantioselectivity is defined by the term $-\Delta_{D,L}(\Delta G)$. Using the Gibbs–Helmholtz Eq. (19), all further thermodynamic data governing enantioselectivity are accessible from Eq. (17) as follows

$$-\Delta_{D,L}(\Delta G) = -\Delta_{D,L}(\Delta H) + T\Delta_{D,L}(\Delta S) = RT \ln\frac{K_D}{K_L} = RT \ln\frac{R'_D}{R'_L} = RT \ln\frac{r_D - r^\circ}{r_L - r^\circ} \tag{19}$$

or as the van't Hoff plot

$$\frac{-\Delta_{D,L}(\Delta G)}{T} = -\frac{\Delta_{D,L}(\Delta H)}{T} + \Delta_{D,L}(\Delta S) = R \ln\frac{K_D}{K_L} = R \ln\frac{R'_D}{R'_L} = R \ln\frac{r_D - r^\circ}{r_L - r^\circ} \tag{20}$$

$-\Delta_{D,L}(\Delta G)$ is independent of the activity a_A of A in S and, hence, also from its concentration (molality). Isothermal measurements at different activities (concentrations) should yield the same value for $-\Delta_{D,L}(\Delta G)$. This requirement has previously been verified in complexation gas chromatography [19,20,34]. Even concentration gradients in the diluted stationary phase would not affect $-\Delta_{D,L}(\Delta G)$.

In chromatography, enantioselectivity is frequently related to the chiral separation factor α.

$$\alpha = \frac{t'_{R(D)}}{t'_{R(L)}} = \frac{k'_{R(D)}}{k'_{R(L)}} = \frac{r_D}{r_L} \tag{21}$$

However, it is important to note that the chiral separation factor α is concentration-dependent when A is diluted in S. By substituting r_D and r_L in Eq. (21) by Eq. (16) it follows that [22]

$$\alpha_{dil} = \frac{K_D a_A + 1}{K_L a_A + 1} = \frac{R'_D + 1}{R'_L + 1} \tag{22}$$

According to Eq. (22), the α_{dil} vs. a_A curves level off at high values of a_A (high concentrations of A in S). The optimum is reached already at low concentrations if chemical association is strong (i.e., Ka_A or $R' \gg 1$) [22]. Thus, while $-\Delta_{D,L}(\Delta G)$ represents a true concentration independent measure of enantioselectivity, the chiral separation factor α is of only practical importance in diluted systems. Its numerical value underestimates the underlying chiral discrimination since the total retention from which it is calculated is the sum of the nonenantioselective physical contribution to retention (r°) and the enantioselective chemical contribution to retention ($r - r^\circ$). It is only the ratio of the latter which leads to enantiomer separation according to Eq. (19), i.e., $(r_D - r^\circ)/(r_L - r^\circ)$. Only in the rare case that r° is very low and association is very strong, i.e., $r \gg r^\circ$, may $-\Delta_{D,L}(\Delta G)$ be approximated from Eq. (23), previously derived for undiluted selectors A [17]

$$-\Delta_{D,L}(\Delta G) = RT \ln\alpha_{undil} \approx RT \ln\frac{t'_{R_D}}{t'_{R_L}} \approx RT \ln\frac{r_D}{r_L} \text{ for } r \gg r^\circ \tag{23}$$

or the van't Hoff plot

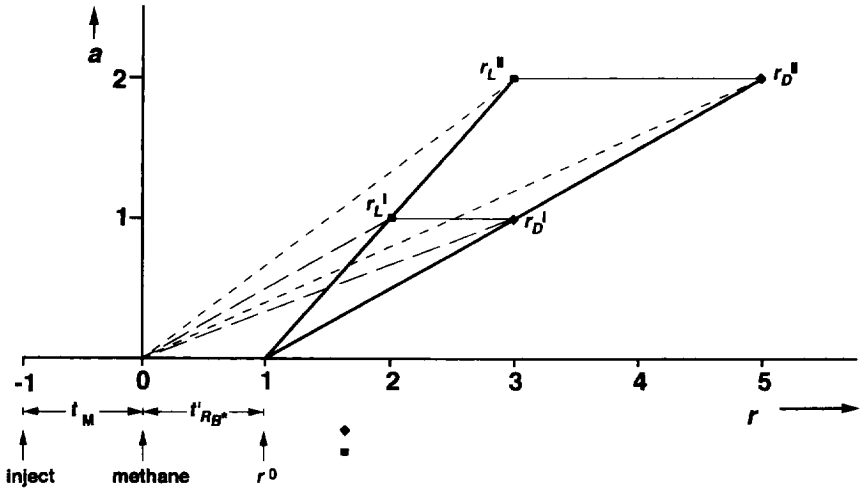


Fig. 6. Schematic representation of the distinction between (i) non-enantioselective contributions to (relative) retention r°, and (ii) enantioselective contributions to (relative) retention, $r_i - r^\circ$ (i = L and D), leading to the constancy of the ratio of R'_D/R'_L as required by Eq. (18). For simplification $t_M = t'_{R_{B*}} = k'_{B*} = 1$ and $R'_D/R'_L = 2$; $r^\circ = 1$ and r_i represent arbitrary numbers. Note that for $r_D = 1.02$ and $r_L = 1.01$ (very low concentration of the selector, not drawn) $\alpha \sim 1$ (implying no enantioselectivity) while $R'_D/R'_L = 2$ (unchanged enantioselectivity as required by Eq. (18)).

$$\frac{-\Delta_{D,L}(\Delta G)}{T} = R\ln\alpha_{undil} \approx R\ln\frac{t'_{R_D}}{t'_{R_L}} \approx R\ln\frac{r_D}{r_L}$$

$$\text{for } r \gg r^\circ \qquad (24)$$

In Fig. 6 a schematic representation of the concept of the retention increment R' based on arbitrary relative retention data r° and r_i (i = D and L) is presented. It is evident that the separation factor $\alpha = r_D/r_L$ contains the nonenantioselective retention r° and is thus rendered concentration-dependent [22], while the ratio $R'_D/R'_L = (r_D - r^\circ)/(r_L - r^\circ)$ is strictly concentration independent.

The concept of the retention increment (previously called the retention increase) R' has originally been developed for the investigation of thermodynamic parameters of molecular association in diluted systems by complexation gas chromatography [17,31] and has later been extended to chiral separations [19–21,34]. The method may also be applied to other types of molecular association, i.e., enantioselective inclusion using cyclodextrin derivatives [22]. While the interaction of saturated hydrocarbons, used as reference compound B*, with metal ions can be neglected in complexation gas chromatography, this may not be the case for cyclodextrin-type selectors. Thus, for inclusion interactions truly inert reference compounds are not available since even unfunctionalized hydrocarbons form weak associates as judged from the observation that the enantiomers of saturated hydrocarbons can be separated on alkylated cyclodextrins [10]. If the reference compound B* possesses itself an inherent retention increment R'_{B*}, however small, with respect to a hypothetical totally inert compound B**, a relationship between the measured retention increment R'

$$R' = \frac{r - r^\circ}{r^\circ} \qquad (25)$$

and the true retention increment R'_{true}

$$R'_{true} = \frac{r_{true} - r^\circ}{r^\circ} \qquad (26)$$

can be derived [22].

The r values (but not the r° values) must be corrected in analogy with Eq. (16)

$$R'_{B*} = \frac{r_{true} - r}{r} \qquad (27)$$

or

$$R'_{true} = \frac{r_{true} - r^{\circ}}{r^{\circ}}$$
$$= (1 + R'_{B*})\frac{r}{r^{\circ}} - (1 + R'_{B*}) + R'_{B*}$$
$$= (1 + R'_{B*})R' + R'_{B*} \quad (28)$$

R'_{B*} is not an inaccessible quantity but can be estimated by comparison of calculated and measured α vs. molality curves [22]. As demonstrated for enantioselective inclusion gas chromatography [22], values for $R'_{B*}=0.1-0.2$ have typically been found for hydrocarbons on permethylated cyclodextrins.

It was inferred recently that the solvent S, e.g., polysiloxanes, may themselves interact with derivatized cyclodextrins [35,36]. Eq. (16) has therefore been modified to account for the nonideal behaviour between A and S [35].

$$R' = K(a_A - K_1 f(a_A) f(a_S)) \quad (29)$$

with K_1 = thermodynamic association constant of selector A and solvent S and $f(a_A)$ and $f(a_S)$ = functions of the activities with an unknown stoichiometry of the equilibria involved.

In the present work, **4** is dissolved in the reasonably apolar polysiloxane SE-54 (dimethyl polysiloxane with 5% phenyl, 1% vinyl) furnishing the reactor columns I and II. Low concentrations have been used because Eq. (16) is valid only at a high dilution of A in S. Moreover, all retention increments R' observed are very large indicating a strong molecular association between **1–3** and **4**. The data obtained showed that corrections as described by Eqs. (27,28) can be neglected in the present investigation.

4. Results and discussion

The pronounced enantioselectivity between the haloethers **1–3** and the cyclodextrin derivative **4** leads to efficient enantiomer separation by gas chromatography in less then 7 min (cf. Fig. 3).

The chiral separation factor α strongly increases in the order

isoflurane (**2**) < desflurane (**3**) < enflurane (**1**)

The separation factor $\alpha > 2$ for **1** can still be increased to $\alpha = 2.7$ at 0°C. This value is one of the largest observed in enantioselective gas chromatography. Yet, as outlined before, $-\Delta_{D,L}(\Delta G)$ rather than the chiral separation factor α should be considered as measure for enantioselectivity. Moreover, according to the Gibbs–Helmholtz Eq. (19), $-\Delta_{D,L}(\Delta G)$ is temperature-dependent and reversals in the sign of this thermodynamic quantity may occur. As expected for an association process, $-\Delta_{D,L}(\Delta H)$ and $\Delta_{D,L}(\Delta S)$ compensate each other in determining $-\Delta_{D,L}(\Delta G)$ [20,21]. Therefore, an isoenantioselective temperature T_{iso} will exist at which enantiomers cannot be separated (peak coalescence) [32].

$$T_{iso} = -\Delta_{D,L}(\Delta H) / -\Delta_{D,L}(\Delta S)$$
$$\text{for} -\Delta_{D,L}(\Delta G) = 0 \quad (30)$$

Below T_{iso}, enantiomer separation is enthalpy-controlled and the D enantiomer is eluted after the L enantiomer while above T_{iso}, enantiomer separation is entropy-controlled and the L enantiomer is eluted after the D enantiomer (change of the elution order, peak inversion) [20,21].

In the present investigations, a temperature range between 0 and 60°C has been selected for **2** and **3**. Above 60°C the peak retentions are too small, both conditions being detrimental to the determination of correct retention times. For the less volatile **1**, peak broadening and peak tailing commences at temperatures below 15°C and therefore data obtained at 0°C are not included in the calculations.

Four volatile reference compounds B*, i.e., *n*-pentane, *n*-hexane, *n*-heptane and diethyl ether were selected. Relative retentions of **1–3** in respect to all four reference compounds B* were determined both on a reference column containing pure polysiloxane S (r°) and on the two reactor columns I and II containing the molal concentrations of **4** in the polysiloxane SE-54 ($r^{(I)}$ and $r^{(II)}$) between 0 and 60°C. A typical set of data is given in Table 1 (the full set of data can be obtained from the authors upon request).

The selection of the reference compounds B* was governed by the following considerations. Since **4** is shielded by a total of 16 *n*-pentyl groups at the two openings of the cavity (cf. Fig. 2), their contribution to the retention of **1–3** in the reactor column is eliminated when *n*-pentane undergoes the same van der Waals interaction and is used as reference

Table 1
Comparison of relative retentions r° measured with the reference column and r measured with reactor column II containing **4** at 30°C, for the enantiomers of **1–3** related to four reference compounds B* and calculated retention increments R'_L, R'_D and $-\Delta_{D,L}(\Delta G)$

	n-Pentane	*n*-Hexane	*n*-Heptane	Diethyl ether
1				
$r^{oa,d}$	1.04	0.37	0.14	0.85
r^a_L	4.90	1.76	0.65	4.09
r^a_D	9.54	3.44	1.26	7.98
R'^b_L	3.68	3.71	3.68	3.48
R'^b_D	8.13	8.18	8.13	8.44
$-\Delta_{D,L}(\Delta G)^c_{30}$ [kJ mol^{-1}]	1.99	1.99	1.99	1.98
2				
$r^{oa,d}$	0.84	0.30	0.11	0.68
r^a_L	2.48	0.89	0.33	2.07
r^a_D	3.17	1.14	0.42	2.65
R'^b_L	1.94	1.95	1.94	2.03
R'^b_D	2.75	2.77	2.75	2.89
$-\Delta_{D,L}(\Delta G)^c_{30}$ [kJ mol^{-1}]	0.88	0.88	0.88	0.87
3				
$r^{oa,d}$	0.16	0.06	0.02	0.13
r^a_L	0.55	0.20	0.07	0.46
r^a_D	0.89	0.32	0.12	0.74
R'^b_L	2.42	2.44	2.43	2.54
R'^b_D	4.53	4.57	4.54	4.72
$-\Delta_{D,L}(\Delta G)^c_{30}$ [kJ mol^{-1}]	1.57	1.57	1.57	1.56

[a] ±1%.
[b] ±2%.
[c] ±0.02.
[d] Obtained on the reference column.

compound B*. This holds true also for *n*-hexane and *n*-heptane since the Kováts plots *log t'* vs. *n* (*n* = number of carbon atoms) for a homologous series of *n*-alkanes were linear on the reference column and on the two reactor columns I and II (cf. Section 2).

In addition, diethyl ether is used as a reference compound to eliminate the contribution of the ether function of **1–3** to the retention increment R' with **4**. The measurements showed that the retention increments R' of **1–3** were essentially independent of the choice of the reference compounds B*. Only diethyl ether showed slightly enhanced values. However, according to Table 1, $-\Delta_{D,L}(\Delta G)$ is not affected by these small deviations. The observation of large retention increments R' indicates a strong molecular association between **1–3** and **4** which cannot be accounted for by the presence of the ether function in **1–3** or by the presence of *n*-pentyl groups in **4**. Most importantly, for $2\times3\times4\times6=144$ measurements, involving two enantiomers of three haloethers **1–3** related to four reference standards at six temperatures, all ratios of the retention increments R' for the two reactor columns I and II, containing different amounts of **4** in SE-54, were identical, i.e., $R'^{(II)}_L/R'^{(I)}_L = R'^{(II)}_D/R'^{(I)}_D = a^{(II)}_A/a^{(I)}_A = 1.64\pm0.04$. According to Eq. (16) the constant factor 1.64 directly reflects the activity ratio $a^{(II)}_A/a^{(I)}_A$ of the two reactor columns I and II, the absolute values being unknown. This important result reiterates the importance of the definition of the retention increment R' and the validity of the basic Eq. (16) [17].

Based upon the validity of Eq. (16) and the simplifications made for its derivation, the r° values at a given temperature should be accessible by extrapolation of the relative retentions $r^{(I)}$ and $r^{(II)}$ obtained on two (or more) reactor columns I and II according to Eq. (18) without resorting, in principle, to measurements on a reference column [19]. Using the measured activity ratio $a^{(II)}_A/a^{(I)}_A$ of the two reactor columns I and II, containing **4** in SE-54, the extrapolation to zero activity yields r° for the enantiomeric pairs (L and D) of **1–3**. Results obtained at 30°C are shown in Fig. 7 as a representative example. By comparison of Fig. 6 and Fig. 7, it is clear that the concept of the retention increment R' correctly describes the present system.

In Table 2, r° values at 30°C for the three haloethers **1–3** in respect to the four reference compounds B* (*n*-pentane, *n*-hexane, *n*-heptane and diethyl ether) obtained (i) by direct measurement, (ii) interpolation of the ln r° vs. $1/T$ linear regression (cf. Fig. 4) and (iii) extrapolation according to Eq. (18) from measurements on the two reactor columns I and II are compared. The reasonably good agreement in most cases (standard deviation 0.5–18%) indicates that r° values can in principle be assessed without the use of a reference column if such a column is not readily available, e.g., if the cyclodextrin derivative is chemically linked to a polysiloxane, i.e., Chirasil-Dex-type stationary phases [33]. Thus, when measurements at two reactor columns I and II, differing in the dilution of the chiral selector

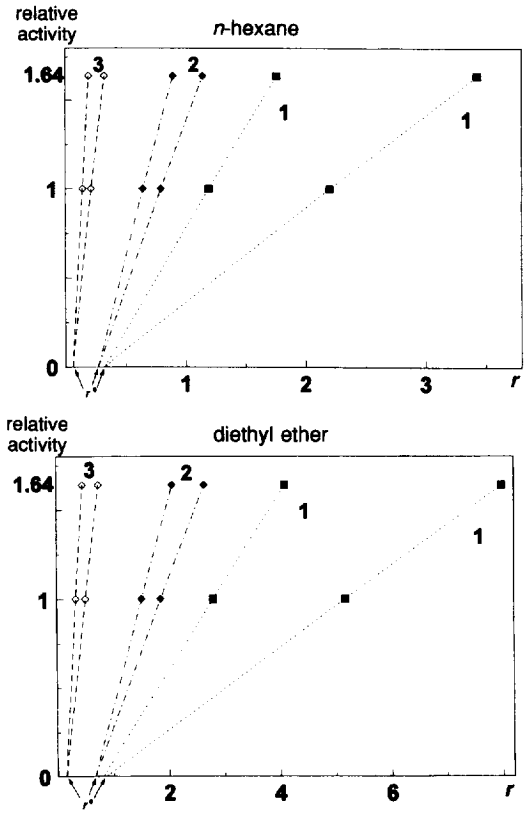


Fig. 7. Extrapolation of relative retentions r of **1–3**, measured for the reactor columns I and II containing **4** in SE-54 with the activity ratio 1:1.64, in respect to two reference compounds *n*-hexane and diethyl ether, to zero activity yielding the relative retention r° on pure SE-54. The plots obtained for *n*-pentane and *n*-heptane were superimposable with that of *n*-hexane within experimental error.

in an achiral environment are performed, Eq. (18) provides a general means to distinguish between enantioselective and nonenantioselective contributions to retention in gas chromatography. Yet, the interpolated values for r° according to Fig. 4 are the most reliable data and they have consequently been used throughout this work.

As a consequence of Eq. (19), representing a ratio, the data for $-\Delta_{D,L}(\Delta G)$ for both reactor columns I and II at six temperatures (0, 15, 30, 40, 50, and 60°C) showed a high precision even when averaged over all four reference compounds B* (standard deviation<0.7%) (cf. Section 2 and Table 3 for reactor column I and Table 4 for reactor column II).

By plotting the Planck function, $-\Delta_{D,L}(\Delta G)/T$, averaged over all four reference compounds B*, vs. the inverse temperature $1/T$, the Gibbs–Helmholtz parameters $-\Delta_{D,L}(\Delta H)$ and $\Delta_{D,L}(\Delta S)$ were obtained separately for the reactor columns I and II at six temperatures (0, 15, 30, 40, 50, and 60°C) (cf. Table 5 and Figs. 8 and 9) according to Eq. (20). All plots obtained for the *n*-alkanes were superimposable.

By plotting the Planck function, $-\Delta_{D,L}(\Delta G)_{mean}/T$, obtained for the enantiomers of **1–3** on the two reactor columns I and II containing **4** in SE-54 with the activity ratio 1.64 in respect to all four reference compounds *n*-pentane, *n*-hexane, *n*-heptane and diethyl ether vs. the inverse temperature, $1/T$, again linear van't Hoff graphs were obtained yielding the totally averaged Gibbs–Helmholtz parameters $-\Delta_{D,L}(\Delta H)_{mean}$ and $\Delta_{D,L}(\Delta S)_{mean}$ (note that the errors involved are much reduced when the data are related to one single activity a_A (cf. Table 5) or even to one single reference compound, Fig. 10).

In the temperature range studied, **1** shows the highest enantioselectivity as expressed by $-\Delta_{D,L}(\Delta G)$. The largest quantities for $-\Delta_{D,L}(\Delta H)$ and $\Delta_{D,L}(\Delta S)$ are observed for desflurane **3**. Thus, when comparing **2** and **3**, substitution of chlorine by fluorine leads to more than a doubling of the thermodynamic quantities. The pronounced enthalpy contribution is accompanied by a strong negative entropy contribution to $-\Delta_{D,L}(\Delta G)$. The large enthalpy term for **3** implies that temperatures as low as possible should be employed for the gas-chromatographic separation of this haloether. At temperatures below −15°C, $-\Delta_{D,L}(\Delta G)$ of **3** will even exceed that of **1**. The ratio between $-\Delta_{D,L}(\Delta H)$ and $\Delta_{D,L}(\Delta S)$ is not constant for **1–3** on **4**, leading to different isoenantioselective temperatures T_{iso} (cf. Eq. (30)) between 350 and 450 K (cf. Table 6). The high volatility of **1–3** precluded thus far the verification of the existence of T_{iso} by the gas-chromatographic experiment above 100°C.

As expected, data for $-\Delta_{D,L}(\Delta G)$ calculated according to Eq. (19) from the ratio of the retention increments R'_D/R'_L are independent within experimental error of the activity (concentration) of the cyclodextrin selector **4** in SE-54 in reactor columns I and II, cf. Table 4 and Figs. 8 and 9. Indeed, with the concept of the retention increment R', only the enantioselective contribution of the selector A is considered and $-\Delta_{D,L}(\Delta G)$ thus refers to the enantio-

Table 2
Comparison of directly measured (on the reference column), interpolated (on the reference column, cf. Fig. 4) and extrapolated (on reactor columns I and II, cf. Eq. (18)) retention data r° for **1–3** and four reference compounds B* at 30°C

	Found	Interpolated via linear regression	Extrapolated via activity ratio (1:1.64)	Δ (inter–vs. extrapolated) in %
1				
n-Pentane	1.022	1.044	0.886	17.8
n-Hexane	0.369	0.374	0.319	17.2
n-Heptane	0.136	0.138	0.118	16.9
Diethyl ether	0.846	0.845	0.841	0.5
2				
n-Pentane	0.828	0.843	0.752	12.1
n-Hexane	0.299	0.302	0.271	11.4
n-Heptane	0.110	0.111	0.099	12.1
Diethyl ether	0.686	0.683	0.672	1.6
3				
n-Pentane	0.189	0.161	0.176	9.1
n-Hexane	0.068	0.058	0.063	9.2
n-Heptane	0.025	0.021	0.023	9.1
Diethyl ether	0.156	0.130	0.158	8.2

Standard deviation 2–20%.

Table 3
Comparison of $-\Delta_{D,I}(\Delta G)$ values of **1–3** obtained on reactor column I containing **4** in SE-54 with four reference compounds B* measured between 0 and 60°C

T (°C)	$-\Delta_{D,I}(\Delta G)$ [kJ mol^{-1}] *n*-Pentane	$-\Delta_{D,I}(\Delta G)$ [kJ mol^{-1}] *n*-Hexane	$-\Delta_{D,I}(\Delta G)$ [kJ mol^{-1}] *n*-Heptane	$-\Delta_{D,I}(\Delta G)$ [kJ mol^{-1}] Diethyl ether
1				
15	2.27	2.27	2.28	2.26
30	2.02	2.02	2.02	2.00
40	1.88	1.88	1.88	1.85
50	1.67	1.64	1.66	1.63
60	1.49	1.47	1.47	1.44
2				
0	1.20	1.21	1.21	1.20
15	1.10	1.09	1.12	1.08
30	0.90	0.89	0.90	0.87
40	0.80	0.80	0.80	0.78
50	0.70	0.67	0.69	0.67
60	0.55	0.54	0.54	0.52
3				
0	2.44	2.45	2.45	2.44
15	2.01	2.00	2.03	1.99
30	1.54	1.54	1.54	1.51
40	1.31	1.31	1.31	1.28
50	1.10	1.07	1.09	1.07
60	0.78	0.77	0.76	0.74

Table 4
Comparison of $-\Delta_{D,I}(\Delta G)$ values of **1–3** obtained on reactor column II containing **4** in SE-54 with four reference compounds B* measured between 0 and 60°C

T (°C)	$-\Delta_{D,I}(\Delta G)$ [kJ mol^{-1}] *n*-Pentane	$-\Delta_{D,I}(\Delta G)$ [kJ mol^{-1}] *n*-Hexane	$-\Delta_{D,I}(\Delta G)$ [kJ mol^{-1}] *n*-Heptane	$-\Delta_{D,I}(\Delta G)$ [kJ mol^{-1}] Diethyl ether
1				
15	2.21	2.22	2.22	2.21
30	1.99	1.99	1.99	1.98
40	1.84	1.84	1.84	1.83
50	1.65	1.65	1.65	1.64
60	1.46	1.45	1.45	1.46
2				
0	1.18	1.18	1.18	1.17
15	1.05	1.05	1.06	1.04
30	0.88	0.88	0.88	0.87
40	0.78	0.78	0.78	0.77
50	0.68	0.68	0.68	0.68
60	0.58	0.57	0.57	0.57
3				
0	2.30	2.31	2.31	2.29
15	2.02	2.03	2.04	2.02
30	1.57	1.57	1.57	1.56
40	1.36	1.35	1.36	1.34
50	1.14	1.14	1.14	1.13
60	0.93	0.92	0.93	0.93

Table 5
Thermodynamic quantities of the enantiomer discrimination of the haloethers **1–3** on the reactor columns I and II containing the cyclodextrin selector **4** in SE-54 for four reference standards

	Column I				Column II			
	$-\Delta_{D,L}(\Delta H)$ [kJ mol^{-1}] *n*-Pentane	$-\Delta_{D,L}(\Delta H)$ [kJ mol^{-1}] *n*-Hexane	$-\Delta_{D,L}(\Delta H)$ [kJ mol^{-1}] *n*-Heptane	$-\Delta_{D,L}(\Delta H)$ [kJ mol^{-1}] Diethyl ether	$-\Delta_{D,L}(\Delta H)$ [kJ mol^{-1}] *n*-Pentane	$-\Delta_{D,L}(\Delta H)$ [kJ mol^{-1}] *n*-Hexane	$-\Delta_{D,L}(\Delta H)$ [kJ mol^{-1}] *n*-Heptane	$-\Delta_{D,L}(\Delta H)$ [kJ mol^{-1}] Diethyl ether
1[a]	7.25	7.39	7.47	7.54	6.99	7.09	7.11	7.01
2[b]	4.18	4.26	4.32	4.32	3.93	3.99	4.01	3.92
3[b]	9.91	10.00	10.07	10.06	8.71	8.80	8.82	8.71
	$\Delta_{D,L}(\Delta S)$ [J K^{-1} mol^{-1}] *n*-Pentane	$\Delta_{D,L}(\Delta S)$ [J K^{-1} mol^{-1}] *n*-Hexane	$\Delta_{D,L}(\Delta S)$ [J K^{-1} mol^{-1}] *n*-Heptane	$\Delta_{D,L}(\Delta S)$ [J K^{-1} mol^{-1}] Diethyl ether	$\Delta_{D,L}(\Delta S)$ [J K^{-1} mol^{-1}] *n*-Pentane	$\Delta_{D,L}(\Delta S)$ [J K^{-1} mol^{-1}] *n*-Hexane	$\Delta_{D,L}(\Delta S)$ [J K^{-1} mol^{-1}] *n*-Heptane	$\Delta_{D,L}(\Delta S)$ [J K^{-1} mol^{-1}] Diethyl ether
1[a]	−17.2	−17.7	−17.9	−18.3	−16.5	−16.9	−16.9	−16.6
2[b]	−10.8	−11.1	−11.3	−11.35	−10.0	−10.3	−10.3	−10.1
3[b]	−27.4	−27.7	−27.9	−27.9	−23.4	−23.7	−23.7	−23.5

[a] Based on measurements at (15, 30, 40, 50, 60°C).
[b] Based on measurements at (0, 15, 30, 40, 50, 60°C).

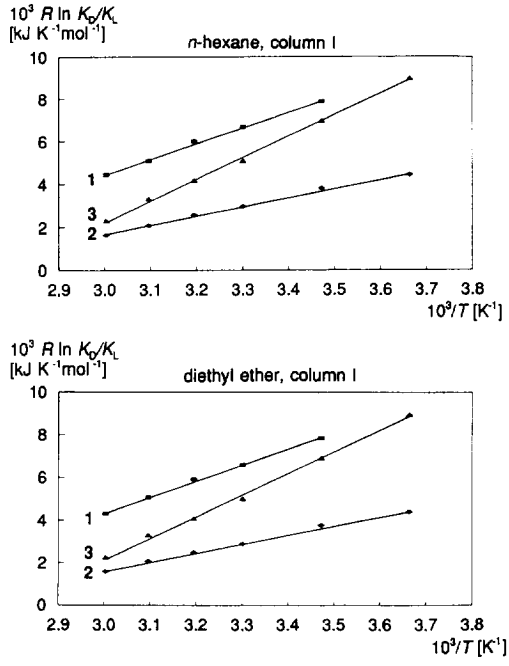


Fig. 8. Van't Hoff plots $-\Delta_{D,L}(\Delta G)/T$ (or $R \ln K_D/K_L$) vs. $1/T$ for the enantiomers of **1–3** on reactor column I containing **4** in SE-54. $-\Delta_{D,L}(\Delta G)$ in respect to the two reference compounds *n*-hexane and diethyl ether. The plots obtained for *n*-pentane and *n*-heptane were superimposable with that of *n*-hexane within experimental error.

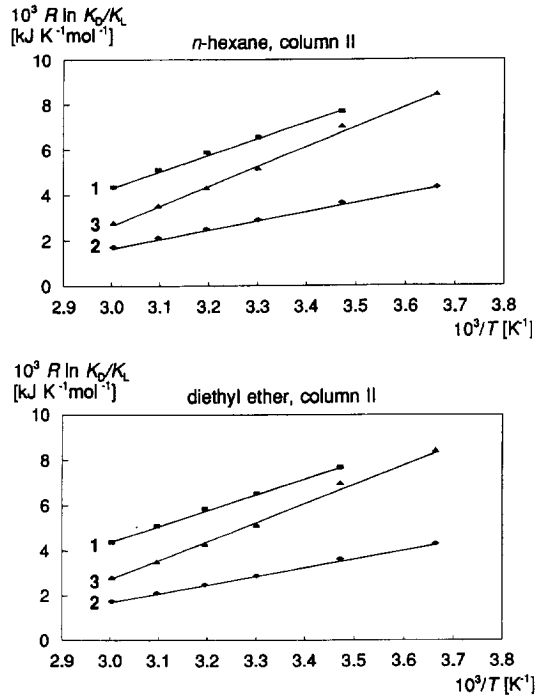


Fig. 9. Van't Hoff plots $-\Delta_{D,L}(\Delta G)/T$ (or $R \ln K_D/K_L$) vs. $1/T$ for the enantiomers of **1–3** on reactor column II containing **4** in SE-54. $-\Delta_{D,L}(\Delta G)$ in respect to the two reference compounds *n*-hexane diethyl ether. The plots obtained for *n*-pentane and *n*-heptane were superimposable with that of *n*-hexane within experimental error.

Table 6
Averaged thermodynamic quantities of the enantiomer discrimination of the haloethers **1–3** on both reactor columns I and II containing the cyclodextrin selector **4** in SE-54 for four reference standards

	1[c]	**2**[d]	**3**[d]
$-\Delta_{D,L}(\Delta H)$ [kJ mol^{-1}][a.I]	7.4±0.2	4.3±0.1	10.0±0.1
$-\Delta_{D,L}(\Delta H)$ [kJ mol^{-1}][a.II]	7.0±0.1	4.0±0.1	8.8±0.1
$-(\Delta_{D,L}(\Delta H))_{mean}$ [kJ mol^{-1}][b]	7.2±0.2	4.1±0.2	9.4±0.6
$\Delta_{D,L}(\Delta S)$ [J K^{-1}mol^{-1}][a.I]	−17.8±0.4	−11.1±0.2	−27.8±0.2
$\Delta_{D,L}(\Delta S)$ [J K^{-1}mol^{-1}][a.II]	−16.7±0.2	−10.2±0.1	−23.6±0.2
$(\Delta_{D,L}(\Delta S))_{mean}$ [J K^{-1}mol^{-1}][b]	−17.2±0.6	−10.6±0.5	−25.7±2.1
T_{iso} [K]	420±40	390±60	365±40

[a.I] Mean for 4 reference compounds, column I.
[a.II] Mean for 4 reference compounds, column II.
[b] Mean for 4 reference compounds on two columns.
[c] Based on measurements at (15, 30, 40, 50, 60°C).
[d] Based on measurements at (0, 15, 30, 40, 50, 60°C).

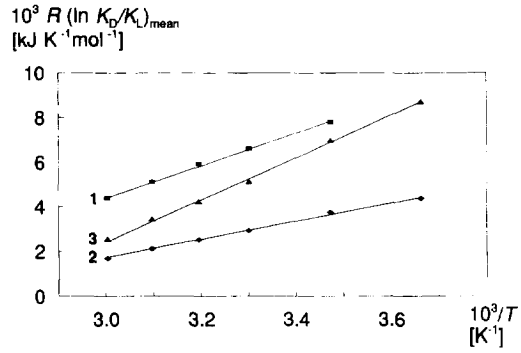


Fig. 10. Van't Hoff plots $-\Delta_{D,L}(\Delta G)_{mean}/T$ (or $R \ln K_D/K_L$) vs. $1/T$ for the enantiomers of **1–3** on the two reactor columns I and II containing **4** in SE-54 with the activity ratio $a_1:a_2 = 1:1.64$. $-\Delta_{D,L}(\Delta G)_{mean}$ is the mean value of all data in respect to all four reference compounds *n*-pentane, *n*-hexane, *n*-heptane and diethyl ether and the two reactor columns I and II.

selectivity of the cyclodextrin selector 4 and not to that of the total stationary phase (A in S). Therefore, only the ratio of the retention increments R'_D/R'_L in Eq. (17) and not the ratio of the retention factors $k'_{R(D)}/k'_{R(L)} = \alpha$ in Eq. (21) is appropriate to quantify enantioselectivity for diluted chiral stationary phases. As outlined before, Eq. (18) accounts for the nonenantioselective contribution to retention arising from the solvent S, i.e., r°, which can directly be measured on the reference column containing the pure polysiloxane SE-54, or can be calculated via Eq. (18) when measurements at two arbitrary concentration of the selector A in the solvent S are performed with two reactor columns (cf. Table 1). Eq. (24), occasionally used in enantioselective gas chromatography employing Chirasil-Val [32] or cyclodextrin derivatives diluted in polysiloxanes [37,38], presupposes the selective association of B with the total stationary phase (A in S) without distinguishing between enantioselective and nonenantioselective interactions. Since the chiral separation factor α_{dil} is dependent on the concentration of A in S [22] the thermodynamic parameters $-\Delta_{D,L}(\Delta G)$, $-\Delta_{D,L}(\Delta H)$ and $\Delta_{D,L}(\Delta S)$ are also rendered concentration-dependent. In this case, reliable data are only obtained if the contribution of the solvent to retention is low, i.e., $r \gg r^\circ$. Only then is Eq. (19) approximated by Eq. (22). Since in this work large α_{dil} values are observed for the enantiomers of **1** on **4** already in high dilution, it appeared worthwhile to compare thermodynamic data calculated from Eqs. (20,24). The results are contained in Fig. 11.

While the van't Hoff plots based on Eq. (20) are identical within experimental error on reactor columns I and II, an unusual dependence on the activity of A in S is observed when Eq. (24) is used. This concentration dependence is even evident despite a strong chemical association, i.e., $r \gg r^\circ$ which tends to approximate Eqs. (20) and (24). The true $-\Delta_{D,L}(\Delta G)$ is clearly underestimated when resorting to Eq. (24) yielding less negative quantities because the nonenantioselective contributions of the solvent and the enantioselective contributions of the selector are not distinguished. The deviation from the correct van't Hoff plot $-\Delta_{D,L}(\Delta G)/T$ (or $R \ln K_D/K_L$) vs. $1/T$ increases at high temperatures and at low cyclodextrin concentrations (reactor column I $\gg$ reactor column II). In both cases r in Eq. (20) is diminished and r° gains in importance. Since Eq.

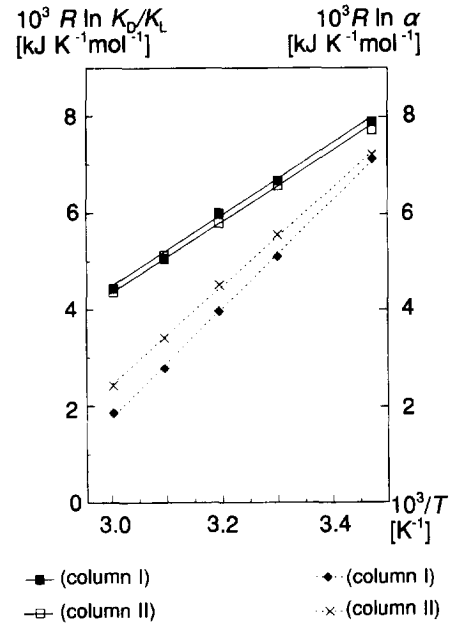


Fig. 11. Comparison of the van't Hoff plots $R \ln K_D/K_L$ vs. $1/T$ according to Eq. (20)) (top) and $R \ln \alpha = R \ln t'_{R_D} t'_{R_L}$ vs. $1/T$ according to Eq. (24)) (bottom) for the enantiomers of **1** and the reactor columns I and II containing **4** in SE-54 between 15°C and 60°C.

(24) disregards the nonenantioselective contribution to retention, r°, identical for the enantiomers, the slope of the van't Hoff plots $R \ln t'_{R(D)}/t'_{R(L)} = R \ln \alpha$ vs. $1/T$ becomes steeper and the apparent quantities $\Delta_{D,L}(\Delta H)$ and, notably, $\Delta_{D,L}(\Delta S)$ become more negative as determined from Eq. (20). Since the nonenantioselective contribution to retention increases when the concentration of the enantioselective cyclodextrin selector is decreased, $-\Delta_{D,L}(\Delta H)$ and $-\Delta_{D,L}(\Delta S)$ will further increase as the concentration of the selector A in the solvent S is reduced and finally approach infinite values for the pure solvent S. This behaviour obviously lacks any physical meaning and reinforces the invalidity of Eq. (24) in assessing thermodynamic data in diluted systems.

The thermodynamic quantity $-\Delta_{D,L}(\Delta G)$ obtained in this work for enflurane **1** and **4** has been corroborated by ^{1}H NMR measurements employing **4** as a shift reagent [13]. It was observed that the proton resonance absorptions of (*S*)-**1** are shifted more downfield than these of (*R*)-**1** in the presence of **4**. Likewise, (*S*)-**1** is eluted after (*R*)-**1** on **4** in the gas-chromatographic experiment. Using a method of determining association constants by NMR advanced by Reinhoudt et al. [39] a ratio of the association constants $K_S/K_R = 2.25$ (mean value for the α- and β-proton of **1** in d_{12}-cyclohexane) was found [40]. According to $-\Delta_{D,L} = RT \ln K_S/K_R$ a value of 2.00 kJ/mol is calculated. Thus, the NMR experiment in d_{12}-cyclohexane at 25°C is in good agreement with the gas-chromatographic experiment giving $-\Delta_{D,L}(\Delta G)_{mean} = 2.08$ kJ/mol in SE-54 at 25°C whereby the individual associations constants K differ between the gas-chromatographic ($K_{(m)}$) and the NMR experiment ($K_{(c)}$) by a ratio of approximately 5:1 due to different experimental conditions.

The thermodynamic quantities measured in this work represent data for extensive chiral recognition as expressed by large experimental chiral separation factors α (cf. Fig. 3). Unlike data featuring very weak enantioselectivities with separation factors $\alpha <$ 1.1 occasionally used for mechanistic considerations [41], the present data are thought to be reliable figures to be verified by molecular calculations. As mentioned before, the high enantioselectivity observed for small linear chiral molecules and large cyclodextrin cavities is surprising and dipole–dipole interactions which extend much more in space than van der Waals contacts may be important. Moreover, the hydrogen atoms in fluoroethers are acidic [42,43] and hydrogen-bonding might be expected. The intuitive assumption that inclusion might not be important can be discounted by the observation that intermolecular Nuclear Overhauser Effects can be observed between the radiated inner protons of the cyclodextrin **4** at C_3 and C_5 and the proton resonance absorptions for both enflurane enantiomers **1** [40]. Hydrogen-bonding interactions between **1** and **4** are responsible for the strong retention increments observed in the gas-chromatographic experiment and large chemical shifts in the NMR experiment. Proton acceptor sites are available within the cyclodextrin torus as glycosidic ether bridges in the neighbourhood of C_3 and C_5.

5. Conclusions

It is concluded that the acquisition of thermodynamic data of enantioselective gas chromatography is only useful when chiral separation factors $\alpha > 1.3$ are involved. In diluted systems, the enantioselective contribution to retention arising from the chiral selector must be distinguished from the nonenantioselective contribution arising from the achiral solvent. The concept of the retention increment (or chemical capacity factor) R' provides a precise vehicle for this requirement. The methodology, outlined in great detail, is applied to the thermodynamics of enantiomer separation of the halo ethers **1–3** by the cyclodextrin selector **4** in polysiloxane SE-54. As required, the data are independent of the activity or concentration of the selector in the solvent.

6. Note added in proof

Meanwhile, the expected concentration-independence of $-\Delta_{D,L}(\Delta G)$, $-\Delta_{D,L}(\Delta H)$ and $\Delta_{D,L}(\Delta S)$ has been corroborated by measurements with a column containing a high concentration of **4** (30%) in SE-54. This reinforces the validity of Eq. (20) even for concentrated solutions. The obtained values for **1** are

as follows: $-\Delta_{D,L}(\Delta G)^{30°C}=1.85$ [kJ mol^{-1}]; $-\Delta_{D,L}(\Delta H)=7.7$ [kJ mol^{-1}]; $\Delta_{D,L}(\Delta S)=-19.3$ [J K^{-1} mol^{-1}] (Reference compound: *n*-hexane).

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