Separation of Enantiomers by Inclusion Gas Chromatography. On the Influence of Water in the Molecular Complexation of Methyl 2-Chloropropanoate Enantiomers and the Modified γ-Cyclodextrin Lipodex-E

Abstract: A profound influence of water has previously been detected in the complexation of the enantiomers of methyl 2-chloropropanoate (MCP) and the chiral selector octakis(3-O-butanoyl-2,6-di-O-pentyl)-γ-cyclodextrin (Lipodex-E) in NMR and sensor experiments. We therefore investigated the retention behaviour of MCP enantiomers on Lipodex-E by gas chromatography (GC) under hydrous conditions. Addition of water to the N_2 carrier gas modestly reduced the retention factors k of the enantiomers, notably for the second eluted enantiomer (S)-MCP.

This resulted in an overall decrease of enantioselectivity -$\Delta_{S,R}(\Delta G)$ in the presence of water. The effect was fully reversible. Consequently, for a conditioned column in the absence of residual water, the determined thermodynamic data, $i.e.$ $\Delta_{S,R}(\Delta H)$ = -12.64±0.08 kJ mol^{-1} and $\Delta_{S,R}(\Delta S)$ = -28.18±0.23 J K^{-1} mol^{-1}, refer to a true 1:1 complexation process devoid of hydrophobic hydration.

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Keywords: Enantioselective inclusion gas chromatography; methyl 2-chloropropanoate; Lipodex-E; influence of water on molecular complexation

Introduction

Nowadays the gas chromatographic separation of enantiomers on modified cyclodextrins reached a high standard and the commercialized technique is used worldwide in many fields of chiral analysis,[1-3] including the chirality experiment in the recent cometary Rosetta mission (Figure 1).[4]

Despite the multitude of successful enantiomeric separations achieved by GC on modified cyclodextrins, little is known on mechanistic aspects of chromatographic chirality recognition process due to a multitude of possible supramolecular host-guest interactions involving $inter$ $alia$ electrostatic and van der Waals forces which preferentially take place internally via inclusion but may also occur externally.[5]
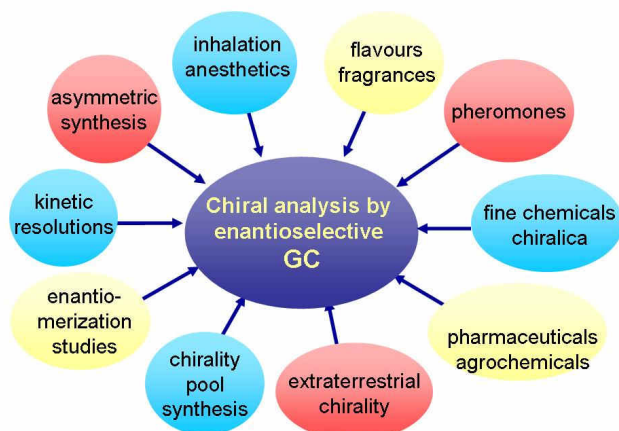
When cyclodextrin derivatives are diluted in polysiloxanes a mixed retention mechanism arises which requires the application of the retention-increment method to differentiate between achiral and chiral contributions to retention.[6] Moreover the thermodynamically controlled enantioselective recognition process is temperature-dependent due to enthalpy/entropy compensation which leads to a reversal of the elution order of the enantiomers above the isoenantioselective temperature T_{iso} = $\Delta\Delta H/\Delta\Delta S$.[6] Hydrophobic hydration is expected when cyclodextrins are associated with water molecules.[7]

In early work emerging from 1983 onwards, Kościelski et al. employed native α-cyclodextrin hydrate in formamide for the enantioseparation of terpenoic hydrocarbons (α- and β-pinene, cis- and $trans$-pinane and carene).[8-10] In a typical application the stationary phase contained α-cyclodextrin (0.79 mol%), 4% of water and lithium nitrate (0.45 g) as stabilizing agent. The water content was determined by thermogravimetric analysis.[11] Later on, glycerol was used as solvent for α-cyclodextrin.[12] Efficiency and temperature stability of the packed column

Figure 1. Pertinent application areas of enantioselective GC employing modified cyclodextrins.

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containing native cyclodextrins are generally low and water was later added directly to the carrier gas for continuous operation.[13]

General gas chromatographic studies suggest that water is eluted even from strongly retentive achiral stationary phases at high temperatures (>100°C).[14] The influence of water on the gas chromatographic properties of stationary phases is important in head-space analysis of aqueous samples. A systematic gas chromatographic study on the influence of water on the enantioselectivity displayed by a 30 m × 0.25 mm i.d. fused silica capillary coated with an 0.25 µm film of 25% heptakis(2,3-di-O-acetyl-6-O-tert-butyldimethylsilyl)-β-cyclodextrin in SPB-20 (20% phenyl/80% dimethylpolysiloxane) showed an increase of the retention factors k for the camphor enantiomers whereas the enantioseparation factor α was reduced from 1.05 to 1.02 up to a partial pressure of water of 140 mm Hg in the N_2 carrier gas at 90°C.[15] Achiral hydroxy compounds showed a decrease of retention and an increase of column performance and peak symmetry with a carrier gas humidity of up to 25 vol % water.[15]

Octakis(3-O-butanoyl-2,6-di-O-pentyl)-γ-cyclodextrin (Lipodex-E) represents one of the most versatile chiral selectors for the gas chromatographic enantioseparation of a multitude of chiral compounds regardless of their functionality.[16,17] At the outset, Lipodex-E was coated on fused silica capillary columns (typically 25 m × 0.25 mm i.d. × 0.25 µm layer thickness) in the undiluted form.[2,16,17] Later on Lipodex-E was applied by dilution (40%) in the silicone OV-1701 (5% cyanopropyl-7% phenyl-dimethylpolysiloxane)[18] and by chemical bonding to dimethylpolysiloxane (Chirasil-γ-Dex) (Figure 2).[19,20]
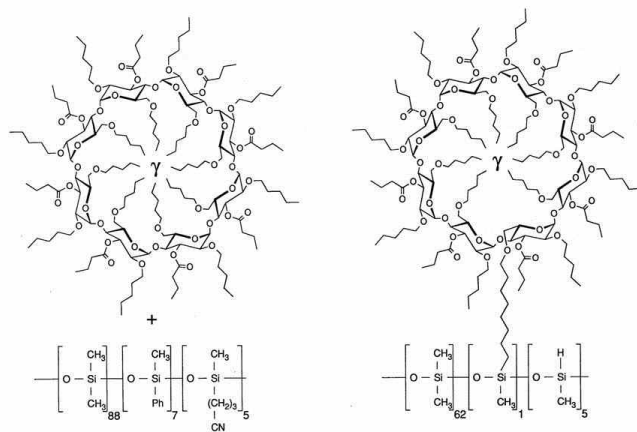

Figure 2. *Left:* Octakis(3-O-butanoyl-2,6-di-O-pentyl)-γ-cyclodextrin (Lipodex-E)[16] diluted in OV-1701.[18,19] *Right:* Octakis(3-O-butanoyl-2,6-di-O-pentyl)-γ-cyclodextrin (Lipodex-E)[16] chemically linked via an octamethylene spacer to polydimethylsiloxane to yield Chirasil-γ-Dex.[19,20]

Enantioseparations by GC employing modified cyclodextrins are usually characterized by low enantioselectivities.[1-3] Yet, due to the high resolving power of capillary GC, very low enantioseparation factors of 1.01 < α < 1.10 are sufficient for quantitative analytical resolutions in a short time. High values of α > 1.5 are only rarely encountered for selectand enantiomers in enantioselective GC. Thus methyl 2-chloropropanoate (MCP) was enantioseparated on Lipodex-E with an enantioseparation factor of α = 2.27 at 70°C.[21] The largest enantioseparation factor of α = 10.6 at 26°C ever

observed in enantioselective GC has been found for 'compound B' (2-(fluoromethoxy)-3-methoxy-1,1,1,3,3-pentafluoropropane), a chiral degradation product of the inhalational anesthetic sevoflurane (1,1,1,3,3,3-hexafluoro-2-(fluoromethoxy)propane), on Lipodex-E diluted in the polysiloxane PS 255.[22] A real conundrum is provided by the observation that the enantioseparation factor drops to α = 2.1 on heptakis(3-O-butanoyl-2,6-di-O-pentyl)-β-cyclodextrin and to α = 1.0 (no enantioselectivity at all!) on hexakis(3-O-butanoyl-2,6-di-O-pentyl)-α-cyclodextrin.[22] A clue to the unexpectedly high enantioselectivity of the γ-congener Lipodex-E may be rationalized by self-inclusion of one 6-O-pentyl group into the cyclodextrin cavity as suggested by NMR measurements and molecular dynamics (MD) calculations.[23] The complexation between single enantiomers of 'compound B' and Lipodex-E and the congener heptakis(3-O-butanoyl-2,6-di-O-pentyl)-β-cyclodextrin was also studied by ^{19}F-NMR spectroscopy in apolar d_{12}-cyclohexane.[24] Heteronuclear NOE measurements proved that inclusion complex formation is taking place with 'compound B' located inside the cavity of the cyclodextrin selectors. However, the study could not confirm the striking difference in enantioselectivity between the β-CD and its γ-CD congener observed by enantioselective GC.[22]

Lipodex-E diluted 1:1 in a poly(dimethylsiloxane) matrix was also used as enantioselective coating in a chiral sensor system based on a capacitive microtransducer.[25,26,27] Interestingly, when the effect of humidity on the chiral recognition process was examined in the sensor device, a significant signal increase upon exposure to water was noted for (S)-MCP but not for (R)-MCP.[28] These findings appear coherent with a recent NMR and molecular dynamics (MD) investigation, where a stronger binding between the chiral host and (S)-MCP alone was observed at long equilibration times (three weeks).[29] Detailed DOSY measurements in the latter study demonstrated that the origin of the phenomenon had a strong thermodynamic contribution.[29]

Because the separation of enantiomers on a chiral chromatographic stationary phase is also thermodynamically driven, it could be envisaged that an analogous positive effect of water can be expected in the GC separation of (R)-MCP and (S)-MCP on the Lipodex-E stationary phase. However, the strong time-dependence of the phenomenon, as evidenced in the NMR experiments, made a comparison doubtful for a fast dynamic process like gas chromatography. Similarly, the importance of orientation effects and dielectric properties of the included molecules in the capacitive chemical microsensors hampered the straightforward prediction of the influence of humidity on the GC performance of the Lipodex-E stationary phase.

In view of the widespread use of enantioselective GC employing modified cyclodextrins and due to the scarcity of studies on the topic noted above, we investigated in detail the retention behaviour of MCP enantiomers on the Lipodex-E stationary phase under 'dry' and 'wet' conditions.

Materials and Methods

Chemicals

(R)-MCP and (S)-MCP, n-nonane (C9), n-decane (C10), and Sicapent® with moisture indicator were purchased from Sigma-Aldrich (Milano, Italy). Copper(II) sulfate pentahydrate was obtained from Carlo Erba (Milano, Italy). Racemic MCP samples were obtained by mixing equal amounts of the pure

enantiomers. The analytical samples were prepared by introducing racemic MCP (0.200 g) and C9 (0.100 g) in a screw-cap vial and flushing the vessel with methane (C1) before sealing. Optionally, C10 (0.100 g) was added for better characterization of the retention of MCP enantiomers in case of overlapping peaks with C9.

Columns

Lipodex-E was synthesized according to König et al.[16] The yellow product was purified by column chromatography over silica gel with ethyl acetate/light petroleum (b.p. 60-90°C) from 1:10 to 1:3 (v/v). Yield: 15% related to the native γ-cyclodextrin employed. $[\alpha]^{20}_D = +69 \pm 5$ (c = 0.9, chloroform). IR and NMR data are reported in [Ref. 30].

Column A. 20 m × 0.25 mm i.d fused silica column coated with 10% Lipodex-E in SE-54 (0.25 µm film thickness).

Column B. 25 m × 0.25 mm i.d fused silica column coated with 5% Lipodex-E in SE-30 (0.25 µm film thickness).

Column C. 20 m × 0.25 mm i.d fused silica column coated with 100% Lipodex-E (0.25 µm film thickness).

Before use, the columns were conditioned overnight at 120°C.

Instruments

Measurements were carried out with a Perkin-Elmer Autosystem XL gas chromatograph equipped with split/splitless injector and flame ionization detector (FID). TotalChrom 6.3.1 software was used for data acquisition and analysis. In order to modulate the moisture content in the carrier, before reaching the GC inlet port the N_2 stream from the supply line was passed through a sturdy plastic tube (*conditioning trap*, 250 mm × 35 mm i.d.) containing either MS-13X or Sicapent® with moisture indicator (40 g, colorless), or $CuSO_4 \cdot 5H_2O$ (120 g). Care was paid to replace the Sicapent® drying agent every time the blue band had reached 2/3 of the trap length. On the contrary, monitoring of the weight loss of the tube filled with $CuSO_4 \cdot 5H_2O$ (≤0.1 g) confirmed the possibility of multiple use of the humidifying trap without the need of replacing the copper(II) salt.

Measurements

If not noted otherwise for thermodynamic measurements between 40 – 80°C, GC analyses were performed isothermally at 40°C by injecting the headspace vapours from the analytical sample in the splitless mode. Injection port and detector were set at 250°C. The experiments were carried out in series of 5-7 entries by switching the two conditioning traps in the order: Sicapent® / $CuSO_4 \cdot 5H_2O$ / Sicapent®. After replacing a trap with the alternative one, the carrier flowrate was adjusted and the system was allowed to equilibrate for 10 minutes before starting the next series of measurements.

$$t'_x = t_x - t_M \quad (1)$$
$$k_x = t'_x / t_M \quad (2)$$
$$r_y = t'_y / t'_{C9} \quad (3)$$
$$R'_y = (r_y / r°) - 1 \quad (4)$$
$$\ln \frac{R'_S}{R'_R} = -\frac{\Delta_{S,R}(\Delta H)}{R \cdot T} + \frac{\Delta_{S,R}(\Delta S)}{R} \quad (5)$$
$$\alpha_{S,R} = \frac{k_{(S)-MCP}}{k_{(R)-MCP}} = \frac{t'_{(S)-MCP}}{t'_{(R)-MCP}} \quad (6)$$

Figure 3. Formulae used in the elaboration of retention data. For the meaning of the various symbols, see the text.

Data elaboration

Figure 3 summarizes the formulae employed for data analyis: t_M is the elution time of the unretained reference compound C1, while t_x and t'_x are the total

and adjusted retention time, respectively, of any other component x in the mixture under examination (either MCP enantiomers or the hydrocarbon internal standard). The subscript y refers to either (R)-MCP or (S)-MCP, with r_y and r° being the adjusted retention time relative to the C9 internal standard over the chiral (Lipodex-E in silicone) and achiral (silicone alone) stationary phases, respectively. R'_y is the *retention increment* of the enantiomers y on the Lipodex-E stationary phase.[6,19,22]

Results and Discussion

Most of the experiments in this study were carried out with Column A, containing 10% Lipodex-E in SE-54 (5%-phenyl-dimethyl-polysiloxane). Measurements were performed isothermally between 40°C and 80°C by injecting the headspace vapours of a mixture containing racemic MCP, C9, C10, and the C1 void volume marker t_M. Analyses were carried out in duplicates, with N_2 as the carrier gas.

In order to separate the enantioselectivity of the chiral selector from achiral contributions of the silicone matrix, the retention increment approach was used.[6,19,22, 31] For this purpose a reference column containing only the silicone phase was employed. Furthermore, the retention factors k were related to those of the inert hydrocarbon reference standards. The relative retentions r are thus independent of all column parameters except the temperature.[6,19,22, 31]

The Retention of MCP Enantiomers on Lipodex-E Under 'Dry' and 'Wet' Conditions

In order to examine the influence of humidity on the GC retention of the MCP enantiomers on the Lipodex-E phase, the measurements were carried out by feeding the GC instrument with N_2 carrier passed through a standard inline moisture scrubber containing either MS-13X or Sicapent® (supported phosphorus(V) oxide) or, alternatively, through a cylinder filled with copper(II) sulphate pentahydrate (p_{H2O} = 7.8 mm Hg at 25°C or 14% relative humidity –rH- at 40°C).[33]

Typical results recorded in these experiments are shown in Figure 4, while Table 1 summarizes average values for a larger set of measurements under headspace analysis conditions and the parameters obtained by the retention increment analysis.

Visual inspection of Figure 4 reveals no obvious correlation between the retention of the hydrocarbon standards, C9 and C10, at the different working conditions. Only a slight *increase* of average t' values on going from 'dry' to 'wet' operation might be suspected for both analytes, but Student's t test suggested the rejection of this hypothesis (H_1) at the 0.0001 confidence level. A similar conclusion was reached for the C1 t_M marker, thus confirming that the findings for the chiral analytes, as discussed below, were not an artefact of the changing instrumental set-up.

By contrast, the experiments revealed that switching between dry and humid carrier caused a statistically significant (Student' t test) change in the adjusted retention time of the MCP enantiomers. In particular, both chiral analytes showed a modest decrease (1.4-2.9%) of their t' values under 'wet' conditions, with the largest reduction being observed for the more strongly retained (S)-MCP enantiomer. The consequence of this latter fact was the decrease of the enantioselectivity $-\Delta_{S,R}(\Delta G)$ in the presence of the humid carrier gas.

Because the observed $\alpha_{S,R}$ values derived from the superposition of the retention effect due to the chiral selector and the achiral silicone phase, an analysis of the available data in terms of retention increments was also performed.
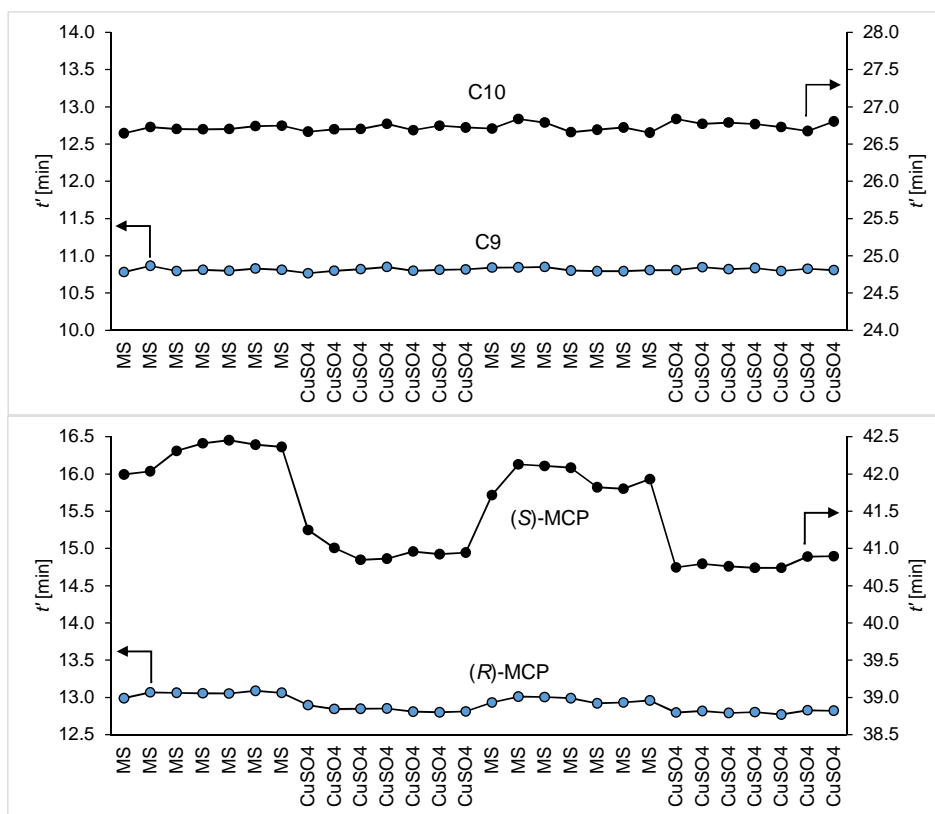
Figure 4. Adjusted retention time *t'* of C9 and C10 hydrocarbon standards (*top*) and MCP enantiomers (*bottom*), as a function of the content of the conditioning trap placed inline to carrier supply. *Conditions*: Column A, 40°C, 25.0 psig N_2. MS: 13X molecular sieve moisture scrubber placed in the carrier gas line. CuSO4: conditioning trap filled with $CuSO_4 \cdot 5H_2O$.

Strictly speaking, a rigorous elaboration of this kind would require 'wet' *r°* values as compared to the available 'dry' ones. However, the observed low sensitivity of *t'* of C9, C10, and (*R*)-MCP (the Lipodex-E weakly interacting enantiomer) to carrier humidity suggests that *r°* should be very close in both cases. Under these assumptions, the *R'$_S$/R'$_R$* ratio showed a trend that was qualitatively identical to the $\alpha_{S,R}$ factor (Table 1). Moreover, this approach allowed the estimation of the reduction of -$\Delta_{S,R}(\Delta G)$ in the order of 30 J mol^{-1} in the humid stream, as calculated from the net interaction of the MCP enantiomers and the pure Lipodex-E selector. As the (*S*)-MCP shows a very strong retention increment *R'$_S$* = 9.752 at 40°C, it can be concluded that water impairs its complexation with the Lipodex-E selector.

Surprisingly, the findings discussed above were in sharp contrast with the behaviour of MCP enantiomers in the sensor and NMR studies mentioned in the Introduction. Also no increase of the retention as reported for camphor enantiomers on heptakis(2,3-di-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin described previously was observed.[15] Therefore, in order to reinforce the GC results, additional measurements were carried out by changing the experimental set-up in various ways. These included: (i) the use of Column B and Column C, that contained the Lipodex-E selector in SE-30 (dimethylpolysiloxane) and in the undiluted (neat) form, respectively; (ii) the direct co-injection of water with the *rac*-MCP sample, under otherwise 'dry' conditions; (iii) the introduction of liquid water in the conditioning trap, as a means to increase the

humidity in the carrier stream and (iv) the extension to a new set of measurements at 50°C (*cf.* electronic supporting information).

Irrespective of all these variations, the outcome of the whole ensemble of new experiments was again the same. The hydrocarbon reference analytes (C9 and C10) proved essentially insensitive to the presence of humidity in the carrier gas, while the MCP enantiomers experienced a reduction of their retention, the latter being significantly larger for (*S*)-MCP than for (*R*)-MCP.

Therefore, all of the GC experiments performed in this study lend support to the conclusion drawn above that (i) the complexation of both MCP enantiomers by the Lipodex-E phase is impaired in the presence of humidity, (ii) this effect is more pronounced for the strongly complexing (*S*)-MCP enantiomer and (iii) almost invariably, the net result is a lower degree of enantioselectivity in the presence of water.

Although the evidence gathered so far do not allow a detailed understanding at the molecular level, it is worth stressing again that the effect of humidity appears to be specific for the MCP enantiomers and not for C9 and C10. The former are known to be included within the cyclodextrin cavity as shown for Lipodex-E[29] and the related selector Lipodex-D (heptakis(3-*O*-acetyl-2,6-di-*O*-pentyl)-β-cyclodextrin),[34] while the apolar hydrocarbon probes obviously do not complex with Lipodex-E as the retention increment approach furnishes identical thermodynamic data for halocarbon enantiomers independent of the choice of the internal standard.[19,22,35] Also the silicone matrix does not play a role as the retention increment approach furnishes identical thermodynamic data also independent of the selector concentration in the achiral matrix.[35]

TABLE 1 Effect of carrier humidity on the adjusted retention times (t'), retention increments (R'), and Gibbs' free-energy differences ($\Delta_{S,R}(\Delta G)$) in the GC separation of C9, (R)-MCP, and (S)-MCP on Column A.[a]

Carrier	t'_{C9} (min)	t'_R (min)	t'_S (min)	$\alpha_{S,R}$	R'_R	R'_S	R'_S / R'_R	$\Delta_{S,R}(\Delta G)$ (KJ mol^{-1})
Dry[b]	10.569	13.265	44.320	3.341	2.218	9.752	4.397	-3.856
	±0.019	±0.018	±0.082	±0.006	±0.005	±0.027	±0.009	±0.006
Wet[c]	10.576	13.154	43.359	3.296	2.189	9.512	4.345	-3.825
	±0.011	±0.014	±0.072	±0.008	±0.002	±0.022	±0.013	±0.008

[a] *Conditions*: 40°C isothermal, 25.0 psig N_2, headspace injection of a mixture of *rac*-MCP and C9; t' values calculated using t_M of C1 standard.
[b] MS-13X or Sicapent® moisture scrubber placed in the carrier gas line. [c] Conditioning trap filled with $CuSO_4 \cdot 5H_2O$ placed in the carrier gas line.

Some of our findings are reminiscent of those of Berezkin *et al.*,[15] who observed the progressive reduction of the enantioseparation factor $\alpha_{S,R}$ for camphor enantiomers on heptakis(2,3-di-*O*-acetyl-6-*O*-tert-butyldimethylsilyl)-β-cyclodextrin when the humidity of the carrier gas was increased up to p_{H2O} = 140 mmHg.

However, the use of 'wet' conditions in that study actually resulted in the *increase* of the net retention of the chiral analytes at 90°C, a phenomenon explained in terms of hydrogen bonding between camphor enantiomers and water molecules within the β-cyclodextrin host.[15] Clearly, this is exactly the opposite of what obtained for MCP enantiomers on Lipodex-E, in spite of the larger cavity of the selector and the lower temperature employed in the present study.

Given the minute change in the $-\Delta_{S,R}(\Delta G)$ values recorded with dry and humidified carrier, respectively, it may be questionable whether any observable effect has to be expected in single theoretical-plate devices like Lipodex-E capacitive sensors.[25-27] Considering also that these devices operate in conditions not far from those of the GC measurements above,[32] the observed signal enhancement, caused by humidity for the (S)-MCP enantiomer,[28] appears therefore particularly intriguing. On the basis of the GC evidence presented herein, it is unlikely that such behaviour would results from simple thermodynamic factors. Accordingly, the involvement of specific orientation-dependant dielectric effects, within the selector-selectand-water assembly in the sensor's active element, could be considered as an alternative working hypothesis for explaining the phenomenon.

Reversibility of the Effect of Water and Practical Aspects

Protection of GC systems from humidity and other contaminants in the carrier stream is a well-established practice in order to prevent hardware failure and erratic results due to stationary phase degradation (Chapter 18.3.7 in Ref. 14). In this regard, cyclodextrin selectors can be particularly prone to irreversible alteration, especially when hydrolytically sensitive fragments -notably in *N*-trifluoroacetylated cyclodextrins- are present in the structure of the chiral host.[36]

Because Lipodex-E is an ester derivative (Figure 2), it was expected that repeated exposure to the humid carrier gas in elevated temperature could eventually result in the alteration of column performance. In spite of this, no hint for an irreversible behaviour was gained in the course of this investigation over almost four months of experimentation. Even if the occurrence of 'memory effects' for long-term usage with humid carrier at higher temperatures cannot be excluded, the present results point therefore to a noticeable stability of the chiral selector.

This conclusion was confirmed in quantitative terms by retention increment analysis of Column A, [6,19,22] carried out just in between the 'dry'-'wet' cycles described above. Previously established $r°$ values were employed for this purpose,[31] with Van't Hoff interpolation of the missing 45°C, 55°C, and 75°C data.

After conditioning the column overnight at 120°C, variable T measurements with dry N_2 carrier (Figure 5) showed the expected Van't Hoff dependence of ln(k) on the temperature reciprocal $1/T$ for the four components (Figure 6, *left*).[37] Separation of the chiral selector effect from the achiral silicone contribution by the anticipated retention increment approach (Figure 6, *right*) allowed to extract the following estimates for the differences in the transfer enthalpy and entropy values for the two MCP enantiomers between the pure chiral selector Lipodex-E and the gas phase: $\Delta_{S,R}(\Delta H)$ = -12.64±0.08 kJ mol^{-1} and $\Delta_{S,R}(\Delta S)$ = -28.18±0.23 J K^{-1} mol^{-1}. Comparison of these figures with those previously obtained for the same phase (10% Lipodex-E in SE-54, $\Delta_{S,R}(\Delta H)$ = -12.6 kJ mol^{-1}, $\Delta_{S,R}(\Delta S)$ = -27.75 J K^{-1} mol^{-1})[31] confirmed that no appreciable change in the thermodynamic properties of the selector had occurred upon exposure to humidity.

Indeed, when one considers that the latter were obtained in a different laboratory, with different columns of different history, the result highlight both the validity of the retention increment approach, for extracting relevant thermodynamic data, and the excellent stability of the chiral phase. In this respect, it is also worth noting that the columns appeared to recover 'dry' properties quite promptly on removal of the humidifying factors (Figure 7). For instance, injection of the mixture of the analytes as soon as 2 min after the replacement of the $CuSO_4 \cdot 5H_2O$ cartridge with the Sicapent® one afforded t' values for each component that were not appreciably different from those obtained after 'dry' conditioning the column for several hours. Similarly, in a series of four experiments in a row with anhydrous carrier gas, the co-injection of water in one run did not cause blurring of the observed 'wet' behaviour, *i.e.*, reduction of t' of MCP enantiomers, to the immediately successive 'dry' run.

Because a similar prompt response of the Lipodex-E column was noticed upon switching from 'dry' to 'wet' conditions, it is evident that, from a kinetic point of view, the conduct of the GC system was profoundly different from that observed in the NMR study recalled in the Introduction.[29] As anticipated, this was not surprising, however, due to the unlikelihood that a phenomenon that requires several days to develop in the NMR tube might have an appreciable counterpart in the short time frame of GC runs. On the other hand, the differences in the experimental conditions (most notably solvent and chiral selector concentration), as well as the very limited perturbation of thermodynamic equilibrium by water probed in the present GC

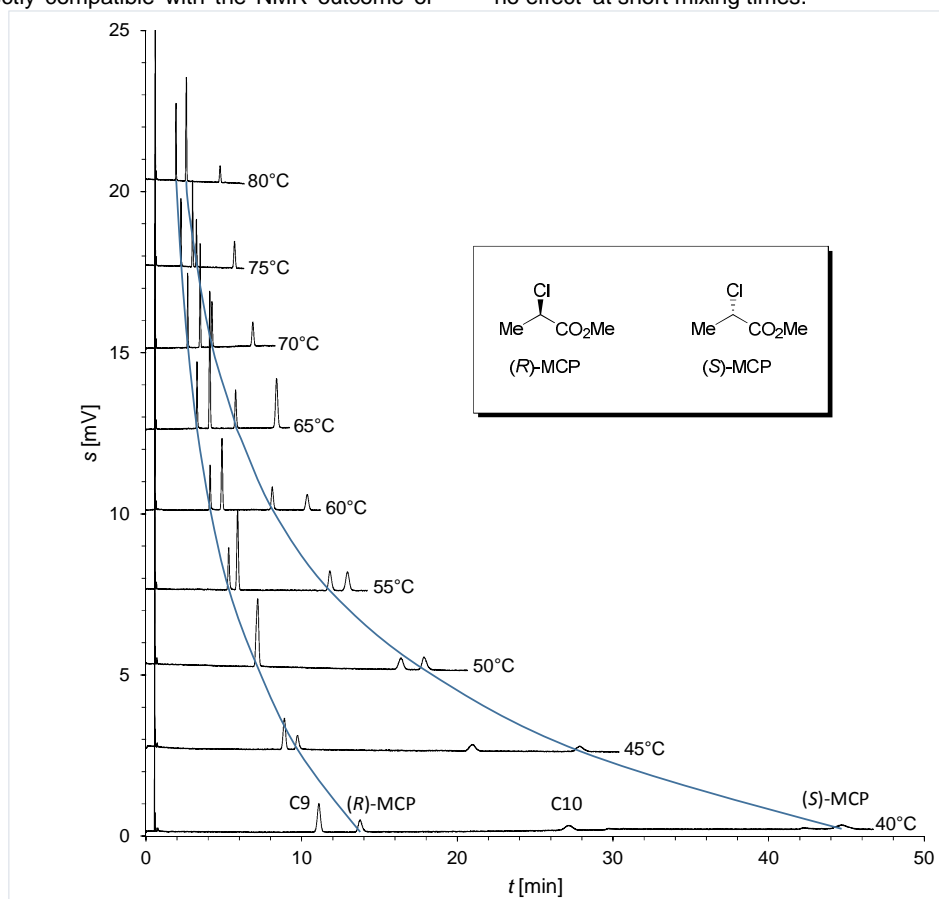study, could be perfectly compatible with the NMR outcome of 'no effect' at short mixing times.

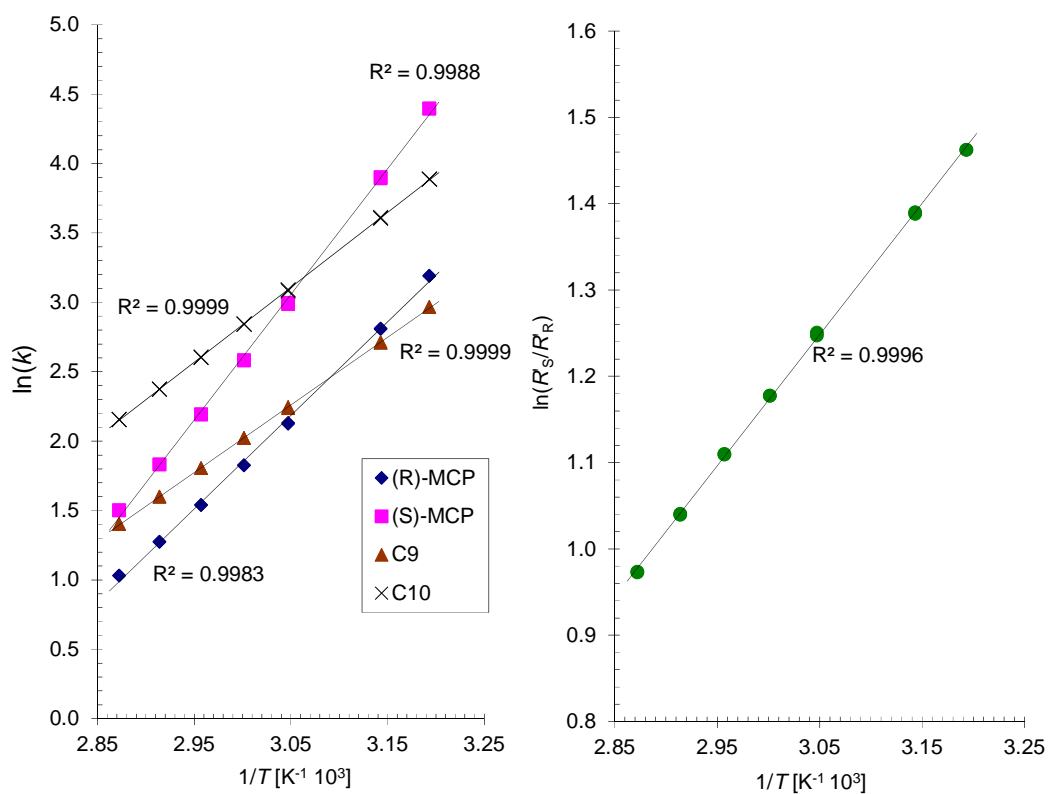Figure 5. Separation of (*R*)-MCP, (*S*)-MCP, C9, and C10 on Column A at various temperatures.

Figure 6. Van't Hoff relationship for the retention factors k of (*R*)-MCP and (*S*)-MCP, C9, and C10 on Column A (*left*) and for the R'_S/R'_R ratio of MCP enantiomers (*right*). *Conditions*: 1 µL headspace volume injected, split ratio = 1:30, 25.0 psig N$_2$.
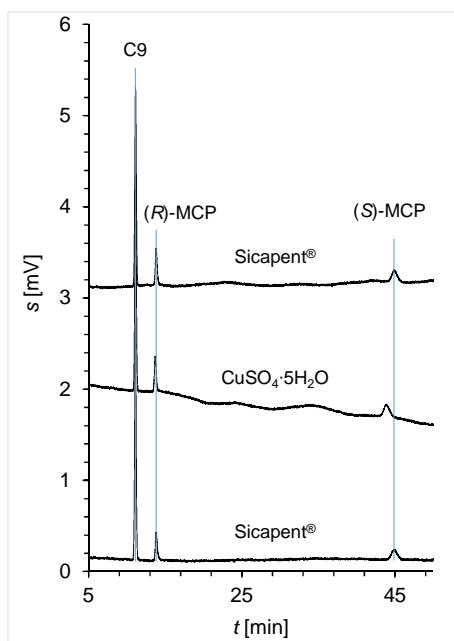
Figure 4. Recovery of 'dry' column properties upon replacing the CuSO₄·5H₂O filled cartridge with the Sicapent® one.

measured Gibbs-Helmholz parameters $\Delta_{S,R}(\Delta H)$ = -12.64±0.08 kJ mol^{-1} and $\Delta_{S,R}(\Delta S)$ = -28.18±0.23 J K^{-1} mol^{-1} as well as a host of previous thermodynamic measurements of enantioselectivity of chiral halocarbons and Lipodex-E safely refer to a 1:1 complexation process devoid of hydrophobic hydration effects.[20,31,33] Also molecular modelling studies and thermodynamic mechanistic approaches advanced in the vast literature on modified cyclodextrin stationary GC phases are not likely to be perturbed by secondary equilibria involving solvents. This does not hold true for the classical enantioselective system of native α-cyclodextrin in formamide where water is required for efficient enantiomeric separation of terpenoids.[8-13]

Acknowledgements

Supporting information

Additional supporting information may be found in the online version of this article at the publisher's website.

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Finally, it is interesting to note that the retention time of (*S*)-MCP was found to undergo changes as large as 1 min on cycling between 'dry' and 'wet' conditions. From the practical point of view, the magnitude of this effect may be important, especially in connection with automatic peak recognition features of modern GC software. At the same time it must be pointed out, however, that no statistically significant shift of peak position was recorded when the separation of MCP enantiomers was carried out by replacing MS-13X with Sicapent® or, eventually, by completely removing the water scrubbing cartridge. Under 'normal' working conditions, which include the use of a N₂ generator and centralized distribution line as in the present investigation, it seems then unlikely that small variations of the humidity content in the carrier gas might have a noticeable effect on the performance of Lipodex-E columns. While reiterating the recommendation of proper set-up of GC instrumentation, this conclusion reinforces again the substantial robustness of the gas chromatographic enantiomer separation methods based on the use of the Lipodex-E chiral selector.

Conclusion

The present study establishes that water affects the molecular complexation of MCP enantiomers and octakis(3-*O*-butanoyl-2,6-di-*O*-pentyl)-γ-cyclodextrin (Lipodex-E) diluted in a silicone matrix in the gas chromatographic experiment. Under hydrous conditions, the enantioselectivity is reduced mainly due to the impaired complexation of the stronger interacting enantiomer. The 'water effect' is fully reversible in a short time. The phenomenon is at odds with NMR and sensor results employing MCP enantiomers and Lipodex-E under essentially non-comparable conditions. Due to the reversibility of the 'water effect', it is important to realize that fully conditioned gas chromatographic columns containing Lipodex-E are clearly devoid of water arising from previous synthesis, storage and purification of the modified cyclodextrin selector. Thus the

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37. It is worth noting that the hydrocarbon standards C9 and C10 followed a better linear relationship than the MCP enantiomers, an observation that is valid for all of the dataset obtained in this

work. Partially, this could be due to the less symmetrical shape of the peaks of the latter, especially at the lower temperatures, which tends to provide somewhat 'noisy' retention time values. Nonetheless, a good fit ($R^2 > 0.998$) was obtained also for the chiral analytes, thus allowing the subsequent thermodynamic analysis to be completed.

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