Enantioseparation of Chiral Organochlorines on Permethylated β- and γ-Cyclodextrin, as well as 1:1 Mixtures of Them

Walter Vetter[1,*], Katja Lehnert[1], and **Georg Hottinger[2]**

[1]Universität Hohenheim, Institut für Lebensmittelchemie, D-70593 Stuttgart, Germany and [2]BGB Analytik KG, Lettenstrasse 97, CH-8134 Adliswil, Switzerland

Abstract

Gas chromatography columns coated with 10% permethylated β- and γ-cyclodextrin in 85% dimethyl–15% diphenyl polysiloxane (β- and γ-PMCD, respectively) and 1:1 mixures are prepared and tested with regard to the enantioseparation of chiral chloropesticides. On the columns with the individual *O*-methylated cyclodextrins (*O*-tCDs), the enantiomers of aaeeee-hexachlorocyclohexane (α-HCH), e-aeee-1,3,4,5,6-pentachlorocyclohex-1-ene (β-PCCH), and e-aaee-1,3,4,5,6-pentachlorocyclohex-1-ene (γ-PCCH), *cis*- and *trans*-chlordane, and *cis*-heptachlor epoxide are separated on both columns, with the exception of the latter being separated only on β-PMCD. On the column coated with 5% β- and 5% γ-PMCD, the resulting separation factor (α) is virtually 1/2 of the arithmetic mean of the elution-dependent separation factors on the individual *O*-tCDs. In case of reversed elution order on β- and γ-PMCD, the enantiomers are not resolved on the mixed columns as is the case with *cis*-chlordane. Likewise, the lower resolution of the γ-PCCH enantiomers on the mixed columns prove the reversed elution order on β- and γ-PMCD without having enantioenriched standards available. On the column coated with 5% β- and 5% γ-PMCD, similar retention times to those observed on both 10% β-PMCD and 10% γ-PMCD are obtained. On the column coated with 10% β- and 10% γ-PMCD, significantly longer retention times are obtained compared with the columns that contain a total of 10% chiral stationary phase (CSP). This indicates that a relevant part of the interaction of the analytes with the chiral selector is non-enantioselective and, thus, only delays the elution of both enantiomers. Moreover, these non-enantioselective interactions prevent a direct comparison of CSPs with different amounts of the chiral selector. However, this is possible by using mixed phases of two CSPs with similar properties. Using this system, it is demonstrated that for the organochlorine compounds studied, no higher separation factor is observed on the mixed CSPs than on the individual *O*-tCD with the higher separation factor. Estimations allow a prediction that enantioseparations of organohalogen compounds can be achieved on columns coated with as little as 1% of the CSP.

* Author to whom correspondence should be addressed: email w-vetter@uni-hohenheim.de.

Introduction

Many pollutants are chiral, but they have been used for decades as racemates. However, when they come in contact with bacteria, enzymes, or other chiral environments, biotransformation in environmental and food samples often results in a strong depletion of one enantiomer (1–3). The use of enantioselective chromatography to determine the enantiomer ratio of individual chiral compounds has become a major development in pesticide research (4,5). Several parameters have been established over recent years that would not have been established with standard (achiral) gas chromatographic (GC) methods (1). The most successful type of chiral stationary phases (CSPs) for the direct GC enantioseparation are O-terminated cyclodextrins (O-tCDs). Introduced in 1987 by Juvancz et al. (6), O-tCDs soon became the standard method for the separation of chiral flavor compounds, drugs, and natural products suitable for chromatography in the vapor state (7–11). In 1989, König et al. (12) widened the range of compounds with the first enantioseparation of the organochlorine pollutant aaeeee-hexachlorocyclohexane (α-HCH). Since then, almost all chiral organohalogens have been successfully separated into enantiomers on O-tCDs (2,3,13). However, selected chiral pollutants have been exclusively separated on O-tCDs that were not fully modified. For instance, racemic compounds of technical toxaphene (CTTs) were to date only enantioseparated on (i) partially tert-butyldimethylsilylated β-cyclodextrin (β-BSCD) (14,15), (ii) randomly modified 2,3-di-O-methyl-6-O-tert-butyldimethylsilyl-β-cyclodextrin (16), (iii) partly ethylated α-cyclodextrin (17), and (iv) polysiloxane-anchored permethyl-β-cyclodextrin (18), a CSP that could not be fully derivatized (19). By contrast, the fully modified and well-defined analogues of items (ii) and (iv) did not separate the enantiomers of any toxaphene congener (20,21).

Two reasons have been suggested for explaining these differences. First, the partly modified cyclodextrins contain several products, of which one (or some) was responsible for the observed enantioseparations. However, β-BSCD (one of the

described O-tCDs) is a mixture of over 20 products (11,22). Assuming equal distribution of the products in a column with 25% CSP diluted in a moderately polar polysiloxane means that only approximately 1–2% of the chiral selector was sufficient for the enantioresolution of CTTs when available in pure form. Second, it has been proposed that individual products in the CSP show intermolecular interactions, which increase the rigidity of the cyclodextrin cavities. In this concept, elevated temperatures lead to increased vibration of the cyclodextrin cavities in the column (13). Thus, the higher the temperature, the more difficult the formation of inclusion complexes becomes. Therefore, any intermolecular interaction (for instance by hydrogen bonds from unmodified OH-groups) between different CD-units that moderates vibrations may increase the enantioselectivity. In this context, it should be noted that chiral organohalogens belong to the class of compounds that require the highest elution temperatures suitable for GC enantioseparation.

Irrespectively of these uncertainties, incompletely modified O-tCDs fall into the group of mixed CSPs. Mixed CSPs have been used previously (23), but their suitability is still debatable (24,25). In this study, a simple system is used, which is two well-described CSPs: permethylated β- and γ-cyclodextrin and 1:1 mixtures thereof. This study investigated the effects of this system on the enantioseparation of organohalogen compounds. It is noted that the measurements were also interesting from a theoretical point of view.

Experimental

Standards

Racemic chiral organohalogen compounds were from LGC Promochem (Wesel, Germany) or Dr. Ehrenstorfer (Augsburg, Germany). Enantioenriched (–)-α-HCH (enantiomer excess 20%) was prepared as previously described (26). Individual standards and mixtures of them were used at concentrations of 80–200 pg/µL using n-hexane for organic trace analysis (Merck, Darmstadt, Germany). Synthesis of pure (> 99.9%) perdeuterated α-HCH (α-PDHCH) was described elsewhere (27).

GC

Separations were performed with a Hewlett-Packard 5890 GC equipped with an electron capture detector (ECD). The injector (one microliter was injected in the splitless mode) and detector temperatures were set at 250°C and 270°C, respectively. Helium (5.0) was used as the carrier gas at a column head pressure of 1.2 bar. Nitrogen (5.0) was used as the make-up gas at 60 mL/min.

Synthesis of heptakis(2,3,6-tri-O-methyl)-β-cyclodextrin (permethylated β-cyclodextrin) (β-PMCD) and octakis(2,3,6-tri-O-methyl)-γ-cyclodextrin (γ-PMCD) was performed according to Schurig et al. (28). Purity was monitored by thin-layer chromatography (ethyl acetate–ethanol, 9:1) and high-temperature GC as described previously (20). No impurities were detected. The pure, lab-made CSPs were diluted in 85% dimethyl–15% diphenylpolysiloxane (PS086) and coated on fused-silica capillary columns (15 m × 0.25-mm i.d., 0.25-µm d_f), according to the method of Blum and Aichholz (11): (i) 10% β-PMCD in PS086 (β-PMCD); (ii) 10% γ-PMCD in PS086 (γ-PMCD); (iii) mixture of 5% β-PMCD and 5% γ-PMCD in PS086 (5β5γ-PMCD); (iv) mixture of 10% β-PMCD and 10% γ-PMCD in PS086 (10β10γ-PMCD).

Investigations took place after increasing the baseline less than three fold the initial value at > 200°C. The GC oven was programmed as follows: 85°C (2 min isothermal), increased 25°C/min to 135°C (20 min isothermal), increased 1°C/min to 185°C, increased 15°C/min to 220°C (20 min isothermal). With this GC oven program the (separated) enantiomers eluted within the slow heating rate of 1°C/min between 24 min (corresponding with 135°C) and 74 min (corresponding with 185°C).

Correctly, α-values were only thermodynamically defined in isothermal runs and also for undiluted CSPs (29,30). α-Values decreased with increasing temperature (in the common case of enthalpy-controlled enantioseparations) so that isothermal elution yields the highest separation factors. However, chiral resolution (R_s), which includes peak widths, often increased with slow heating rates. In this presentation, α-values were calculated by relating the reduced retention times of the enantiomers with each other. Therefore, the values in this study were labelled $\alpha_{prog,dil}$ (i.e., separation factors obtained on polysiloxane-diluted chiral stationary phases, as obtained in temperature-programmed analysis) to distinguish them from thermodynamic α-values. The $\alpha_{prog,dil}$-values represent no correct absolute numbers. However, when the same amount of CSP(s) in the polysiloxane was used, the relative numbers will be comparable. Note that diluted CSPs have become standard nowadays; thus, the absolute α-values are not obtained.

Results and Discussion

Permethylated β- and γ-cyclodextrin were known to enantioseparate only a limited number of chiral organochlorines (21,31,32). Among the tested compounds, α-HCH, cis- and trans-chlordane, cis-heptachlor epoxide, and β-pentachlorocyclohexene (β-PCCH) and γ-pentachlorocyclohexene (γ-PCCH) (Figure 1) were at least resolved on one of the O-tCDs (Table I). By contrast, the columns did not separate any of the tested atropisomeric PCBs nor any compound of technical toxaphene,

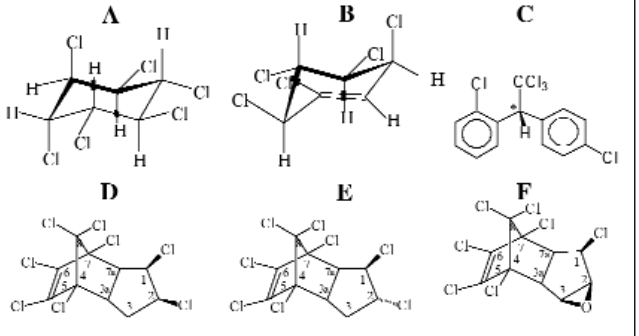

Figure 1. Structures of α-HCH (A), β-PCCH (B), *o,p'*-DDT (C), *cis*-chlordane (D), *trans*-chlordane (E), and *cis*-heptachlor epoxide (F).

which was in agreement with previous studies (21).

The enantiomers of α-HCH were enantioseparated on both individual columns, providing a better enantioresolution on γ-PMCD (Figure 2). Moreover, (+)-α-HCH eluted first from β-PMCD and second from γ-PMCD (33). On the 5β5γ-PMCD mixture, both enantiomers were enantioseparated as well. Because of the reversed elution order of enantiomers on the individual columns, they showed different R_s values. The retention times on the 5β5γ-PMCD were comparable with those on the individual columns (Table I). By contrast, the enantiomers eluted significantly later from the 10β10γ-PMCD column, indicative of a higher overall polarity of the column (described later). The same results were obtained for the perdeuterated α-HCH, which eluted slightly earlier than the native compound (Table I).

A very similar behavior was observed for β-PCCH (Figure 3). However, because of the lower difference in the R_s values on both individual columns, the enantioresolution of β-PCCH on 5β5γ-PMCD was worse than was obtained for α-HCH (Figure 3C). On the 10β10γ-PMCD column, the enantioresolution was better than on the 5β5γ-PMCD column (Figures 3C and 3D). This was either because of the longer retention times or because of the lower enantioselectivity on the 5β5γ-PMCD column.

Enantiomers of cis-chlordane eluted in reversed order from β- and γ-PMCD and showed similar R_S values (Table I). Thus, the enantiomers coeluted on the mixed β- or γ-PMCD columns (Figures 4E–4H). The enantioselectivity was a simple additive, and the higher retainment of one enantiomer on one part of the column was evened out on the other part of the column, which was not unexpected. For trans-chlordane enantiomers, the peak resolution was comparable as well as on β- and γ-PMCD (Figure 4). However, trans-chlordane enantiomers eluted in the same order from β- and γ-PMCD. Therefore, $\alpha_{prog,dil}$ on the 5β5γ-PMCD column was virtually the same as on the individual columns (Figure 4G). Consequently, each column was responsible for half of the enantioseparation, as seen in equation 1:

$$\alpha_{prog,dil,M} = |(c_1 [\alpha_{prog,dil,1} - 1] + a([c_2 \alpha_{prog,dil,2}] - 1)| + 1 \quad \text{Eq. 1}$$

where $\alpha_{prog,dil,1}$ and $\alpha_{prog,dil,2}$ are the α-values of the individual enantiomers, c_1 and c_2 are the contributions of the respective phases (here $c_1 = c_2 = 0.5$), a = 1 if both enantiomers elute in the same order and a = (–1) if both enantiomers elute in reversed order; and $\alpha_{prog,dil,M}$ is the predicted $\alpha_{prog,dil}$-value on the mixed phase (M).

This appears plausible in view of the fact that the analyte will statistically be found in interaction with half of the reduced retention time (t_R') on each column, respectively. This generally points to a reduced enantioseparation efficiency on mixed O-tCDs compared with individual O-tCDs. For instance, the calculated $\alpha_{prog,dil,M}$ of cis-chlordane on 5β5γ-PMCD was 1.001, which was in very good agreement with the observed coalescence of the enantiomers. Therefore, equation 1 was suitable to determine the relative elution order of enantiomers. For γ-PCCH, no enantioenriched standards were available but the $\alpha_{prog,dil,M}$ value on the 5β5γ-PMCD column of:

$$|[½ (0.035 + (–1) 0.012)]| + 1 = 1.0115 \quad \text{Eq. 2}$$

matched the observed value of 1.012, which confirms that both enantiomers eluted in reversed order from β- and γ-PCCH (Table I).

It has been mentioned that α-values were only defined for (i) isothermal elutions (2) and (ii) for undiluted O-tCDs (30). Because the polysiloxane also retains the compounds (both enantiomers to the same degree), the retention times were (to a first order of estimation) delayed. However, when the same

Table I. Retention Times (t_R) and $\alpha_{prog,\,dil}$ Values* on the Four Columns Investigated

O-tCD in PS086:	10% β-PMCD		10% γ-PMCD		5% β-PMCD–5% γ-PMCD		10% β-PMCD–10% γ-PMCD	
Compound	t_R (min)	$\alpha_{prog,\,dil}$*	t_R (min)	$\alpha_{prog,dil}$*	t_R (min)	$\alpha_{prog,dil}$*	t_R (min)	$\alpha_{prog,dil}$*
β-PCCH	31.37 / 32.32	1.031	30.07 / 30.63	1.019	32.00 / 32.14	1.004	39.28 / 39.52	1.006
γ-PCCH	13.02 / 13.45	1.035	12.97 / 13.11	1.012	13.60 / 13.75	1.012	16.23 / 16.51	1.018
α-HCH	34.16 / 34.66	1.015	34.79 / 36.20	1.042	35.86 / 36.37	1.015	41.24 / 41.93	1.017
α-PDHCH	33.67 / 34.19	1.016	34.17 / 35.60	1.042	35.17 / 35.70	1.015	40.51 / 41.21	1.018
cis-Heptachlor epoxide	66.04	1.000	67.52 / 68.17	1.010	68.06 / 68.43	1.005	72.87 / 73.35	1.007
trans-Heptachlor epoxide	67.49	1.000	69.33	1.000	69.87	1.000	74.80	1.000
cis-Chlordane	72.59 / 72.98	1.005	73.57 / 74.09	1.007	74.47	1.000	76.64	1.000
trans-Chlordane	71.77 / 72.39	1.009	72.40 / 72.84	1.006	73.17 / 73.70	1.007	76.36 / 76.60	1.003
Heptachlor	49.29	1.000	50.71	1.000	51.37	1.000	55.92	1.000
o,p'-DDT	79.71	1.000	79.53	1.000	80.15	1.000	81.95	1.000
B8-1413†	80.15	1.000	80.09	1.000	80.62	1.000	82.93	1.000
B9-1679†	83.76	1.000	83.68	1.000	84.34	1.000	85.82	1.000
PCB 95	68.97	1.000	67.59	1.000	69.67	1.000	73.30	1.000
PCB 132	69.74	1.000	66.73	1.000	69.40	1.000	74.57	1.000
PCB 149	78.41	1.000	77.93	1.000	78.67	1.000	80.08	1.000
PCB 171	88.08	1.000	87.70	1.000	88.67	1.000	91.20	1.000
PCB 174	86.91	1.000	87.11	1.000	87.24	1.000	89.59	1.000

* Only relative α-values (see Results and Discussion section).
† Only two out of 10 toxaphene congeners are shown (no enantioseparation in any case).

amount of polysiloxane was used, the relative $\alpha_{prog,dil,M}$ values were comparable. Thus, the 5β5γ-PMCD column could be compared with the β- and γ-PMCD columns, respectively. On the other hand, the (relative) $\alpha_{prog,dil}$-values could not be compared with those on the 10β10γ-PMCD column because the retention times (and, thus, elution temperatures) were different compared with the three other columns (Table I). For instance, the coeluting enantiomers of cis-chlordane were less delayed on 5β5γ-PMCD than on 10β10γ-PMCD (Figures 4C and 4D). Higher retention times on the 10β10γ-PMCD column were also found for compounds that were not enantioresolved (e.g., atropisomeric PCBs and CTTs, Table I). Thus, interaction of any analyte with a CSPs was not restricted to enantioselective interaction. A good proportion of interaction must also be related to non-enantioselective association of the analyte with the O-tCD. Any investigation of the molecular origin was beyond the possibilities that the data provided. However, one key point was surely the inherent polarity of cyclodextrin. Typically, the O-tCD is more polar than the achiral part of the column (34). Literature data indicate that increasing the amount of β-PMCD from 10% to 20% increases the McReynolds constant from 815 to 858 (35). These values are based on dilution in 65% dimethyl–35% diphenyl polysiloxane, which alone has a slightly higher McReynolds constant than PS086. This effect clarifies that the thermodynamic definition of the α-value, which relates the overall mechanism of interaction to be enantioselective, cannot be correct in real chromatography because an increase in the retention was directly connected with a reduction of the α-value. This also suggests that enantioselectivity in GC was higher than when calculated from the experimentally determined separation factor on O-tCD.

The enantiomers of cis-heptachlor epoxide were separated on γ-PMCD, but they were not separated on β-PMCD (Figure 5). On the mixed CSP, the enantiomers were resolved, but the resolution was significantly decreased on the 5β5γ-PMCD in comparison with γ-PMCD. The calculated $\alpha_{prog,dil,M}$ value of 1.005 ($c_1 = c_2 = 0.5$, equation 1) matched the value measured 5β5γ-PMCD (Table I). 5β5γ-PMCD contains half of the amount of the chiral selector suitable for enantioseparation (γ-PMCD), and the observed effect fully agrees with equation 1. Thus, the lower amount of γ-PMCD in the 5β5γ-PMCD column explains the reduced enantioresolution. Note that such comparisons were only possible with the mixed columns because simple diluted columns with different amounts of CSP led to completely different retention times and elution temperatures. Assuming the same non-enantioselective effect on both β- and γ-PMCD (this appears justified given the fact the 5β5γ-column had virtually identical retention times) equation 1 will be valid as well when the amount of the resolving CSP was subsequently be decreased (for cis-heptachlor epoxide: decreasing factor c_2) and substituted with a non-enantioselective part that had the same polarity (in this case, increasing c_1). Thus, in the case of the separation of α-HCH on γ-PMCD, less than 1% of the CSP would be enough for a sufficient separation of the enantiomers.

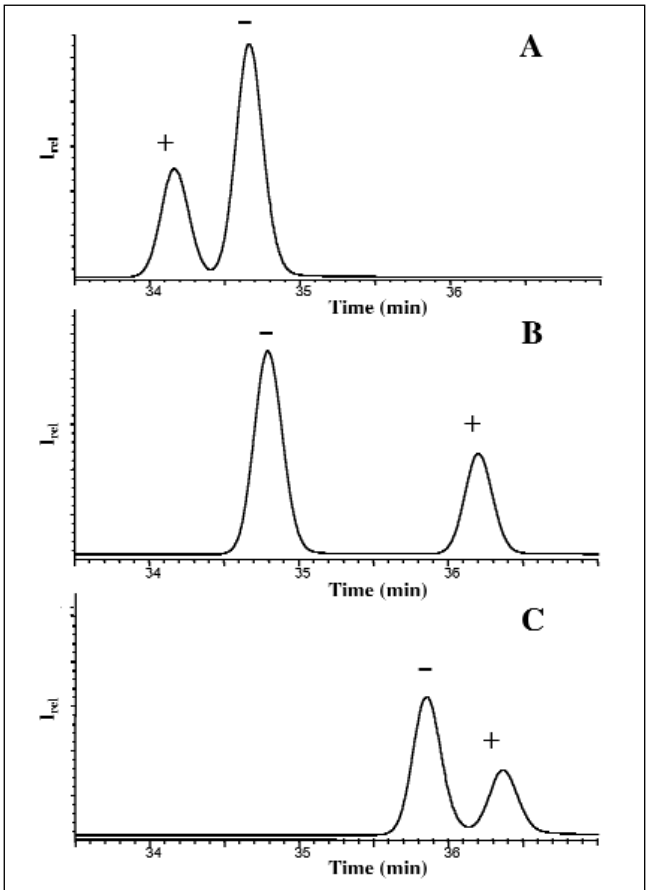

Figure 2. Enantioseparation of enantioenriched (–)-α-HCH on β-PMCD (A), γ-PMCD (B), and the mixed chiral stationary phase consisting of 5% β- and 5% γ-PMCD (C). Relative intensity (I_{rel}).

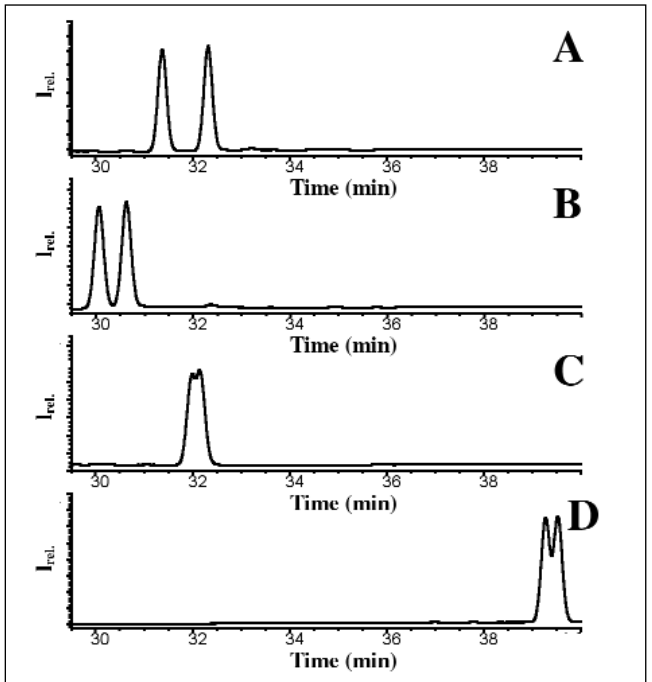

Figure 3. Enantioseparation of β-PCCH on β-PMCD (A), γ-PMCD (B), and the mixed chiral stationary phases consisting of 5% (C) or 10% of both β- and γ-PMCD (D). Relative intensity (I_{rel}).

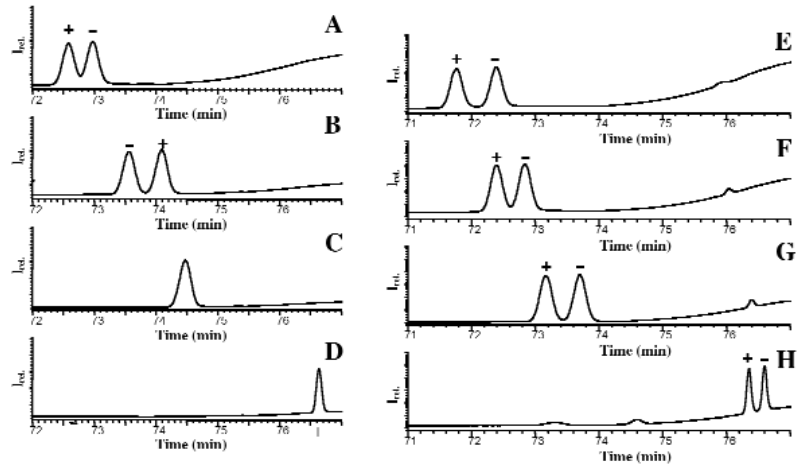

Figure 4. Enantioseparation of *cis*-chlordane (left panels) and *trans*-chlordane (right panels) on β-PMCD (A and E), γ-PMCD (B and F), and the mixed chiral stationary phases consisting of 5% (C and G) or 10% of both β- and γ-PMCD (D and H). Relative intensity (I_{rel}).

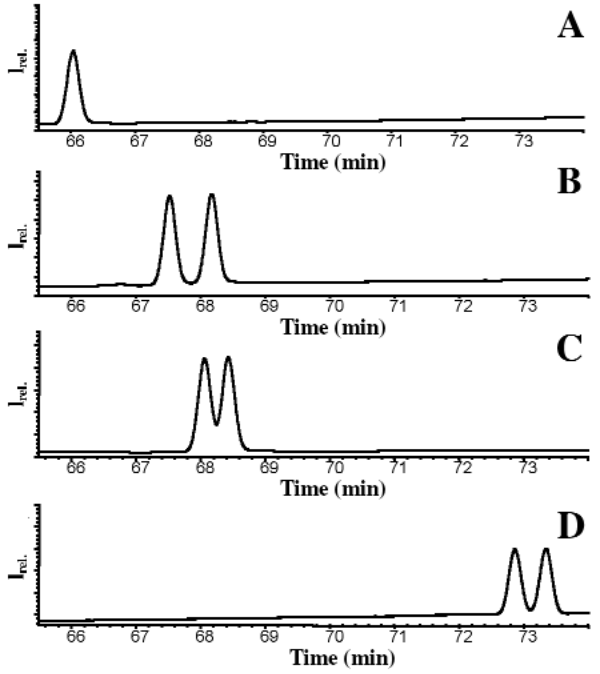

Figure 5. Enantioseparation of *cis*-heptachlor epoxide on β-PMCD (A), γ-PMCD (B), and the mixed chiral stationary phases consisting of 5% (C) or 10% of both β- and γ-PMCD (D). Relative intensity (I_{rel}).

Conclusion

The data shows that in the case of β- and γ-PMCD columns and polyhalogenated compounds, mixed columns had no considerable effect on the enantioselectivity, which is in contrast with other studies with different CSPs and compounds (36-38). There was no example where enantioselectivity directly results from the mixing of the O-tCDs. The highest $\alpha_{prog.,dil}$ value obtained on the four columns returned to the individual O-tCD (β- or γ-PMCD), which showed the best resolution. When one part of the mixed column showed no enantioselectivity, the observed α-values were lower than on the neat column with higher enantioselectivity. When the amount of the O-tCD was reduced (5β5γ- vs 10β10γ-PMCD), the lower separation factor was because of the lower overall amount of the suitable O-tCD. When the amount was kept constant (10β10γ vs 10β- or 10γ-PMCD), the contribution of (unnecessary) O-tCD significantly increased the retention, which was directly connected with a lowering of the α-value. The examples also confirmed that very low amounts of a suitable O-tCD (~ 1%) will be enough for sufficient enantioresolution (decrease of c_1 and setting $\alpha_{prog.,\,dil,2}$ = 1.000 or vice versa in equation 1). This could, in fact, explain the high enantioselectivity of the impure tert-butyldimethylsilylated cyclodextrins, which contain approximately 20 compounds or more. The goal of recent studies of the composition of β-BSCD (22) and β-TBDM (21) did not take into account such small amounts of special minor products, which need to be studied according to the data obtained in this study. It is also clear that different parts in the mixed columns may yield peak coalescence to the reversed enantioselectivity obtained with the parts, as is observed for cis-chlordane (described earlier). Furthermore, varied contribution of the CSPs may lead to peak reversals in the resulting column, as was observed for β-BSCD (2). In the case of the complex β-BSCD, further research should, therefore, be directed into the analysis of minor products in this mixed phase. The results on the much simpler mixed PMCD columns illustrate that focusing on minor products may lead to improved CSPs for the enantioseparation of toxaphene compounds.

References

1. W. Vetter and K. Bester. "Gas chromatographic enantioseparation of chiral pollutants-techniques and results". In *Chiral Analysis*, K.W. Busch and M.A. Busch, Eds, Elsevier, Amsterdam, the Netherlands, 2006.
2. W. Vetter and V. Schurig. Enantioselective determination of chiral organochlorines in biota on modified cyclodextrins by gas chromatography. *J. Chromatogr. A* **774:** 143–75 (1997).
3. R. Kallenborn and H. Hühnerfuss. *Chiral Evironmental Pollutants.* Springer, Berlin Heidelberg, Germany, 2001.
4. S.D. Richardson. Water analysis: Emerging contaminants and current issues. *Anal. Chem.* **75:** 2831–57 (2003).
5. V. Schurig. Chiral separations using gas chromatography. *Trends Anal. Chem.* **21:** 647–61 (2002).
6. Z. Juvancz, G. Alexander, and J. Szejtli. Permethylated β-cyclodextrin as stationary phase in capillary gas chromatography. *J. High Resolut. Chromatogr.* **10:** 105–107 (1987).
7. W.A. König. *Gas Chromatographic Enantiomer Separation with Modified Cyclodextrins.* Hüthig, Heidelberg, Germany, 1992.
8. V. Schurig and H.-P. Nowotny. Separation of enantiomers on diluted permethylated β-cyclodextrin by high-resolution gas chromatography. *J. Chromatogr.* **441:** 155–63 (1988).
9. W. Armstrong, W.Y. Li, and J. Pitha. Reversing enantioselectivity in capillary gas chromatography with polar and nonpolar cyclodextrin derivative phases. *Anal. Chem.* **62:** 214–17 (1990).
10. A. Dietrich, B. Maas, V. Karl, P. Kreis, D. Lehmann, B. Weber, and

A. Mosandl. Stereoisomeric flavor compounds. Part LV: stereodifferentiation of some chiral volatiles on heptakis(2,3-di-*O*-acetyl-6-*O*-tert-butyldimethylsilyl)-β-cyclodextrin. *J. High Resolut. Chromatogr.* **15:** 176–79 (1992).
11. W. Blum and R. Aichholz. Gas chromatographic enantiomer separation on tert-butyldimethylsilylated β-cyclodextrin diluted in PS-086. Simple method to prepare enantioselective glass capillary columns. *J. High Resolut. Chromatogr.* **13:** 515–18 (1990).
12. W.A. König, R. Krebber, and P. Mischnick. Cyclodextrins as chiral stationary phases in capillary gas chromatography. Part V: octakis(3-*O*-butyryl-2,6-di-*O*-pentyl)-β-cyclodextrin. *J. High Resol. Chromatogr.* **12:** 732–38 (1989).
13. W. Vetter. Enantioselective fate of chiral chlorinated hydrocarbons and their metabolites in environmental samples. *Food Rev. Int.* **71:** 113–82 (2001).
14. H.-R. Buser and M.D. Müller. Isomeric and enantiomeric composition of different commercial toxaphenes and chlorination products of (+)- and (–)-camphenes. *J. Agric. Food Chem.* **42:** 393–400 (1994).
15. R. Kallenborn, M. Oehme, W. Vetter, and H. Parlar. Enantiomer selective separation of toxaphene congeners isolated from seal blubber and obtained by synthesis. *Chemosphere* **28:** 89–98 (1994).
16. U. Klobes, W. Vetter, B. Luckas, and G. Hottinger. Enantioseparation of compounds of technical toxaphene (CTTs) on 35% heptakis(6-*O*-tert.-butyldimethylsilyl-2,3-di-*O*-methyl)-β-cyclodextrin diluted in OV1701. *Chromatographia* **47:** 565–69 (1998).
17. A. Jaus and M. Oehme. Enantioselective behaviour of ethylated β-cyclodextrins as GC stationary phases for chlorinated pesticides and phase characterisation by HPLC. *Chromatographia* **50:** 299–304 (1999).
18. W. Vetter and B. Luckas. Enantioselective fate of persistent and partly degradable toxaphene components in high trophic level biota. *Chemosphere* **41:** 499–506 (2000).
19. V. Schurig. Personal communication to W. Vetter. March, 2000.
20. W. Vetter, U. Klobes, B. Luckas, and G. Hottinger. Use of 6-*O*-tert-butyldimethylsilylated β-cyclodextrins for the enantioseparation of chiral organochlorines. *J. Chromatogr. A* **846:** 375–81 (1999).
21. S. Ruppe, W. Vetter, B. Luckas, and G. Hottinger. Application of well-defined β-cyclodextrins for the enantioseparation of compounds of technical toxaphene and further organochlorines. *J. Microcol. Sep.* **12:** 541–48 (2000).
22. M. Oehme, L. Müller, and H. Karlsson. High-resolution chromatographic test for the characterisation of enantioselective separation of organochlorine compounds. Application to tert.-butyldimethylsilyl β-cyclodextrin. *J. Chromatogr. A* **775:** 275–85 (1997).
23. T. Zhang and E. Francotte. Chromatographic properties of composite chiral stationary phases based on cellulose derivatives. *Chirality* **7:** 425–33 (1995).
24. S. Allenmark. A note concerning chromatography on mixed chiral stationary phases. *Enantiomer* **4:** 67–69 (1999).
25. P.A. Levkin, A. Ruderisch, and V. Schurig. Combining the enantioselectivity of a cyclodextrin and a diamide selector in a mixed binary gas-chromatographic chiral stationary phase. *Chirality* **18:** 49–63 (2006).
26. W. Vetter, U. Klobes, B. Luckas, G. Hottinger, and G. Schmidt. Determination of (+/–) elution orders of chiral organochlorines by HPLC with a chiral detector and enantioselective gas chromatography. *J.—Assoc. Off. Anal. Chem.* **81:** 1245–51 (1998).
27. W. Vetter and B. Luckas. Synthesis, isolation, and chromatography of perdeuterated ±-1,2,3,4,5,6-hexachlorocyclohexane. *J. High Resolut. Chromatogr.* **18:** 643–46 (1995).
28. V. Schurig, M. Jung, D. Schmalzing, M. Schleimer, J. Duvekot, J.C. Buyten, J.A. Peene, and P. Mussche. CGC enantiomer separation on diluted cyclodextrin derivatives coated on fused silica columns. *J. High Resolut. Chromatogr.* **13:** 470–74 (1990).
29. V. Schurig. Enantiomer separation by gas chromatography on chiral stationary phases. *J. Chromatogr. A* **666:** 111–29 (1994).
30. M. Jung, D. Schmalzing, and V. Schurig. Theoretical approach to the gas chromatographic separation of enantiomers on dissolved cyclodextrin derivatives. *J. Chromatogr.* **552:** 43–57 (1991).
31. W.A. König, D. Icheln, T. Runge, B. Pfaffenberger, P. Ludwig, and H. Hühnerfuss. Gas chromatographic enantiomer separation of agrochemicals using modified cyclodextrines. *J. High Resolut. Chromatogr.* **14:** 530–36 (1991).
32. H.-R. Buser, M.D. Müller, and C. Rappe. Enantioselective determination of chlordane components using chiral high-resolution gas chromatography-mass spectrometry with application to environmental samples. *Environ. Sci. Technol.* **26:** 1533–40 (1992).
33. R.L. Falconer, T.F. Bidleman, and D.J. Gregor. Air-water gas exchange and evidence for metabolism of hexachlorocyclohexanes in Resolute Bay, N.W.T. *Sci. Total Environ.* **160/161:** 65–73 (1995).
34. Z. Juvancz and P. Petersson. Enantioselective gas chromatography. *J. Microcol. Sep.* **8:** 99–114 (1996).
35. Anonymous. Supelco capillary GC columns. *Supleco Bulletin* **875c** (1999).
36. S. Tamogami, K.-I. Awano, M. Amaike, Y. Takagi, and T. Kitahara. Development of an efficient GLC system with a mixed chiral stationary phase and its application to the separation of optical isomers. *Flavour Fragrance J.* **16:** 349–52 (2001).
37. A. Ruderisch, J. Pfeiffer, and V. Schurig. Mixed chiral stationary phase containing modified resorcinarene and β-cyclodextrin selectors bonded to a polysiloxane for enantioselective gas chromatography. *J. Chromatogr. A* **994:** 127–35 (2003).
38. M. Bayer and A. Mosandl. Improved gas chromatographic stereodifferentiation of chiral main constituents from different essential oils using a mixture of chiral stationary phases. *Flavour Fragrance J.* **19:** 515–17 (2004).

Manuscript received November 28, 2005;
revision received May 31, 2006.