Towards more reproducible separations of polychlorinated compounds with modified cyclodextrins: Necessary requirements and present status

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Introduction

The enantioselective separation of polychlorinated compounds has gained an increased interest in recent years. Examples of chiral pesticides are α-hexachlorcyclohexane, o,p'-DDT, some chlordane congeners and most toxaphene compounds. The originally racemic enantiomer ratio in the technical products is usually changed by biogenic processes. This alteration allows to detect degradation by such processes in the environment and transport between compartments.

High resolution gas chromatography (HRGC) using mixtures of polysiloxanes with modified β-cyclodextrins is most frequently used for the enantioselective separation of chiral pesticides. It could be shown that capillaries based on heptakis(2,3,6-O-t-butyl-dimethylsilyl)-β-cyclodextrin (TBDMS-CD) are able to separate nearly all chiral pesticide compounds of interest [1,2]. However, due to steric interactions this β-cyclodextrin is only randomly derivatised, and a not very well defined mixture is formed consisting of homologues with 7 to 11 t-butyl-dimethylsilyl groups [2]. Therefore, the achieved separation of both home-made and commercially available capillaries vary very much between columns [2]. For example, only one column in our laboratory was able to separate nearly all toxaphene congeners which are available as pure standards. In addition, even moderate changes in the applied temperature program had a significant influence on the achieved separation.

Due to the reasons given above, it was found desirable to replace TBDMS-CD by another β-cyclodextrin of comparable separation properties having a well-defined composition and, correspondingly, a better separation reproducibility. Therefore, a systematic search for a substitute was started in our group. It is based on the following three main elements:

- Systematic variation of the substituents at position 2, 3 and 6 as well as of the size of the cavity (α-, β- or γ-cyclodextrin as basic structure).
- Purity test of the modified cyclodextrins with a newly developed method combining HPLC separation with light scattering detection. If necessary, a further control can be carried out by HPLC-MS.
- Test of prepared HRGC capillaries by an improvement of the recently presented test mixture for the evaluation of enantioselective separations of polychlorinated pesticides [2].

The systematic study of the suitability of different cyclodextrins for the separation of chiral chlorinated pesticides was split into two different approaches. The first one varies the size of the

cavity and the substituents (same substituents at all positions) in a systematic way as outlined above. The second one is based on the recently published results that (2,3-O-dimethyl-6-O-t-butyl-dimethylsilyl)-β-cyclodextrin (2,3M6T-CD) shows separation properties for chlordane compounds which are similar to those of TBDMS-CD [3]. The bulky group at the 6 position is obviously of advantage. The change of the rest at the 2 and 3 position as well as the size of the cavity are then further possibilities to gain selectivity.

This paper presents first results of the systematic approach mentioned above and makes a proposal concerning alternatives to TBDMS-CD for the reproducible separation of chiral chlordane compounds.

Material and Methods

Reference compounds and solutions: Pure crystalline compounds of cis-(cs-CD), trans-chlordane (tr-CD), cis-(cs-HEP), trans-heptachlorexpoxide (tr-HEP), o,p'-DDT and Toxaphene (Tox) no. 50 were obtained from Promochem (Wesel). Racemic, enantiopure and/or enantiomer-enriched U82, MC5, MC7 and MC8 were isolated from technical chlordane as described in [4]. Enantiomer-enriched α-hexachlorcyclohexane (HCH) was a gift of Dr. W. Vetter, University of Jena, Germany. The mixture for testing the enantioselective separation of HRGC capillaries (abbreviated as CHIROTEST X) contained the following concentrations dissolved in hexane: α(-)-HCH, 33 pg/µl; α(+)-HCH, 34 pg/µl; (+)-U82, 109 pg/µl; (-)-U82, 48 pg/µl; (-)-tr-HEP, 70 pg/µl; (+)-tr-HEP, 61 pg/µl; (-)-tr-CD, 50 pg/µl; (+)-tr-CD, 127 pg/µl; (-)-cs-CD, 208 pg/µl; (+)-cs-CD, 165 pg/µl; (+/-)-o,p'-DDT, 368 pg/µl and (+/-)-Tox 50, 143 pg/µl.

β-cyclodextrin derivatives: PMCD and 2,3M6T-CD were obtained as crystalline compounds from Macherey-Nagel (Switzerland). Perethyl-β-cyclodextrin (PECD) was a gift of Dr. Markus Müller, Swiss Federal Research Station, Wädenswil, Switzerland.

Puritiy control of β-cyclodextrin derivatives: A quaternary Hewlett-Packard HP 1050 pump was connected to a PL-EMD 960 light scattering detector (Polymer laboratories, Shropshire, UK). The output signal was recorded with a HP 3390 A integrator. The detector temperature was 50 °C (for 2,3DM6T-CD 40 °C) and compressed air at a flow rate of 3,5 l/min was applied. Separations were carried out on a 125 mm long, 2 mm ID column packed with Nucleosil 100-5, 5 µm particle size C18 phase (Macherey-Nagel). For PMCD a gradient was used from methanol/-water (50:50) to 100 % methanol within 5 min. PECD was controlled with a gradient from methanol/water (90:10) to 100 % methanol within 15 min. 2,3M6T-CD was separated with a gradient from methanol/tetrahydrofuran (95:5) to methanol/tetrahydrofuran (80:20) within 7 min.

HRGC separation capillaries: A commercially available TBDMS-CD capillary of 30 m length, 0,25 mm ID and a film thickness of 0,25 µm of 25% TBDMS-CD in OV-1701-OH was used (BGB-Analytik, Switzerland). A 30 m long, 0,25 mm ID capillary coated with 0,25 µm of 20% permethylated β-cyclodextrin (PMCD) in 35%-diphenyl-65%-dimethylpolysiloxane (SPB-35) (trade name Betadex 120) was purchased from Supelco (Buchs, Switzerland). Capillaries of 10 to 25 m length, 0,25 mm ID coated with a film thickness of 0,26 µm of 50% 2,3M6T-CD in OV-1701 vinyl were prepared by Macherey-Nagel (Switzerland) on request.

HRGC-separations: Separations were carried out on a Varian 3800 gas chromatograph equipped with a electron capture detector and a split/splitless injector. The injector temperature was 250 °C and the detector temperature 280 °C. N_2 was used as a make-up gas at a flow rate of 30 ml/min. Alternatively, negative ion chemical ionization (NICI) mass spectrometry was used employing the same system and conditions as described in [4].

Results and discussion

As reported before, capillaries based on TBDMS-CD diluted in e.g. OV-1701-OH are able to separate all chlordane and most toxaphene congeners as well as o,p'-DDT and α-HCH into enantiomers. However, this almost universal stationary phase has some severe drawbacks due to its variable composition. Enantiomer elution orders change between batches as well as the ability to separate selected chlordane and toxaphene congeners [2]. Furthermore, the separation is extremely sensitive to changes in the temperature program. Figure 1 shows the separation of two chlordane metabolites, oxy-chlordane and trans-heptachlorepoxide applying different temperature programs. Beside a dramatic change in the enantioselective behavior, a further difficulty is observed caused by the overlap of pairs of enantiomers. This problem can often be solved by using tandem columns [5].

Müller et al. have proposed to use (2,3-O-dimethyl-6-O-t-butyl-dimethylsilyl)-β-cyclodextrin (2,3M6T-CD) as a substitute of the ill-defined TBDMS-CD for the separation of chlordane congeners [3]. However, no information was given about the purity of the applied batch.

Based on own experiences and differences in reported enantioselectivies of the same stationary phase, the most possible reason for the observed deviations is a variable composition/-purity of the applied cyclodextrin. Therefore, based on the work of Deege et al. [6], an improved HPLC method was developed which allowed a comprehensive purity control of derivatized cyclodextrins. Due to missing UV-absorption of the cyclodextrins, light scattering detection was used. Figure 2 shows the separations of some cyclodextrin derivatives. Permethylated β-cyclodextrin is the most frequently used stationary phase in enantioselective HRGC and is normally of high purity (see Figure 2A). However, other peralkylated cyclodextrins such as perethyl-β-cyclodextrin (Figure 2B) can consist of a complex mixture and are difficult to clean. Such an insufficient purity is not necessarily detectable by the usually applied control methods such as NMR. As Figure 2C shows, 2,3M6T-CB obtained from Macherey-Nagel contained no measurable amounts (≥ 1 %) of other compounds when the eluent described under experimental was employed. This was additionally confirmed by electrospray mass spectrometry. Beside the isotope clusters typical for 98 carbon atoms of $[M+H]^+$, $[M+NH_4]^+$ and $[M+K]^+$ no further masses with an abundance higher than the general background (ca. 1 % relative intensity) could be observed (see [2] for details).

A first step of the more systematic study of the enantioselective separation properties of modified cyclodextrins was to propose alternatives to TBDMS-CD for the separation of chlordane congeners. Of particular interest is a complete isomer and enantiomer separation of cis- and trans-chlordane, U82, MC5, MC6, MC7 and the metabolites oxy-chlordane, cis- and trans-heptachlorepoxide. As shown earlier, the enantiomer ratio for the two first compounds was different between male and female cod [7]. Furthermore, U82 is the most abundant octachloro congener in marine mammals and humans [4]. Table 1 summarizes the achievable enantiomer separations of the available alternatives so far and compares them with those obtained on the best *TBDMS-CD capillaries.*

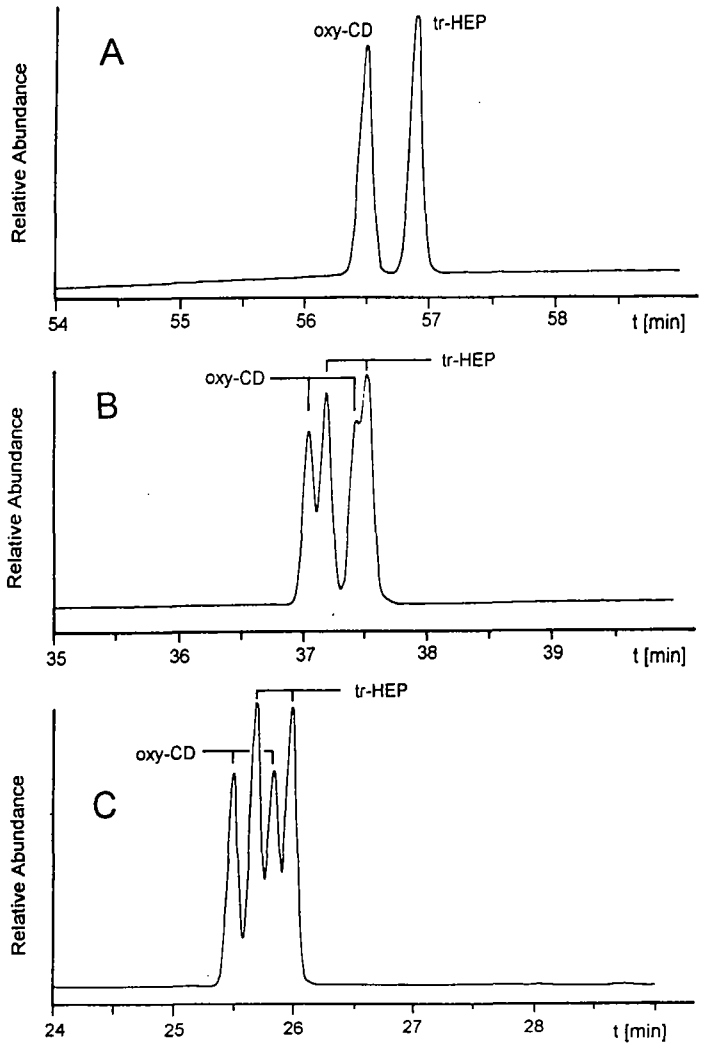

Figure 1: Influence of the temperature program on the separation of oxy-chlordane (oxy-CD) and trans-heptachlorepoxide (tr-HEP) on TBDMS-CD. Initial 60°C, 2 min; final 220 °C. A: 15 °C/min to 120 °C, then 2 °C/min. B: 15 °C/min to 120 °C, then 4 °C/min. C: 15 °C/min to 150 °C, then 7 °C/min.

As shown in Table 1, so far, none of the alternatives are able to replace TBDMS-CD. However, as Table 1 and the chromatogram of the CHIROTEST X in Figure 3 show, 2,3DM6T-CD is suitable for the separation of chlordane congeners and α-HCH. Compared to ref. 3, even U82 could be reasonably resolved. However, a partial overlap of the enantiomers of cis- and trans-chlordane (see Figure 3 and [3]) makes its use as part of a tandem column desirable. On the

opposite, Betadex 120 separates only four chlordane congeners into enantiomers. Nevertheless, its advantage is no interference between cis- and trans-chlordane enantiomers as already reported by Bidleman et al. [8]. Therefore, a combined use of both columns allows a separation of chlordane isomers without employing more complex tandem columns with longer analysis times.

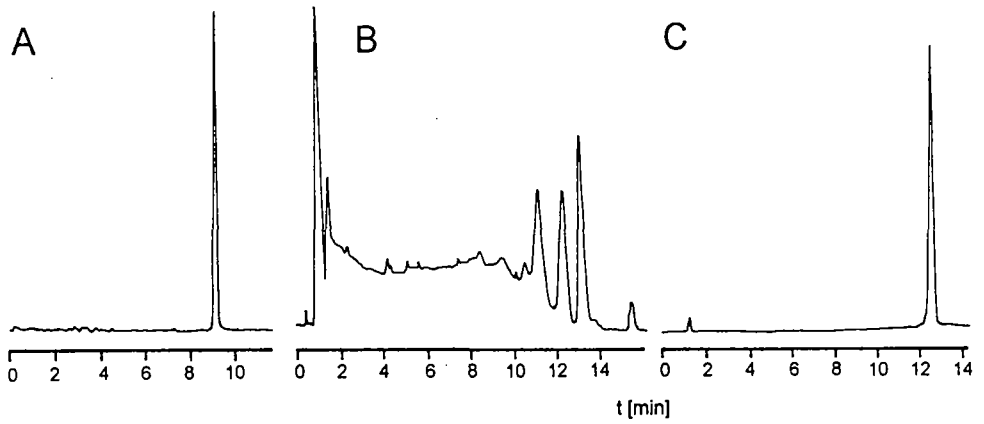

Figure 2: Purity control of permethylated β-cyclodextrin (A), perethylated β-cyclodextrin (B) and 2,3DM6T-CD (C) by HPLC light scattering detector. For separation conditions, see experimental.

Table 1: Best achievable enantiomer resolution R_S of chlordane congeners and major metabolites by different β-cyclodextrin-based stationary phases. n.r.: not resolved

Compound	R_S of column:		
	TBDMS-CD	Betadex 120	2,3DM6T-CD, 16 m long
U82	1.0[a]	n.r.[c]	0.77[d]
tr-CD	3.3[a]	0.8[c]	0.85[f]
cs-CD	1.2[a]	1.3[c]	2.3[e]
MC5	3.6[a]	0.9[c]	3.0[e]
MC7	1.7[a]	3.6[c]	7.3[e]
oxy-chlordane	1.8[a]	n.r.[c]	0.98[e]
cs-HEP	1.9[a]	n.r.[c]	0.62[e]
tr-HEP	1.5[a]	n.r.[c]	0.98[d]
α-HCH	1.0[b]	1.2[d]	8.6[e]
o,p'DDT	1.2[b]	n.r.[d]	n.r.[e]
Tox 50	1.5[b]	n.r.[d]	n.r.[e]

[a] 60 °C, 2 min, 15 °C/min to 150 °C, 7 °C/min to 220 °C, isothermal. [b] 100 °C, 2 min, 5 °C/min to 220 °C, isothermal. [c] 60 °C, 2 min, 30 °C/min to 160 °C, 1 °C/min to 220 °C, isothermal. [d] 60 °C, 2 min, 20 °C/min to 120 °C, 1 °C/min to 220 °C, isothermal. [e] 60 °C, 2 min, 20 °C/min to 120 °C, 2 °C/min to 220 °C, isothermal. [f] 60 °C, 2 min, 20 °C/min to 100 °C, 1 °C/min to 220 °C, isothermal.

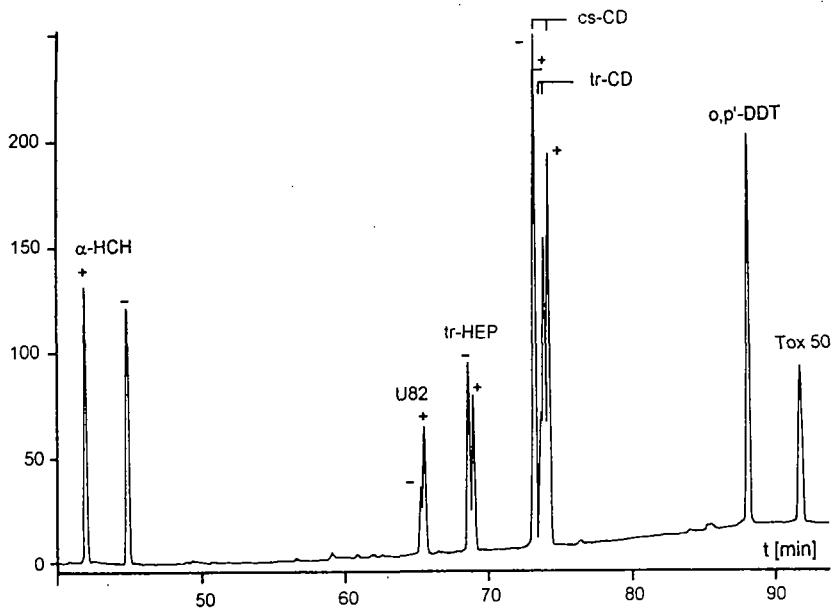

Figure 3: Separation of the CHIROTEST X on 2,3DM6T-CD (25 m capillary). Separation conditions were: 60 °C, 2 min, 20 °C/min to 120 °C, 1 °C/min to 200 °C, isothermal.

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