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Short communication

Enantioselective analysis of chiral polychlorinated biphenyls in sediment samples by multidimensional gas chromatography–electron-capture detection after steam distillation–solvent extraction and sulfur removal

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Abstract

Enantioselective analysis of the chiral polychlorinated biphenyls (PCBs) 95, 132 and 149 in river sediment samples was carried out by multidimensional gas chromatography with an achiral–chiral column combination and electron-capture detection. The investigations revealed the PCB congeners analysed to be present racemic within experimental error. A micro simultaneous steam distillation–solvent extraction device was used as extraction method. This technique proved to be a versatile tool for the extraction of organochlorine compounds from several matrices and was used for the extraction of PCBs from sediment samples. The removal of interfering elementary sulfur was carried out by the treatment with copper powder during the extraction.

Keywords: Enantiomer separation; Steam distillation–solvent extraction; Polychlorinated biphenyls; Sulphur; Copper powder; Organochlorine compounds

1. Introduction

Polychlorinated biphenyl (PCB) contamination in the environment was first reported in 1966 [1]. Due to their toxicity, persistence and bioaccumulation, PCBs are an important class of priority pollutants. One of the major environmental sinks for these pollutants is the sediment and therefore this matrix plays an important role in the environmental monitoring.

Until now, little attention has been paid to the inherent chirality of PCB congeners. Seventy-eight out of 209 congeners are axially chiral in their nonplanar conformations and 19 form stable enantiomers (atropisomers) due to restricted rotation around the central carbon–carbon bond at ambient temperatures [2]. At least nine of the conformationally stable chiral PCBs are present in commercial PCB formulations and are expected to accumulate in the environment.

As pointed out before for other chiral pollutants [3,4], biotic processes (uptake, metabolism, excretion) may be selective for enantiomers whereas abiotic processes such as chemical (oxidation, hydrolysis), distribution (adsorption, desorption), transport and photochemical processes

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are identical for both enantiomers and, consequently, enantiomeric ratios will not be changed. Enantioselective discrimination of chiral pollutants in environmental samples may give additional information on possible degradation pathways and may allow a distinction to be made between enzymatic biotic and non-enzymatic abiotic processes. The development of derivatized cyclodextrins as chiral stationary phases enables the determination of enantiomeric ratios of chiral pollutants, even at trace levels, by capillary gas chromatography [3,5,6]. Investigations were performed, e.g., for chiral pesticides, such as α-hexachlorocyclohexane (HCH) [7], pesticides of the chlordane group [4,8] and toxaphene congeners [9,10].

The gas chromatographic separation of most of the chiral PCB congeners has been achieved on different cyclodextrin phases [11–13]. Recently, we described the use of multidimensional gas chromatography (MDGC) with an achiral–chiral column combination for the enantiomer separation of chiral PCBs of commercial mixtures [14] and the application of this method to the enantioselective analysis of chiral PCBs in human milk samples [15]. In the present work this method is applied to the enantioselective determination of chiral 2,2′,3,5′,6-pentachlorobiphenyl (PCB 95), 2,2′,3,3′,4,6′-hexachlorobiphenyl (PCB 132), 2,2′,3,4′,5′,6-hexachlorobiphenyl (PCB 149) in sediment samples.

Classical sample preparation methods for sediment samples involve solid–liquid extraction (Soxhlet or ultrasonic extraction) followed by a clean-up procedure. The disadvantages of these methods are the need for large solvent volumes and the non-selective extractions. In recent years, beside supercritical fluid extraction (SFE), steam distillation–solvent extraction (SDE), in which the compounds are distilled and extracted into organic solvents simultaneously, has achieved much attention. Since SDE extraction was introduced by Likens and Nickerson in 1964 [16], different modifications [17] and miniaturizations [18,19] have been developed to decrease the solvent volume and to improve performance. Micro devices have been applied to environmental analysis, e.g., for extraction of organochlorine pesticides and PCBs at sub-ppb level [20,21]. To our knowledge, we applied this method for the first time to the extraction of PCBs from sediments.

A major problem in sediment analysis is the presence of elementary sulfur. The concentration of sulfur depends on the origin of the sediment and can be as high as several percent. Elementary sulfur is easily dissolved by the extraction methods and can cause significant interference in the further analytical procedure. In GC–electron-capture detection (ECD) analysis of the extract, the presence of sulfur at high concentrations may deteriorate separations and saturate the detector. Traditionally, treatment with metallic copper and mercury or with tetrabutylammonium sulfite during or after the extraction have been the most popular methods [22,23]. Mercury is the most efficient reagent but creates waste problems. We tried the removal of sulfur by the treatment with metallic copper during the SDE extraction.

The purpose of this work is to screen the enantiomeric composition of chiral PCB congeners present in sediment samples by MDGC after SDE extraction and to evaluate the possibility of removing sulfur during the extraction procedure.

2. Experimental

2.1. Samples

River sediment samples were taken in 1991 from the river Elsenz (tributary of river Neckar, Southern Germany).

2.2. Simultaneous steam distillation–solvent extraction (SDE)

Sample extraction was carried out using a Chrompack micro SDE apparatus in the low density solvent configuration. A 5-g sample of sediment material (freeze-dried) was extracted after addition of 50 ml of tap water, *n*-pentane (2 ml, Fluka, Buchs, Switzerland) was used as extraction solvent. Tap water instead of deionized water was used because of the frequently

found trace amounts of ECD-sensitive components that leach off the ion-exchange resins [17]. Extraction was carried out according to our previous experience under the following conditions: sample heating bath temperature, 149°C; solvent heating bath temperature, 67°C; coolant temperature, −15°C and extraction time, 1 h. The final extract was evaporated to dryness under a nitrogen stream and redissolved in 100 μl of *n*-hexane (Riedel-de-Haen, Seelze, Germany). An aliquot of 0.5–1.0 μl was used for chromatographic analysis. Blank extractions of 50 ml of tap water were carried out between successive sediment extraction.

For the removal of elementary sulfur 5 g of sediment material was mixed with 4 g of copper powder and SDE extraction was carried out as described before. The copper powder was pretreated prior to use as follows [24]: 20 g of copper powder was washed with 200 ml of deionised water, 200 ml of acetone and 200 ml of *n*-hexane. The remaining solvent was evaporated on the Rotavap and the powder kept under nitrogen.

2.2.1. Recovery for PCBs

The extent of recovery for the SDE extraction was checked with the technical PCB mixture Clophen A60 (Dr. Ehrenstorfer, Augsburg, Germany). A 50-μl volume of the Clophen A60 solution (1 ng/μl) was added to 50 ml of tap water, thus resulting in a Clophen A60 concentration of 1 ng/ml (1 ppb). SDE extraction was carried out as described above. The final extract was evaporated to dryness, redissolved in 1 ml of *n*-hexane and 20 ng of PCB 54 were added as internal standard. A calibration mixture was prepared by dissolving 50 μl of the Clophen A60 solution in 2 ml of *n*-pentane, evaporating to dryness and adding 1 ml of *n*-hexane and 20 ng of PCB 54 as internal standard.

2.3. Multidimensional gas chromatography

Gas chromatography was performed with a Siemens Sichromat-2 MDGC system (Mannheim, Germany) equipped with a flame ionization detector (FID) and with a ^{63}Ni–ECD (make-up gas nitrogen). Column switching was achieved with a pneumatically controlled six-port valve (Valco). Peak broadening was minimized by cooling the first part of the second column with air precooled with liquid nitrogen, thus focusing the cut fraction.

The first (achiral) column was a 30 m × 0.22 mm I.D. fused-silica capillary column coated with a 0.1 μm film of DB-5. The second (chiral) column was a 10 m × 0.25 mm I.D. fused-silica capillary column coated with a 0.2 μm film of immobilized Chirasil-Dex [25,26]. Hydrogen was used as carrier gas at 1 bar for the first column and at 0.4 bar for the second column. The first column was connected to the FID (250°C) and the second column to the ECD (280°C).

The temperature of the first column was maintained at 60°C for 3 min after on-column injection, then programmed at 20°C/min to 180°C, which was held for 15 min, and finally programmed at 20°C/min to 250°C.

The temperature program for the second column depended on the analysis performed. For PCB 95 the temperature was maintained at 100°C until the transfer was made (15.0 min), then increased to 145°C at 10°C/min. For PCB 132 the temperature was maintained at 100°C until the transfer was made (20.2 min), then increased to 170°C at 10°C/min. For PCB 149 the temperature was maintained at 100°C until the transfer was made (19.2 min), then increased to 155°C at 10°C/min.

For the determination of the recovery, the Siemens Sichromat-2 was used and the DB-5 column was directly connected to the ECD. The chromatographic conditions were chosen as described above.

The efficiency of the sulfur removal was tested using a Dani 3900 gas chromatograph (Monza, Italy) equipped with a ^{63}Ni–ECD (make-up gas nitrogen). A 25 m × 0.25 mm I.D. fused-silica capillary column coated with a 0.25 μm film of SE-54 was used. Helium was used as carrier gas at 1 bar. The temperature was maintained at 60°C for 3 min after on-column injection, then programmed at 20°C/min to 200°C, which was held for 15 min, and finally programmed at 15°C/min to 240°C.

3. Results and discussion

A micro SDE device has been applied to the extraction of PCBs from sediment samples for the first time. In order to study the extent of recovery for the extraction of PCBs by this device under the applied conditions, extractions of water spiked with the technical PCB mixture Clophen A60 were carried out. The chromatograms are shown in Fig. 1. Recovery values between 90–100% were obtained for the marked peaks; i.e. PCB 66/95, 84/92, 118/149, 105/132/153. The recovery for the extraction of PCBs from sediment is presumably lower but this does not affect the determination of the enantiomeric ratio which is the prime object of this work.

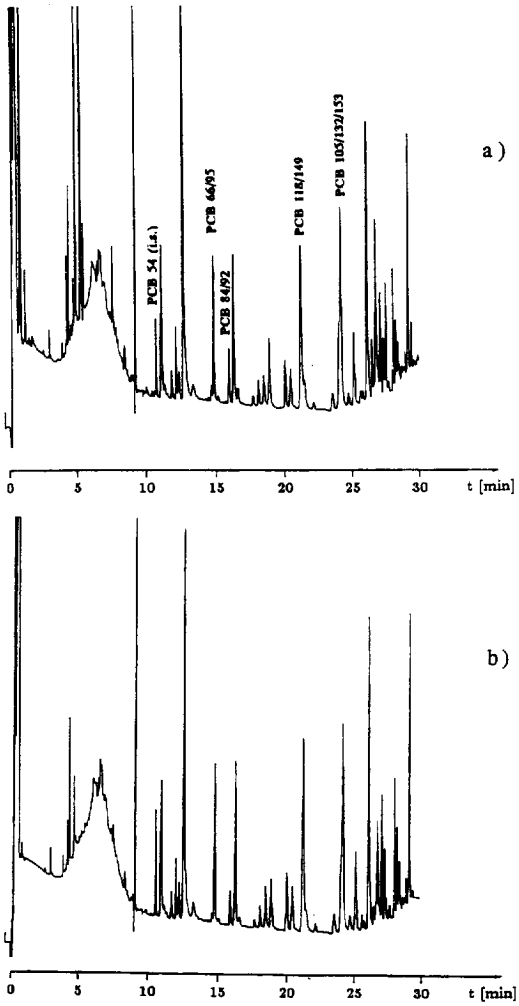

Fig. 1. GC–ECD chromatograms of: (a) SDE extract of water spiked with the technical mixture Clophen A60; (b) calibration mixture used as reference. See experimental section for chromatographic conditions.

The presence of elementary sulfur is a major problem in sediment analysis, because it can cause significant interference in the further GC–ECD analysis. In MDGC this interference and saturation of the detector is less severe, because only minute cut portions of the eluate are transferred to the ECD. Yet, in the present case the presence of sulfur disturbed the analysis of PCB congener 95 and a sulfur clean-up procedure was mandatory. The treatment with copper powder during the SDE extraction proved to be an efficient method for the sulfur removal, as ECD chromatograms before and after the treatment demonstrate (Fig. 2). Thus, micro SDE extraction is a useful technique for the extraction of polychlorinated biphenyls from sediment samples. The advantages are the requirement of only small amounts of solvent as well as the short analysis time because no further clean-up steps are necessary. If there is any elementary sulfur present, it can easily be removed during the extraction procedure by treatment with copper powder.

The MDGC method described recently [14] has been used for the determination of the enantiomeric ratios of the chiral PCB congeners 95, 132 and 149 in river sediment samples. The investigations of five samples revealed the PCB congeners analysed to be present as a racemate within experimental error (Fig. 3). Racemization during the extraction could be excluded by carrying out the same extraction procedure with one single enantiomer of PCB 132 (enantiomeric rato, ER = 0.08 before and 0.07 after the extraction). The single enantiomers were obtained by semipreparative HPLC on a Chirasil-Dex column [27].

This is in contrast to the findings of Benicka et al., who reported an enantiomeric enrichment of PCB 95 (ER = 0.5–0.7) in river sediment samples [28]. A possible explanation for this difference could be the different origin of the sediment samples, especially the depth from which the

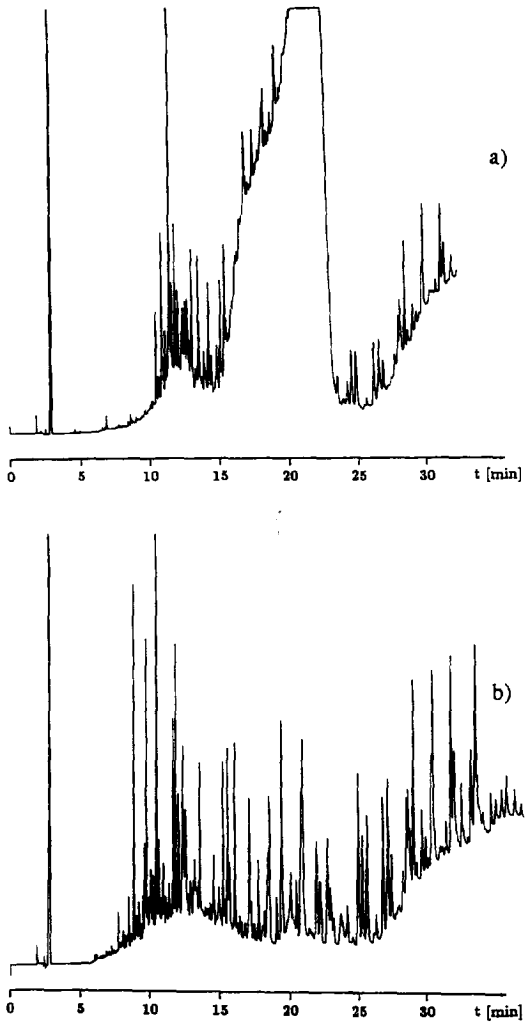

Fig. 2. GC–ECD chromatograms of the SDE extracts from sediment samples (a) before and (b) after the sulfur clean-up. See experimental section for chromatographic conditions.

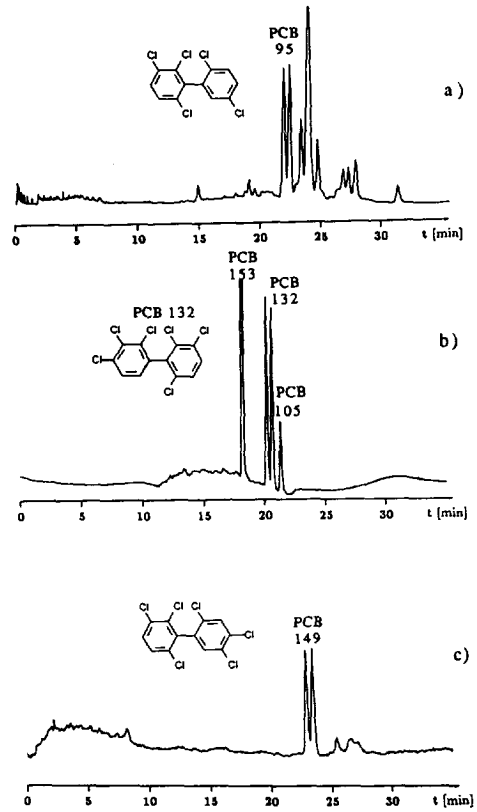

Fig. 3. MDGC–ECD chromatograms of PCB fractions from sediment samples, demonstrating the separation of the enantiomers of (a) PCB 95; (b) PCB 132; (c) PCB 149. Non-labeled peaks were not identified. See experimental section for chromatographic conditions.

samples were taken, as this is directly correlated with the time of the PCB imission [29].

Assuming that biotic processes often proceed enantioselectively the present results could indicate that very little or no biological degradation of these compounds takes place for the higher chlorinated PCB congeners, as at least four chlorine atoms are necessary to form stable chiral PCBs. The results, however, cannot rule out degradation by abiotic processes, such as chemical and photochemical transformations. Another possibility would be that the degree of degradation in nature is too low to be measured. Enantioselective degradation by a multitude of microorganisms involving opposite stereoselectivities leading to a randomized racemic mixture may also be considered [30]. The method described here may find useful applications in clarifying the above mentioned notions by further detailed degradation studies.

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