Supercritical fluid chromatography of polychlorinated biphenyls on packed columns

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ABSTRACT

The supercritical fluid chromatographic behaviour of polychlorinated biphenyls using carbon dioxide and nitrous oxide as mobile phases was studied on packed columns. Factors affecting the retention and separation of these compounds were investigated and an application to sediment samples is described.

INTRODUCTION

Mixtures of polychlorinated biphenyls (PCBs) have been produced and used commercially since 1929. PCBs have had multiple uses and from 1929 to 1972 about 500 000 tons of PCBs were manufactured [1]. In recent years, PCBs have become widespread pollutants [2–4].

High-resolution gas chromatography (GC) is the method of choice for the determination of PCBs. Therefore, only a few investigations have been made on the determination of PCBs by high-performance liquid chromatography (HPLC) [5,6] and there appear to be no reports on the determination of PCBs by supercritical fluid chromatography (SFC). The separation of polycyclic aromatic hydrocarbons (PAHs) by SFC on different kinds of packed [7,8] and capillary columns [9] is a well investigated and established method. SFC has advantages over HPLC for the analysis of PAHs when the same kind of columns are used. The advantage of SFC is that supercritical fluid possess solvating properties similar to those of a liquid, and the solute diffusion coefficients are more than two orders of magnitude greater than those found in liquids. Therefore, comparable efficiencies to HPLC can be obtained in shorter analysis times [8].

In many complex solid and liquid samples, such as river sediments, fly ashes and waste motor oils, PAHs are accompanied by PCBs and other aromatic compounds. In this work, we investigated the retention characteristics of PCBs on cyanopropyl and octadecylsilane (ODS) columns using carbon dioxide (CO_2) and nitrous oxide (N_2O) as eluents. A very simple, rapid and reproducible method for the determination of PCBs and PAHs in sediments is presented.

EXPERIMENTAL

A schematic diagram of the chromatographic system is shown in Fig. 1. Liquid CO_2 or N_2O (both from Westfalen, Münster, F.R.G.) is supplied from pressure vessels to an ISCO SFC-500 syringe pump (Colora, Lorch, F.R.G.) through a stainless-steel tube fitted with a 5-μm in-line filter (Valco Instruments, Houston, TX, U.S.A.). It is possible to draw liquid CO_2 from the pressure vessel into the SFC pump by an eductor (siphon) tube, but to obtain liquid N_2O it is necessary to place the N_2O pressure vessel upside down. Cooling the pump head to 0°C enhances the pumping efficiency. The mobile phase (N_2O or CO_2) is delivered from the pump and preheated by passage through a coil placed in the oven, a commercial gas chromatograph (Model 5890, Hewlett-Packard, Ratingen, F.R.G.), in front of the injector. The Valco CI4W injection valve with a 0.5-μl internal sample rotor is mounted on the top of the oven and connected to a microbore SFC column (20 or 25 cm × 1 mm I.D.). The column outlet is connected with a variable-wavelength UV detector (Model LCD 501, Gamma Analysen Technik, Bremerhaven, F.R.G.) via a short piece of 25-μm I.D. fused-silica tubing to minimize the dead volume. The high-pressure microbore flow cell has a path length of 2 mm and a volume of 250 nl. The wavelength of detection was 235 nm. It is possible to optimize the sensitivity by programming the wavelength for each peak, but this is not relevant to this discussion. The outlet of the UV cell is connected to a restrictor to control and maintain supercritical conditions. The restrictors are made from 10-μm I.D. fused-silica tubes and are thermostated.

The chromatographic columns are of 1 mm I.D. and vary in length from 20 cm for columns packed with Deltabond ODS (Raytest, Straubenhardt, F.R.G.) to 25 cm
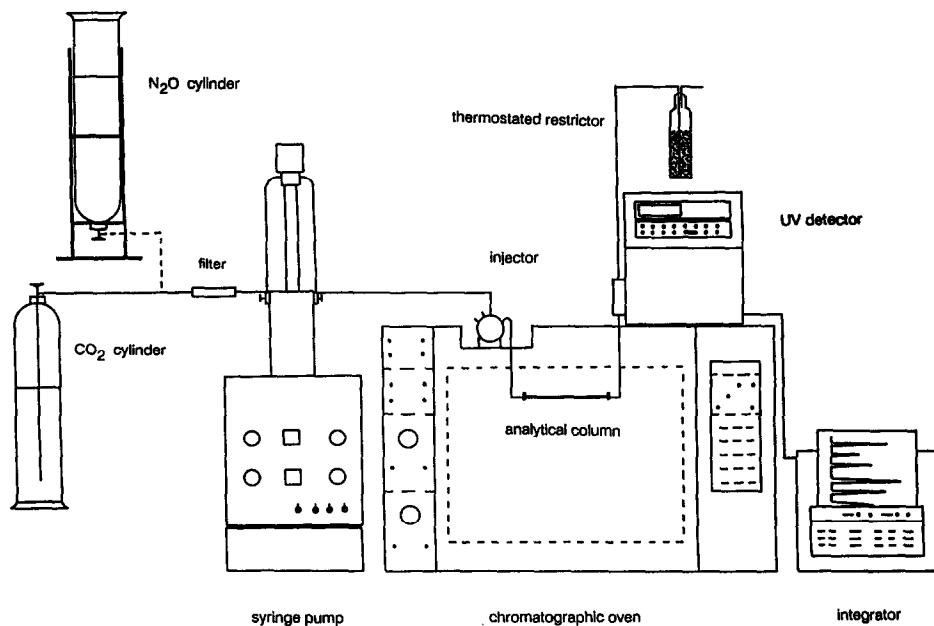

Fig. 1. Schematic diagram of the supercritical fluid chromatograph.

for columns packed with Spheri-5 cyanopropyl (Applied Biosystems, Weiterstadt, F.R.G.).

The reference compounds were obtained from Amchro (Sulzbach, F.R.G.) and Promochem (Wesel, F.R.G.) and named according to Ballschmiter and Zell [10]. The U.S. Environmental Protection Agency (EPA) PCB standard mixture (Amchro) used contained the following: (1) PCB 1, 50 μg/ml; (2) PCB 5, 50 μg/ml; (3) PCB 29, 50 μg/ml; (4) PCB 50, 100 μg/ml; (5) PCB 87, 100 μg/ml; (6) PCB 154, 100 μg/ml; (7) PCB 188, 150 μg/ml; (8) PCB 200, 150 μg/ml; (9) PCB 209, 250 μg/ml. The *ortho*-substituted PCB congeners used were PCB 1, PCB 4, PCB 16, PCB 54, PCB 104, PCB 136, PCB 188, PCB 199 and PCB 209, each at 35 μg/ml, and the non-*ortho*-substituted PCB isomers were PCB 3, PCB 15, PCB 37, PCB 77, PCB 126, PCB 169 (each at 35 μg/ml).

Samples of 5 g of the sediment were extracted with 50 ml of hexane using sonication at 100 W for 30 min. After sonication, the extract was filtered and cleaned using solid-phase extraction tubes filled with silica gel and a strong cation exchanger (both from Baker, Gross-Gerau, F.R.G.). The eluates were evaporated to 1 ml under nitrogen.

RESULTS AND DISCUSSION

Effects of column packing, mobile phase and density on retention

The density of the mobile phase determines retention and resolution in SFC and is therefore the most important parameter for optimizing a separation in SFC. Density programming during an analytical run is similiar to temperature programming in GC or programming of eluent composition in HPLC. At low density, the solubility of a substrate is low, but it increases with increasing density. In the same way the elution power increases and consequently the capacity factor, k', decreases [11].

The influence of density on the retention of decachlorobiphenyl is demonstrated in Fig. 2. The capacity factor of decachlorobiphenyl on two different stationary phases
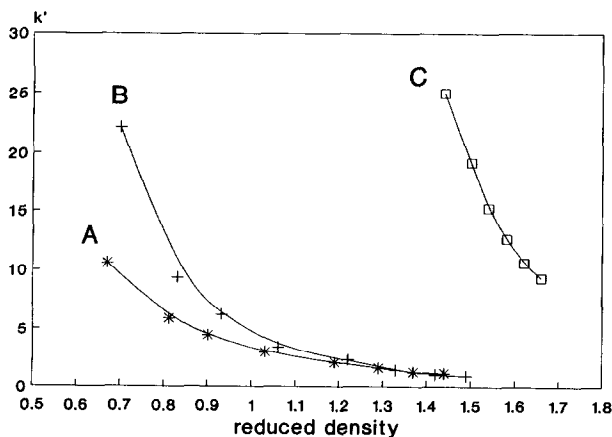

Fig. 2. Capacity factor *vs.* reduced density for decachlorobiphenyl at the same reduced temperature T_R = 1.06. (A) Cyanopropyl column, CO_2; (B) cyanopropyl column, N_2O; (C) ODS column, CO_2.

(ODS and cyanopropyl) is plotted against reduced density, d_R ($d_R = d_E/d_C$, where d_E is the experimental density and d_C the critical density), for a constant reduced temperature, T_R ($T_R = T_E/T_C$, where T_E is the experimental temperature and T_C the critical temperature) and for two different mobile phases (CO_2 and N_2O). The slope of the decrease is not the same for the different mobile and stationary phases. The value of the capacity factor is mainly determined by the interaction between the substrate and the stationary phase. There is no significant difference in elution order between CO_2 and N_2O for a given stationary phase (curves A and B), and the curves coincide at higher densities. On the ODS phase (curve C), the interaction between the stationary phase and the substrate is stronger than that on the cyanopropyl phase, so on ODS phases a higher density is needed to elute the PCBs.

The weight and size of the PCB congeners are the main parameters controlling the elution order in SFC. In addition, the retention is determined by the shape and the electron configuration of the molecule. The two phenyl rings of a biphenyl molecule can rotate freely around their common axis. The conformation with lowest potential energy is a coplanar orientation of the two rings, because the π-electron can delocalize over the two rings. A substituent in a *meta-* or *para*-position or one substituent in an *ortho*-position has no influence on the free rotation about the common axis and the molecule is still coplanar. A further chlorine substituent in an *ortho*-position restricts the coplanar orientation of the rings and according to the number of chlorine atoms in *ortho* positions the degree of out-of-plane orientation increases [1,6].

For these investigations the PCBs were divided into two groups: non-*ortho*-substituted coplanar PCB congeners and *ortho*-substituted PCB congeners. Fig. 3 illustrates the plot of the capacity factor, k', for some PCBs *vs.* the number of chlorine substituents on an ODS column. Curve A represents data for non-*ortho*-substituted PCB congeners (PCBs 3, 15, 37, 77, 126 and 169) and curve B represents data of the maximum *ortho*-substituted PCBs (PCBs 1, 4, 16, 54, 104, 136, 188, 199 and 209) within a group of chloro homologues. All the other PCB congeners within a group of chloro homologues appear between those mentioned above. The coplanar PCB congeners are retained more strongly than the non-planar congeners within a group of
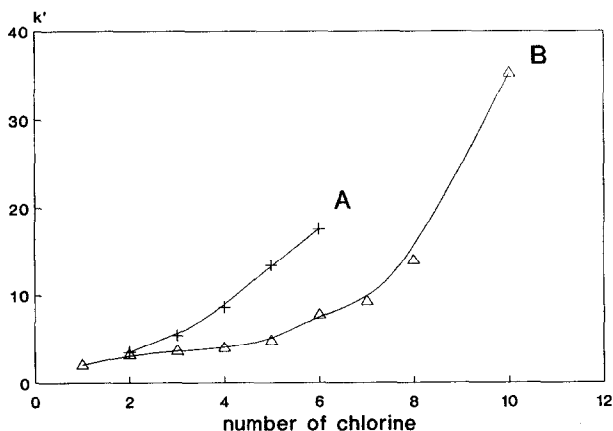

Fig. 3. Capacity factor *vs.* number of chlorine substituents on the ODS column. (A) Non-*ortho*-substituted PCBs; (B) *ortho*-substituted PCBs.

chloro homologues and there is a nearly linear relationship between k' and the number of chlorine substituents for the coplanar PCBs congener (curve A). For the *ortho*-substituted PCB congeners, there is no linear relationship between k' and the number of chlorine substituents (curve B). Both curves coincide for the dichloro homologues.

The difference in retention on an ODS phase between the *ortho*-substituted and non-*ortho*-substituted PCB isomers is caused by an increasing degree of out-of-plane orientation of the *ortho*-substituted PCB isomers and consequently by a weakening of the Van der Waals interaction between the molecule and the C_{18} chains of the stationary phase. This effect is enhanced by a chlorine in a *meta* position. A chlorine atom in a *meta* position pushes the chlorine which is in an *ortho* position towards to the C–C bonding between the two rings and consequently the degree of out-of-plane orientation increases [12].

Similar results were obtained on a cyanopropyl column, as can been seen in Fig. 4. Curve A represents data obtained for the non-*ortho*-substituted PCBs (PCBs 3, 15, 37, 77, 126 and 169) and curve B for the *ortho*-substituted PCBs (PCBs 1, 4, 16, 54, 104, 136, 188, 199 and 209). The cyanopropyl phase is a polar bonded phase and can be used for both normal- and reversed-phase chromatography. In spite of the polar parts of the stationary phase, polar interactions are negligible and the retention can be pictured as a reversible association process between the ligand anchored to the stationary phase and the solute molecule. The coplanar isomers are retained more strongly than *ortho*-substituted isomers because of stronger interactions of the coplanar isomers with the stationary phase. There is a linear relationship between k' and the number of chlorine atoms for the non-*ortho*-substituted isomers. However, for the *ortho*-substituted isomers there is no such linear relationship. The increase in k' for the *ortho*-substituted isomers on a cyanopropyl column is smaller than that on an ODS column, and decachlorobiphenyl is eluted as fast as the coplanar hexachloro isomer (PCB 169). Hence the interactions between solute and stationary phase decrease with increase in the degree of out-of-plane orientation of the isomer. These parameters compensate for the influence of molecular mass on the elution order.
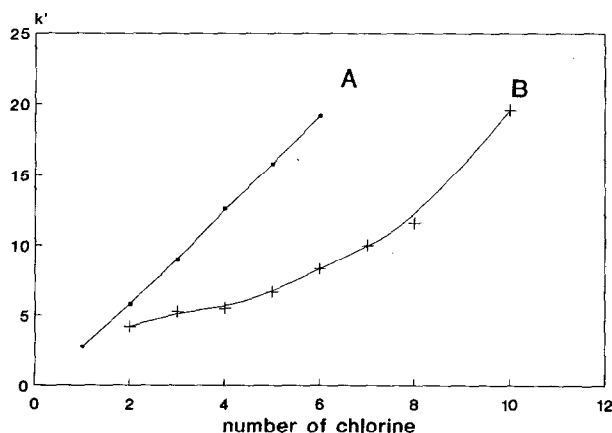

Fig. 4. Capacity factor *vs.* number of chlorine substituents on the cyanopropyl column. (A) Non-*ortho*-substituted PCBs; (B) *ortho*-substituted PCBs.
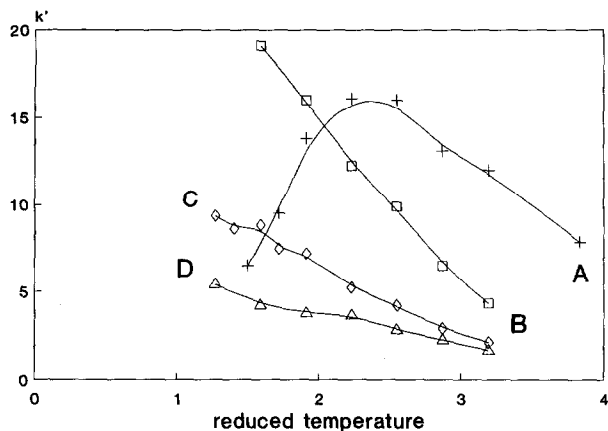
Fig. 5. Capacity factor s. reduced temperature on the cyanopropyl column at various densities: (A) 0.369; (B) 0.417; (C) 0.496; (D) 0.580 g/ml.

The results demonstrate that the size and shape of the PCB isomers control retention in SFC. This agrees well with results obtained for HPLC separations by other workers [5,6,12].

Effect of temperature on retention and resolution

The influence of temperature on the capacity factor at constant density can be explained by the Van 't Hoff equation [13,14]. The plots of ln k' vs. $1/T$ are straight lines and the enthalpies of substrate transfer from the mobile phase to the stationary phase were determined from these plots [15]. Graphs of k' vs. T_R at constant density are shown in Fig. 5 for CO_2 and N_2O as mobile phases and the substrate decachlorobiphenyl. The capacity factor decreases monotonously with increasing temperature above T_C (curves B, C and D). However, for low densities there was first an increase in
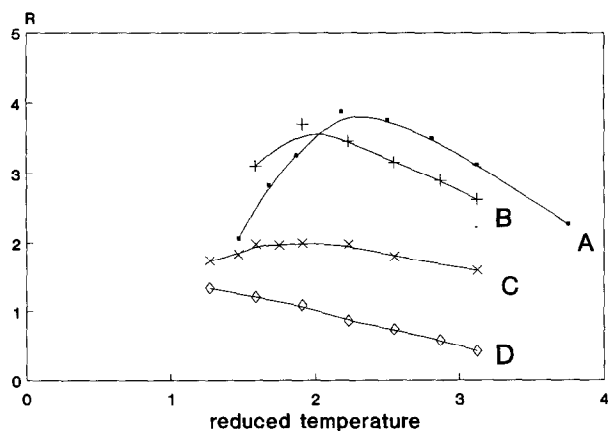

Fig. 6. Mean resolution vs. reduced temperature on the cyanopropyl and ODS column at various densities. (A)–(D) as in Fig. 5.

k', decreasing beyond a maximum value for k' (curve A). The shape of the curve is similar to those obtained for k' *vs.* T at constant pressure [15]. Moreover, for the mean resolution, we observed a maximum, illustrated in Fig. 6. The mean resolution, R_m, is

$$R_m = \sum_{1}^{n} R/n$$

where n is the number of pairs of neighbouring peaks. R_m was plotted *vs.* T_R at various densities for the PCB congeners of the EPA test mixture. The temperature of the maximum of k' is the same as that for the maximum of R_m. The tendency of R_m to form a maximum, at constant densities, has been reported [13,14], but up to now there has
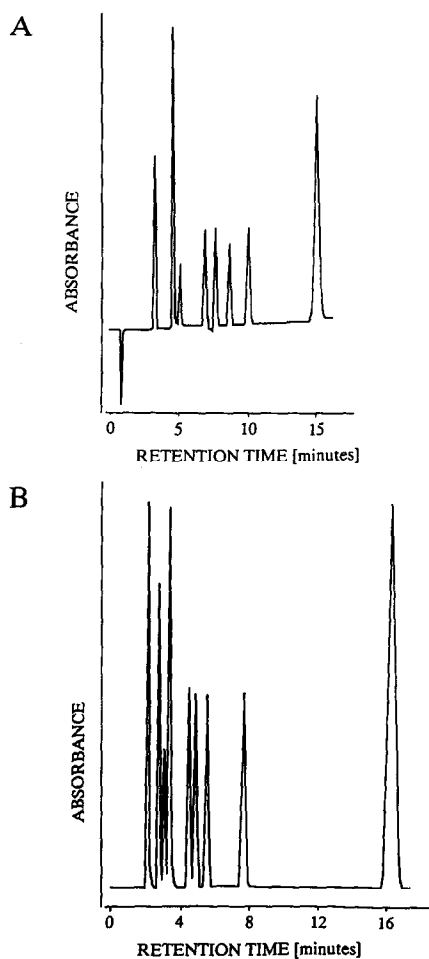

Fig. 7. Separation of EPA PCB mixture with CO_2. (A) On the cyanopropyl column at 0.366 g/ml and 80°C; (B) on the ODS column at 0.744 g/ml and 44°C. Peaks from left to right (order of elution): (A) PCBs 1, 29 + 5, 50, 154, 188, 87, 199, 209; (B) PCBs 1, 29, 5, 50, 154, 188, 87, 199, 209.
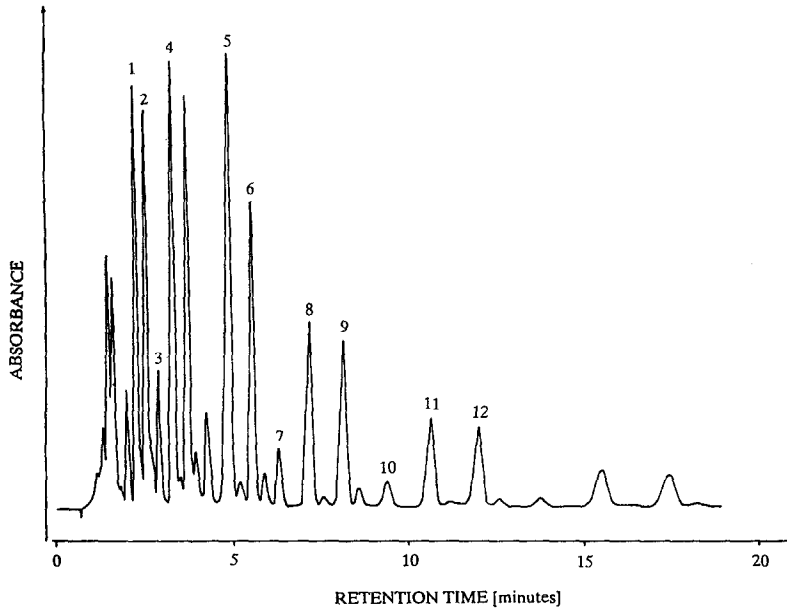
Fig. 8. Chromatogram of a sediment extract obtained on the cyanopropyl column at a CO_2 inlet pressure of 110 bar and 80°C. Peaks: 1 = acenaphtene; 2 = fluorene; 3 = PCB 1; 4 = anthracene; 5 = PCB 28; 6 = PCB 52; 7 = PCB 50; 8 = PCB 154; 9 = PCB 87; 10 = fluoranthene; 11 = PCB 153; 12 = PCB 180.

been no satisfactory explanation. We found that a maximum only appears at densities $d_E < d_C$ (curves A and B), as opposed to densities d_E near d_C, where the curve shows more of a plateau than a maximum (curve C), and for densities $d_E > d_C$ R_m decreases constantly with increasing temperature (curve D).

Fig. 7 compares the best separation of the EPA mixture obtained on an ODS column (B) with the best separation obtained on a cyanopropyl phase; in the latter instance it is not possible to separate PCBs 5 and 29. However, the selectivity of the cyanopropyl column is greater for the higher PCBs and therefore we chose a cyanopropyl column for the rapid analysis of a sediment extract.

Fig. 8 demonstrates the rapid elution and detection within 16 min of PCBs and PAHs in a sediment extract using a cyanopropyl column. The solutes range from acenaphtene eluted at 3 min to PCB 188 at 12 min.

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