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Retention characteristics of some selected halogenated environmental pollutants in silica and bonded normal-phase liquid chromatography

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Abstract

Seven bonded stationary phases and unmodified silica were investigated regarding their retention properties towards a number of halogenated environmental pollutants including 26 individual polychlorinated biphenyl congeners. No simple retention–structure relationship was found for the polychlorinated biphenyls but a number of phases demonstrated similar selectivities towards the investigated congeners. The retention of compounds with aliphatic carbons were substantially influenced by the presence of hydrogens possessing acidic properties due to inductive effects of adjacent chlorine or oxygen atoms. Solute molecule ether oxygens were shown to promote retention by hydrogen bonding only on silica and phenylpropyl silica. Polybrominated diphenyl ethers demonstrated a complex retention behaviour on the different stationary phases. Indication was found that bromine may be directly involved in the retention mechanism.

1. Introduction

Halogenated environmental pollutants include a number of compound groups who in themselves contain numerous of homologues and isomers. Most methods of determination in complex matrices include one or more liquid chromatographic fractionation steps. Clean-up of chlorinated environmental pollutants performed by chromatographic fractionation traditionally utilise open columns packed with adsorbents like silica, aluminium oxide or Florisil. In recent years the use of high-performance liquid chromatography (HPLC) for clean-up has increased. HPLC provides a much better defined system with higher resolution compared to the packed open columns. It is also possible to choose between various chemically modified bonded stationary phases with different retention properties. A number of HPLC stationary phases have been used in clean-up procedures applied for the analysis of polychlorinated biphenyls (PCBs). Silica has been used in the clean-up procedure for the analysis of PCBs in biota, sediment and atmospheric particles [1], cyanopropyl-modified silica for the determination of PCBs in fish [2], aminopropyl-modified silica for the clean-up of cod liver oil [3] and nitrophenylpropyl-modified

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silica in the determination of PCBs in transformer oil [4]. However, there is a rather limited knowledge regarding the retention behaviour of individual halogenated compounds as well as the numerous groups of halogenated compounds, especially regarding the bonded phases. Silica is the most investigated stationary phase [1,5–10]. Both degree of chlorination and substitution pattern affect the retention order of individual chlorinated biphenyl (CB) congeners. CB congeners with the highest degrees of substitution tend to have a weak retention while retention is stronger for slightly substituted aromatic compounds [6,7]. Anyhow, no general explanation has been found yet for the retention order of all congeners. A number of properties that are recognised as important in liquid chromatography of halogenated compounds are linearity, area, planarity, electron density, ionisation potential, polarisability and dipole moment [10,11]. In recent years electron-donor and electron-acceptor (EDA) phases utilised in both reversed- [12] and normal-phase [13,14] mode have gained some interest. The chemistry of EDA stationary phases has been reviewed by Nondek [15] and Sander and Wise [16].

In the present study the retention properties of a selected number of halogenated environmental pollutants on seven chemically bonded stationary phases and unmodified silica have been investigated.

2. Experimental

2.1. Chemicals

The individual CBs were 18 congeners that have been recommended for analysis by the Nordic Council of Ministers [17]: 2,4,4′-trichlorobiphenyl (CB-28), 2,4,2′,4′-tetrachlorobiphenyl (CB-47), 2,5,2′,5′-tetrachlorobiphenyl (CB-52), 3,4,3′,4′-tetrachlorobiphenyl (CB-77), 2,4,5,2′,5′-pentachlorobiphenyl (CB-101), 2,3,4,3′,4′-pentachlorobiphenyl (CB-105), 2,3,4,5,4′-pentachlorobiphenyl (CB-114), 2,4,5,3′,4′-pentachlorobiphenyl (CB-118), 3,4,5,2′,3′-pentachlorobiphenyl (CB-122), 3,4,5,3′,4′-pentachlorobiphenyl (CB-126), 2,3,4,5,2′,4′,5′-hexachlorobiphenyl (CB-138), 2,4,5,2′,4′,5′-hexachlorobiphenyl (CB-153), 2,3,4,5,3′,4′-hexachlorobiphenyl (CB-156), 2,3,4,3′,4′,5′-hexachlorobiphenyl (CB-157), 2,4,5,3′,4′,5′-hexachlorobiphenyl (CB-167), 3,4,5,3′,4′,5′-hexachlorobiphenyl (CB-169), 2,3,4,5,2′,3′,4′-heptachlorobiphenyl (CB-170) and 2,3,4,5,2′,4′,5′-heptachlorobiphenyl (CB-180) [18]. Eight additional congeners were tested for possible use as internal standards in future analytical procedures for PCBs: 3,5,3′-trichlorobiphenyl (CB-36), 2,3,3′,5′-tetrachlorobiphenyl (CB-58), 3,4,5,4′-tetrachlorobiphenyl (CB-81), 2,4,6,2′,5′-pentachlorobiphenyl (CB-103), 3,4,5,3′,5′-pentachlorobiphenyl (CB-127), 2,4,5,2′,4′,6′-hexachlorobiphenyl (CB-154), 2,3,4,5,3′,4′,5′-heptachlorobiphenyl (CB-189) and 2,3,4,5,6,2′,4′,6′-heptachlorobiphenyl (CB-204). The individual CB congeners were purchased from Ultra Scientific (North Kingstown, Richmond, USA) except CB-189 which was synthesised at the Department of Environmental Chemistry, Stockholm University [19]. A PCB mixture was made by mixing equal amounts of the technical products Aroclor 1221, 1232, 1242, 1248, 1254 and 1260 (Monsanto, MO, USA). Biphenyl was obtained from Merck (Darmstadt, Germany).

The organochlorine pesticides were US Environmental Protection Agency (EPA) reference materials consisting of 1,1-dichloro-2,2-bis(4-chlorophenyl)ethene (*p,p′*-DDE), 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (*p,p′*-DDT), 1,1-dichloro-2,2-bis(4-chlorophenyl)-ethane (p,p′-DDD), *p,p′*-methoxychlor, trans-nonachlor, aldrin, endrin, dieldrin, heptachlor, heptachlorepoxide, oxychlordane, α- and γ-chlordene, α- and γ-chlordane, α-, β-, γ- and δ-hexachlorocyclohexane, hexachlorobenzene, Toxaphene and Mirex.

Polychlorinated naphthalenes (PCNs) were investigated as a mixture of equal amounts of the technical formulations Halowax 1014, 1051 and 1099 (Koppers, Pittsburgh, PA, USA). Chlorinated paraffins (CPs) consisted of the technical formulations Hüls 40 (C_{18}–C_{26}, 40% Cl) and Hüls 70 (C_{10}–C_{13}, 70% Cl) (Hüls, Germany) mixed in the proportions 1:1. Polybrominated diphenyl ethers (PBDEs) were investigated as the technical formulations Bromkal 70-5, 79-8 and 82-0 (Chemische Fabrik Kalk, Germany). Bromkal 70-5 has been shown to consist of three major compounds: 2,4,2′,4′-tetrabromodiphenyl ether and two pentabromodiphenyl ethers [20]. Bromkal 79-8 consists of hepta-, octa-. nona- and decabrominated diphenyl ethers [21]. In Bromkal 82-0 the main component is decabromodiphenyl with a small amount of nonabrominated diphenyl ether [21]. Diphenyl ether and 2,4,2′,4′-tetrabromodiphenyl ether were synthesised at the Department of Environmental Chemistry, Stockholm University. Decabromodiphenyl ether originated from Fluka, Germany.

All standard substances were dissolved in either glass-distilled HPLC-grade hexane (Rathburn, UK) or pesticide-grade heptane (Lab-Scan, Dublin, Ireland).

2.2. Stationary phases

Seven bonded normal-phase HPLC stationary phases were characterised. Four columns were obtained from Macherey–Nagel, Germany: nitrophenylpropyl silica (Nucleosil-NO_2, 200 × 4 mm, 5 μm), dimethylaminopropyl silica (Nucleosil-$N(CH_3)_2$, 200 × 4 mm, 5 μm), diol silica (Nucleosil-$(OH)_2$, 250 × 4 mm, 7 μm) and phenylpropyl silica (Nucleosil-C_6H_5, 250 × 4 mm, 7 μm). The other columns were cyanopropyl silica (LiChrosorb-CN, 250 × 4 mm, 5 μm; Merck, Germany), aminopropyl silica (μBondapak-NH_2, 300 × 3.9 mm, 10 μm; Waters, MA, USA) and 2-(1-pyrenyl)ethyldimethylpropyl silica (Cosmosil-PYE, 150 × 4.6 mm, 5 μm; Nacalai Tesque Japan). A column with unmodified silica was also included in the study (μPorasil, 150 × 4.6 mm 10 μm; Waters).

2.3. Instrumentation

The HPLC system consisted of a pump system (Model 590, Waters), an electrically actuated switching valve for reversing the column flow, a UV detector (SPD-2AS, Shimadzu, Japan) and an injector (Model 7125, Rheodyne, CA, USA) equipped with a 200-μl loop. The detector was operated at 225 nm when analysing aromatic compounds, and at 205 nm for all other substances. HPLC-grade hexane (Rathburn) was used as mobile phase at a flow-rate of 1.0 ml/min. The separations were performed at ambient temperature. Pentane (HPLC grade, Rathburn) was used to determine the dead volume of all the HPLC columns. The injected amount varied between 50 and 500 ng for individual compounds and between 500 ng and 5 μg for the technical mixtures.

Capillary GC was used to analyse the fractions collected from the HPLC column outlet. The gas chromatograph was a Varian 3400 (Walnut Creek, CA, USA) equipped with a split/splitless injector and an electron capture detector. The column was a DB-5 (30 m × 0.25 mm, 0.25 μm, J & W Scientific, USA). Hydrogen was used as carrier gas with a column head pressure of 8 p.s.i. (1 p.s.i. = 6894.76 Pa). A make-up gas flow-rate of 30 ml/min of nitrogen was applied to the detector. Temperature settings were as follows: injection port 250°C, detector 310°C, column oven was kept at 80°C for 2 min, followed by a linear temperature increase of 10°C/min up to a final temperature of 310°C which was kept for 10 min. Chromatographic data were registered, stored and processed by an ELDS 900 chromatography data system (Chromatography Data Systems, Svartsjö, Sweden).

2.4. Methodology

The retention properties of compounds like chlorinated paraffins and hexachlorocyclohexanes (HCHs) that have no or little UV absorbance, even at 205 nm, were evaluated by collecting fractions at the HPLC system outlet and subsequently analysing them by GC–electron-capture detection. As a major interest was the isolation of PCBs for application in future analytical procedures, fractions were defined according to the elution of these compounds. A mixture consisting of technical Aroclor products (see *Chemicals* above) was injected. At the time of the last UV response, the flow was reversed. A single back-flush peak (BF) was then obtained containing all the material with stronger retention than the PCBs. Two fractions were collected. The first (F1) consisted of the material eluting between the dead volume and the time of flow reversal and the second (BF) contained the material in the back flush peak. Both fractions were analysed by GC–electron-capture detection. Estimation of the amounts of compounds found in the fractions was done by comparing the peak areas. To keep track of the variations in retention, CB-118 was injected as every second to tenth injection. The increased frequency was applied when solutes with long retention time were injected. During the experiments the relative standard deviation of the CB-118 retention time was found to be less than 2%.

3. Results and discussion

3.1. Polychlorinated biphenyls

Capacity factors (k') of individual CBs were determined for the different stationary phases and are shown in Table 1. In Fig. 1 the capacity

Table 1
Capacity factors for some individual CB congeners

CB	k'							
	PYE	Cyano	Diol	Phenyl	DMA	Amino	Nitro	Silica
Biphenyl	0.33	0.65	0.63	0.71	0.88	1.08	1.48	2.04
28	0.65	0.42	0.51	0.37	0.61	0.85	0.96	0.76
36	0.73	0.46	0.52	0.32	0.62	0.77	0.98	0.67
47	0.57	0.37	0.50	0.35	0.57	0.77	0.84	0.66
52	0.66	0.51	0.52	0.40	0.55	0.79	0.93	0.75
58	0.74	0.48	0.66	0.43	0.91	0.96	1.05	0.74
77	2.04	0.67	0.81	0.50	1.18	1.67	1.79	0.89
81	1.93	0.55	0.56	0.41	0.59	1.13	1.35	0.76
101	0.71	0.42	0.46	0.35	0.53	0.71	0.81	0.61
103	0.53	0.40	0.42	0.33	0.42	0.62	0.72	0.59
105	1.36	0.59	0.81	0.56	1.25	1.52	1.53	0.89
114	1.17	0.44	0.47	0.35	0.44	0.81	0.99	0.63
118	1.16	0.45	0.48	0.35	0.48	0.84	1.01	0.64
122	1.04	0.55	0.86	0.50	1.36	1.30	1.40	0.88
126	2.72	0.61	0.65	0.44	0.74	1.41	1.62	0.81
127	1.70	0.46	0.44	0.33	0.34	0.82	1.01	0.56
138	0.98	0.47	0.59	0.39	0.68	1.02	1.07	0.71
153	0.82	0.36	0.37	0.29	0.30	0.58	0.71	0.51
154	0.54	0.33	0.36	0.27	0.31	0.62	0.61	0.46
156	1.61	0.48	0.53	0.37	0.54	0.96	1.15	0.64
157	1.74	0.53	0.70	0.43	0.87	1.27	1.37	0.78
167	1.37	0.41	0.41	0.32	0.33	0.70	0.89	0.53
169	3.47	0.57	0.55	0.37	0.42	1.22	1.45	0.67
170	1.28	0.49	0.68	0.40	0.85	1.19	1.21	0.72
180	1.04	0.38	0.40	0.30	0.32	0.66	0.79	0.49
189	1.91	0.43	0.42	0.34	0.33	0.89	1.05	0.53
204	0.41	0.26	0.33	0.23	0.26	0.39	0.49	0.35

The k' values were obtained using hexane as mobile phase at a flow-rate of 1 ml/min and a UV detector set at 225 nm. The variation in retention time as tested by repetitive injections of CB-118 was < 2%. The stationary phases are abbreviated as follows: PYE = 2-(1-pyrenyl)-ethyldimethylpropyl silica; Cyano = cyanopropyl silica; Diol = diolpropyl silica; Phenyl = phenylpropyl silica; DMA = dimethylaminopropyl silica; Amino = aminopropyl silica; Nitro = nitrophenylpropyl silica.

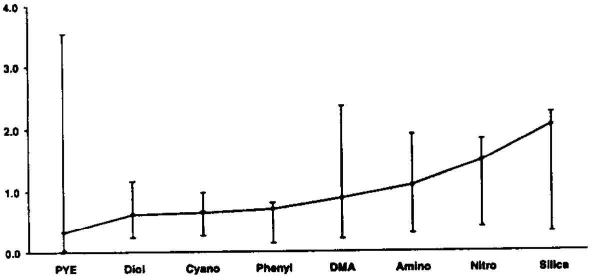

Fig. 1. Retention interval of the commercial PCB mixtures (vertical lines). The capacity factors of biphenyl are shown by diamonds linked together with a horizontal solid line. Column abbreviations as in Table 1. Y-axis represents k'.

factor of biphenyl (linked together with a horizontal line) and the retention interval of the mixture of technical PCB formulations (vertical lines) are plotted for each stationary phase. In general retention was weak. Only the 2-(1-pyrenyl)-ethyldimethylpropyl, nitrophenylpropyl, aminopropyl and dimethylaminopropyl silica phases exhibited $k' > 1$ for any CB congener. The influence on retention of the chloro substitution of biphenyl showed a large variation between the phases. On unmodified silica the introduction of chlorine substituents into biphenyl decreased retention with a factor as large as 5.8 and all investigated CBs had a shorter retention than biphenyl. Similar retention properties were demonstrated by the phenylpropyl silica. Retention was weaker on this column compared to the unmodified silica but the selectivity towards the investigated CBs were similar on both columns with a coefficient of correlation >0.96 for the linear regression. There were no similarities to the retention behaviour of the 2-(1-pyrenyl)-ethyldimethylpropyl silica demonstrating that there is no significant charge transfer interaction between the single aromatic nucleus on the phenylpropyl group of the phase and the CBs. Thus, the phenylpropyl silica behaved like deactivated unmodified silica towards the investigated CBs. The diolpropyl-modified silica demonstrated a similar selectivity towards CBs as the phenylpropyl silica with a linear regression coefficient >0.95, but the retention of biphenyl was within the retention interval of the CBs. However, the correlation between diolpropyl silica and unmodified silica was only 0.92.

A totally opposite selectivity towards CBs was demonstrated by the 2-(1-pyrenyl)ethyldimethylpropyl silica phase (PYE). When chlorine was incorporated into the biphenyl solute molecule, retention increased up to 10.5 times. The effect was most pronounced for the coplanar, non-*ortho* CB-77, CB-126 and CB-169. Retention on the PYE stationary phase is highly influenced by solute planarity and this phase has been shown to be excellent for isolating coplanar non-*ortho* CBs and mono-*ortho* CBs from the bulk of PCBs in technical mixtures [13]. Retention is mainly attributed to a charge transfer interaction mechanism were the delocalised aromatic π-electrons of the pyrenyl group of the stationary phase act as electron donors, and the aromatic nuclei of the CB solute molecules having decreased π-electron densities are acting as electron acceptors [13].

Retention of unsubstituted aromatic hydrocarbons on aminopropyl silica is due to a mechanism involving charge-transfer interaction between the n-electrons of the amino nitrogen and the π-electrons of the solute [22]. The same retention mechanism is assumed for the dimethylaminopropyl silica. These two phases demonstrated different selectivities towards the investigated CB congeners. Retention was in general weaker on the latter phase and the coefficient of correlation for a linear regression was <0.85. This difference in retention properties of these two stationary phases is attributed to steric hindrance of the solute–stationary phase interaction excerted by the methyl groups of the dimethylaminopropyl silica.

The CBs exhibited similar retention orders on the nitrophenylpropyl, aminopropyl and cyanopropyl silica phases. Capacity factors for the investigated CBs obtained from the aminopropyl and cyanopropyl phases were plotted *versus* capacity factors from the nitrophenylpropyl phase. Linear relationships were obtained with regression correlation coefficients better than 0.98 and 0.97 respectively. This implies that a similar retention mechanism may be involved regarding these three stationary phases.

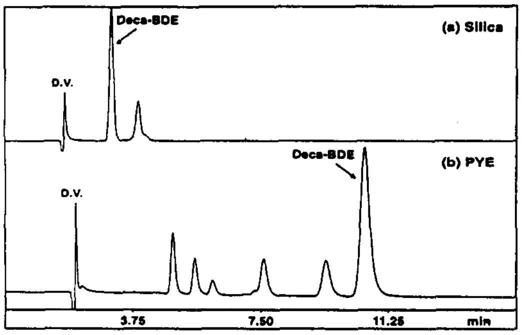

Fig. 2. HPLC chromatogram of the technical PBDE mixture Bromkal 79-8 on a silica (a) and a PYE column (b). D.V. = Dead volume; Deca-BDE = decabromodiphenyl ether.

3.2. Hexachlorobenzene and polychlorinated naphthalenes

Hexachlorobenzene and the chlorinated naphthalenes exhibit short retention times on all investigated stationary phases except PYE. The stronger retention on this phase is attributed to the importance of solute planarity for the solute–stationary phase interaction [13].

3.3. Polybrominated diphenyl ethers

The brominated diphenyl ether (BDE) congeners demonstrate a complex retention behaviour. On both unsubstituted silica and phenylpropyl-modified silica the retention of BDEs decreased by the introduction of bromine in the diphenyl ether molecule (Fig. 2a). A study of retention of BDEs in the system silica gel/*n*-hexane has earlier been performed by De Kok *et al.* [21]. They concluded that "retention tends to decrease with an increasing number of substituents, but this rule is not strictly adhered to". The decrease in retention can be explained in terms of an inductive effect of the bromine substituents decreasing the electron density on the ether oxygen and in the aromatic π-electron nucleus in the solutes. Retention could be reduced by steric hindrance of the solute–stationary phase interaction by the bulky bromine substituents as well. In this study a decrease in retention with an increased number of bromine substituents was also observed on the dimethylaminopropyl silica phase. This effect is attributed to the methyl groups of the stationary phase shielding the interaction between the amino nitrogen and the solute molecule. For all the other bonded stationary phases a reversed behaviour was observed. Retention increased with increasing bromine content (Table 2). The effect was especially pronounced on the nitropropyl-, aminopropyl- and PYE columns. This retention behaviour has previously been found regarding polybrominated biphenyls (PBBs) on aluminium oxide [7]. In contrast to the other bonded phases phenylpropyl silica, as the unsubstituted silica, demonstrated a retention of the unsubstituted

Table 2
Capacity factors for diphenyl ether, and intervals of capacity factors of peaks in technical PBDE mixtures

Compound	k'							
	PYE	Cyano	Diol	Phenyl	DMA	Amino	Nitro	Silica
Diphenyl ether	0.35	0.70	0.67	1.22	0.90	1.05	1.65	4.22
2,4,2′,4′-Tetra-BDE	1.17	0.80	0.92	0.67	0.99	1.93	2.28	1.72
Deca-BDE	5.02	0.89	1.24	0.77	0.70	5.25	6.31	0.95
Bromkal 70-5	1.18–1.95	0.74–1.04	0.78–0.91	0.59–0.86	0.65–1.00	1.74–1.93	2.10–2.27	1.29–1.67
Bromkal 79-8	1.83–5.05	0.70–1.16	0.97–1.24	0.64–0.78	0.70–1.09	2.60–5.46	3.17–6.33	0.94–1.39
Bromkal 82-0	2.46–5.01	0.78–1.16	1.10–1.24	0.70–0.78	0.70–0.89	3.68–5.15	4.42–6.31	0.94–1.39

The average degree of bromination is increased in the order 70-5, 79-8 and 82-0 (see Experimental). The k' values were obtained using hexane as mobile phase at a flow-rate of 1 ml/min and a UV detector set at 225 nm. The variation in retention time as tested by repetitive injections of CB-118 was <2%. Column abbreviations as in Table 1.

diphenyl ether that was stronger than for the bromosubstituted derivatives. In the case of the nitrophenyl phase all the PBDEs in the technical formulations detected by UV where shown to elute after the PCB fraction. This implies that the two groups can be completely separated. The PYE column was shown to provide the best selectivity regarding separation of individual BDE congeners (Fig. 2b). Compared to chlorination of biphenyl the effect of bromination of diphenyl ether had a much stronger influence on retention. Bromine have stronger steric effects on the solute–stationary phase interaction due to its size, when compared to chlorine. It also exerts a smaller inductive effect on an aromatic ring system due to its lower electronegativity. Both are properties that decrease interaction with the stationary phase. Since retention increases with increasing bromine substitution, this leads to the conclusion that the increased retention is due to interaction between the stationary phase and the bromine atoms in the solute molecules. This could be explained by the high polarisability of bromine.

3.4. *Chlorinated environmental pollutants containing aliphatic carbons*

Compared to chlorinated aromatic compounds, a totally different effect on retention is observed when chlorine is introduced into an aliphatic compound. If a chlorine atom is bonded to a carbon with a hydrogen attached to it, there will be a polarisation of the bond and the hydrogen will obtain an acidic character. The inductive effect of the chlorine is additive, so when adding more chlorine substituents to a carbon atom the bond polarisation increases. The effect may extend over more than one carbon atom and reach hydrogens attached to other adjacent carbon atoms. The influence on retention of hydrogens with acidic character have been observed earlier regarding chloro-added polycyclic aromatic hydrocarbons [23]. The present study shows that this kind of acidic hydrogens have a strong influence on the chromatographic retention of environmental pollutants containing aliphatic carbons in their structure (Table 3). The low retention of aldrin and Mirex, two compounds without acidic hydrogens in their structures, enhance the fact that it is not the chlorine atom by itself that causes the retention when bonded to an sp^2-hybridised carbon. The acidic hydrogen effect on retention is demonstrated for a number of compounds, *i.e.* chlordanes, hexachlorocyclohexanes and chlorinated paraffins all exhibited strong retention.

All HCH isomers have six hydrogen atoms with acidic character. By fractionation and subsequent GC analysis of the HPLC effluent it was shown that the four tested HCH isomers eluted after the PCB fraction on all columns except the PYE. The strong retention of HCH is explained by means of the polarised carbon–hydrogen bonds yielding acidic hydrogens being involved in the main retention mechanism. This shall be compared to cyclohexane that eluted with the dead volume and hexachlorobenzene which exhibited k' values ≤ 0.40 on all the stationary phases except PYE. On the PYE column α- and γ-HCH eluted in the PCB fraction, while β- and δ-HCH eluted after. Fractionation of CPs confirmed a strong retention caused by solute acidic hydrogens on all columns except the PYE on which the CPs eluted entirely within the PCB fraction. Acidic hydrogens obviously have only a small influence on retention on a PYE column compared to the other stationary phases.

For the studied chlordane compounds, α- and γ-chlordane and α- and γ-chlordene, the capacity factors increase with an increased number of acidic hydrogens (Table 3). This additivity is well demonstrated by the shift in capacity factor for p,p'-DDT and the related compounds p,p'-DDE and p,p'-DDD (Table 3). On all the investigated stationary phases the elution order was the same: p,p'-DDE, p,p'-DDT and p,p'-DDD. p,p'-DDE has no hydrogen bonded to an aliphatic carbon in the vicinity of any of the chlorine substituents. Hence, the major retention mechanism for p,p'-DDE involves interaction with the aromatic nuclei. For p,p'-DDT, long-range inductive effects of the three chlorine atoms in the 1 position affect the hydrogen in the 2 position of the ethane group. p,p'-DDD finally has one hydrogen atom affected by two adjacent

Table 3
Capacity factors of some organochlorine compounds

Compound	k'							
	PYE	Cyano	Diol	Phenyl	DMA	Amino	Nitro	Silica
HCB	2.21	0.20	0.20	0.25	0.14	0.25	0.40	0.23
PCNs	0.48–21.3	0.22–0.56	0.14–0.66	0.10–0.70	0.13–1.00	0.24–0.98	0.40–1.34	0.05–1.10
PCBs	0.03–3.76	0.25–0.96	0.23–1.16	0.13–0.80	0.20–2.33	0.28–1.87	0.41–1.83	0.31–2.22
p,p'-DDE	0.63	0.56	0.53	0.40	0.55	1.02	1.07	0.91
p,p'-DDT	1.03	1.04	1.09	0.65	1.75	3.16	2.63	1.75
p,p'-DDD	2.57	2.22	2.22	1.26	4.47	9.42	6.80	4.23
α-Chlordene	0.64	0.58	0.59	0.48	0.60	1.00	1.05	1.19
γ-Chlordene	0.68	0.72	0.76	0.56	0.94	1.39	1.38	1.36
α-Chlordane	0.72	1.18	1.28	0.83	2.19	3.91	2.83	2.62
γ-Chlordane	1.55	1.28	1.54	0.86	2.97	5.63	3.56	2.77
Heptachlor	0.44	0.47	0.51	0.39	0.54	0.85	0.87	0.97
Transnonachlor	0.73	1.04	0.98	0.56	1.35	4.64	2.62	1.74
Aldrin	0.36	0.35	0.40	0.29	0.41	0.35	0.64	0.63
Endrin	1.07	2.55	3.26	5.94	4.87	5.81	11.3	41.4
Dieldrin	0.69	2.93	3.88	8.89	7.88	7.78	13.2	53.4
Oxychlordane	0.55	0.83	0.86	0.76	0.99	2.34	1.96	3.16
Heptachloroepoxide	0.62	2.13	2.20	3.31	3.57	5.75	7.88	17.5
p,p-Methoxychlor	1.27	7.43	10.1	>50	34.4	22.9	48.9	>50

The k' values were obtained using hexane as mobile phase at a flow-rate of 1 ml/min and a UV detector set at 225 nm for aromatic compounds and 205 nm for non-aromatic compounds. PCBs and PCNs are commercial mixtures with various degrees of chlorination. The variation in retention time as tested by repetitive injections of CB-118 was $<2\%$. Column abbreviations as in Table 1.

chlorine atoms and one affected by long-range effects. For p,p'-DDT and p,p'-DDD the main retention mechanism involves interaction with the acidic hydrogens or the polarised carbon–hydrogen bond.

The components of Toxaphene, a technical product of polychlorinated camphenes, are spread out in a wide retention interval on the different stationary phases. The camphene structure makes it possible for different chlorinated derivatives to have none or many hydrogens with acidic character.

3.5. Halogenated environmental pollutants containing epoxy or ether groups

Oxygen is more electronegative than chlorine and its presence in the molecular structure induce a similar effect on retention as chlorine. The major influence on retention on the chemically bonded phases involve solute acidic hydrogens interacting with the stationary phase. As shown by the comparison of the retention of biphenyl and diphenyl ether (Tables 1 and 2), hydrogen bonding interaction involving solute ether oxygens does not substantially contribute to retention on any of the bonded phases except phenylpropyl silica. Even the aminopropyl silica did not show any increased retention due to hydrogen bonding with solute ether oxygen. However, on silica a strong retention caused by hydrogen bonding to this kind of oxygen atom was demonstrated.

On all columns except PYE, the highest capacity factor of all the tested compounds was obtained by p,p-methoxychlor (Table 3). This molecule is similar in structure to p,p'-DDT but has the p-chlorine atoms on the phenyl groups substituted by methoxy groups. The oxygen atom in the substituent group is supposed to have a similar influence on the six adjacent hydrogens as is demonstrated by chlorine.

For the compound groups aldrin/endrin/dieldrin and heptachlor/heptachlorepoxide a large increase in capacity factor can be attributed to epoxy groups yielding hydrogens with acidic character (Table 3). Heptachlorepoxide has two acidic hydrogens adjacent to the epoxy group. Oxychlordane is obtained by substituting one of these acidic hydrogens with a chlorine atom. On the bonded phases this yielded a decrease in retention due to the removal of one of the acidic hydrogens. However, on silica oxychlordane exhibit an extremely short retention compared to other compounds containing an epoxy or ether group (Table 3). This can be explained by a decreased strength of hydrogen bonding to this solute oxygen that might be caused by the adjacent chlorine atom counteracting interaction with the silica.

4. Conclusions

When analysing retention data from the different columns it becomes evident that there is no simple structure–retention relationship to be found for the CBs. The nitropropyl, aminopropyl and cyanopropyl phases exhibit similar selectivity towards the investigated CBs, thus implying a similar mechanism of retention. Carbon–hydrogen bond polarisation by adjacent chlorine or oxygen atoms yield hydrogen substituents with an acidic character. These were shown to have a major influence on normal-phase liquid chromatography retention of environmental pollutants having aliphatic carbons in their structure. In contrast to chlorine the retention behaviour of bromine-containing aromatic compounds indicated that bromine itself may be directly involved in the retention mechanism.

For the purpose of clean-up and group isolation of PCBs it was clearly demonstrated that none of the investigated columns were able to provide a complete separation of PCBs from all of the other investigated groups of halogenated environmental pollutants. The best isolation of a PCB fraction would be obtained by using the silica, nitropropyl silica or aminopropyl silica stationary phases. It was also shown that the PYE was the only investigated stationary phase that selectively retained the co-planar toxic CBs from the other CB congeners.

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References

[1] G. Petrick, D.E. Schulz and J.C. Duinker, *J. Chromatogr.*, 435 (1988) 241.

[2] H. Hyvönen, T. Auvinen, M.-L. Riekkola and K. Himberg, *J. Microcol. Sep.*, 4 (1992) 123.

[3] M. Schantz, R. Parris, S. Wise, H. Won and R. Turle, *Chemosphere*, 24 (1992) 1687.

[4] V. Nero and R. Hudson, *Anal. Chem.*, 56 (1984) 1041.

[5] U.A.Th. Brinkman, J.W.F.L. Seetz and H.G.M. Reymer, *J. Chromatogr.*, 116 (1976) 353.

[6] U.A.Th. Brinkman, A. de Kok, G. de Vries and H.G.M. Reymer, *J. Chromatogr.*, 128 (1976) 101.

[7] U.A.Th. Brinkman and G. de Vries, *J. Chromatogr.*, 169 (1979) 167.

[8] A. Gillespie and S. Walters, *J. Liq. Chromatogr.*, 9 (1986) 2111.

[9] E. Storr-Hansen, M. Cleeman, T. Cederberg and B. Jansson, *Chemosphere*, 24 (1992) 323.

[10] R.F. Rekker, G. de Vries and G.J. Bijloo, *J. Liq. Chromatogr.*, 12 (1989) 695.

[11] P.G. Seybold and J. Bertrand, *Anal. Chem.*, 65 (1993) 1631.

[12] K. Kimata, K. Hosya, T. Arakai, N. Tanaka, E.R. Barnhart, L.R. Alexander, S. Sirimanne, P.C. McClure, J. Grainger and D.G. Patterson, Jr., *Anal. Chem.*, 65 (1993) 2502.

[13] P. Haglund, L. Asplund, U. Järnberg and B. Jansson, *J. Chromatogr.*, 507 (1990) 389.

[14] L.C. Sander, R.M. Parris and S.A. Wise, *Anal. Chem.*, 63 (1991) 2589.

[15] L. Nondek, *J. Chromatogr.*, 373 (1986) 61.
[16] L.C. Sander and S.A. Wise, *CRC Crit. Rev. Anal. Chem.*, 18 (1987) 299.
[17] U. Ahlborg, A. Hanberg and K. Kenne, *Risk Assessment of Polychlorinated Biphenyls, Nord 1992:26*, Nordic Council of Ministers, Stockholm, 1992, p. 84.
[18] R. Fischer and K. Ballschmitter, *Fresenius' Z. Anal. Chem.*, 335 (1989) 20.
[19] G. Sundström, *Acta Chem. Scand.*, 27 (1973) 600.
[20] G. Sundström and O. Hutzinger, *Chemosphere*, 3 (1976) 187.
[21] J.J. de Kok, A. de Kok and U.A.Th. Brinkman, *J. Chromatogr.*, 171 (1979) 269.
[22] D. Karlesky, D.C. Shelley and I.M. Warner, *J. Liq. Chromatogr.*, 6 (1983) 471.
[23] U. Nilsson and A. Colmsjö, *Chromatographia*, 32 (1991) 334.