Short communication

Retention behaviour of polychlorinated biphenyls on polysiloxane-anchored C_{60} in gas chromatography

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Abstract

The synthesis of a fullerene-containing stationary phase by chemically linking C_{60} to an aminopropylpolysiloxane is described. The stationary phase was applied in gas chromatography to the separation of polychlorinated biphenyl (PCB) isomers with different degrees of substitution in the ortho-position (non-ortho to tetra-ortho substituted). The retention behaviour of several hexachlorobiphenyls was studied systematically by determining the retention increment, R'. An increasing R' with decreasing chlorine substituents in the ortho-position of the molecule was observed, i.e. this stationary phase shows increased affinity with increased planarity of the PCB molecule. © 1998 Elsevier Science B.V. All rights reserved.

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1. Introduction

C_{60} or buckminsterfullerene is the smallest stable fullerene molecule and consists of twelve isolated pentagons and twenty hexagons [1,2]. The properties of fullerene C_{60}, such as thermal and oxidation stability, are favorable prerequisites for its use as a stationary phase in gas chromatography (GC). The acceptor properties of C_{60} may provide interesting selectivities in chromatography.

Jinno et al. [3] and Saito et al. [4] evaluated the use of essentially pure C_{60} as a stationary phase and the use of a C_{60} bonded silica phase in liquid chromatography for the separation of polycyclic aromatic hydrocarbons (PAHs). Stalling et al. [5,6] described the application of fullerenes, which are covalently linked to the surface of support materials, in high-performance liquid chromatography (HPLC) for the separation of aromatic compounds, e.g. PAHs and polychlorinated biphenyls (PCBs). Recently, Bianco et al. [7] described molecular recognition studies using a silica-bound fullerene derivative in HPLC.

In GC, the use of glass capillary columns, which were coated with pure C_{60} using the high-pressure static method, has previously been reported [8]. These columns have been applied to the separation

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of *n*-alkanes, PAHs (with and without functional groups) and methyl esters of fatty acids.

In the present work, we describe a fullerene stationary phase that was prepared by the chemical linking of C_{60} via an amide bond to polysiloxane. The properties and the suitability of this stationary phase in GC have been studied with aromatic compounds, viz. PCBs.

2. Experimental

2.1. Materials

PCB standards were obtained as isooctane solutions (10 ng/μl) from Dr. Ehrenstorfer (Augsburg, Germany). 1-Bromoeicosane was obtained from Aldrich (Steinheim, Germany).

2.2. Synthesis of the fullerene stationary phase

Synthesis of 3 (Fig. 1): To a solution containing 31.5 mg (31.5 μmol) of 1,2-cyclohexylamino-carbonyl-(*N*)-succinimidyloxycarbonyl - 1,2 - dihydro-[60]fullerene[1] (2) [9] in 200 ml toluene, 49.5 mg (40 μmol) of the polysiloxane (1) were added. After stirring the reaction mixture for two days, the solvent was removed and the residue was washed with water and methanol.

2.2.1. Spectroscopic data of 3

UV–Vis (CH_2Cl_2): λ_{max}=261, 325, 361 sh, 428, 481 br.
^{1}H-NMR (250 MHz, C_2HCl_3, 25°C): δ=2.05 (m; CH), 1.87 (m; CH_2), 1.83 (m; CH_2), 1.70 (m; CH_2), 1.34 (m; CH_2), 0.83 (m; CH_2), 0.57 (m; CH_2), 0.05 (s; CH_3).
^{13}C-NMR (62.9 MHz, $CHCl_3$, 25°C): δ=152.68, 145.25, 145.22, 145.08, 144.90, 144.75, 144.66, 144.60, 143.84, 143.09, 143.05, 143.02, 142.93, 142.20, 141.11, 141.03, 138.67, 137.03, 68.18, 57.19, 43.73, 32.50, 30.34, 29.68, 29.43, 26.45, 25.58, 25.34, 24.61, 23.25, 14.72, 1.76, 1.59, 1.00, 0.41.

2.3. Column preparation

Fused-silica capillary columns (10 m×0.25 mm I.D.) were heated at 250°C for 2 h at a slow flow-rate of hydrogen and were subsequently coated without further deactivation with a 0.4% solution of the stationary phase in diethyl ether by the static method [10], yielding a film thickness of 0.25 μm.

The columns were conditioned at 190°C overnight.

2.4. Gas chromatography

GC was performed with a Dani 3900 chromatograph (Monza, Italy) equipped with a [63]Ni electron-capture detector (280°C; make-up gas, nitrogen) and
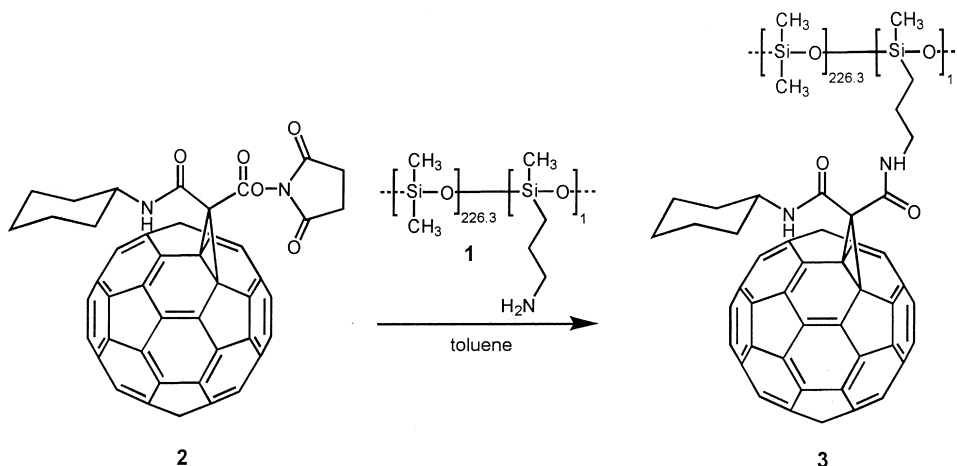

Fig. 1. Synthesis of compound **3**.

a split injector (250°C). Helium (99.996%) was used as the carrier gas. Retention times were determined using a Shimadzu C-R6A integrator.

Volumes (0.1 μl) of the solutions mentioned in Section 2.1 were injected at a split ratio of 1:50. The chromatographic separation was carried out at 190°C.

2.5. Determination of net retention times and retention increments, R'

Retention increments were determined as described for complexation GC [11]; all measurements were performed in duplicate.

The first eluted peak resulting from the solvent (or its components) was considered to represent the dead time, t_M, at a column temperature of 190°C and was used to calculate the net retention times, t'.

The relative net retention, r_0, of a solute with respect to a reference standard was calculated from the ratio, t'_0 (selectand)/t'_0* (standard) obtained on the reference column. The relative net retention, r, of a solute with respect to a reference standard was calculated from the ratio t' (selectand)/t'* (standard) obtained on the reactor column. The retention increment, R', was calculated according to $R' = (r - r_0)/r_0$.

3. Results and discussion

The C_{60}-containing stationary phase was synthesized via the direct coupling of the polyaminopropyl(methyl)siloxane precursor, 1, with the reactive ester, 2 (Fig. 1) [9]. A quantitative conversion (TLC) of 2 into the fullerene-containing stationary phase, 3, was achieved after stirring for two days in toluene at room temperature.

The fullerene stationary phase described above was coated onto fused-silica capillary columns using the static method [10]. These columns were used in GC mode to separate PCB isomers with different degrees of substitution in the ortho-position (mono-ortho- to tetra-ortho-substituted hexachlorobiphenyls: PCB 132, PCB 136, PCB 138, PCB 149, PCB 155, PCB 156, PCB 158 and PCB 159).

Within the group of isomers, a strong increase of the net retention time, t', was observed in going from tetra-ortho- to mono-ortho-substituted PCBs. To describe this retention behaviour systematically in thermodynamic terms, the retention increments, R', were determined.

The concept of the retention increment, R', was developed for GC stationary phases, which consist of selector molecules diluted in a polysiloxane, to study the thermodynamics of molecular association between the selector and selectand. This concept was applied to stationary phases containing diluted transition metal β-diketonates [12,13] or cyclodextrin derivatives [14].

R' is experimentally accessible from the relative retention data, r (column with selector bonded to solvent, reactor column) and r_0 (column with pure solvent, reference column) of the selectand with respect to an inert reference compound [14] (see Section 2). R' represents the fraction of associated to non-associated selectand in the stationary phase. Thus, R' is a quantitative measure of the strength of interaction between the selector (in this instance, fullerene C_{60}) and the selectand (in this instance, PCB).

In this work, the reactor column contained polysiloxane-bonded C_{60}. For the reference column, pure aminopropyldimethylsiloxane was used, but with acetylated amino groups. Thus, the reactor and reference columns were coated with comparable stationary phases, and only the C_{60} moieties were replaced by ethyl groups.

1-Bromoeicosane was used as the reference compound, because it (i) is electron-capture detection sensitive, (ii) shows an appropriate retention time at the applied temperature, (iii) should exhibit no interaction with the fullerene molecules.

Fig. 2 shows the elution order of the selected hexachlorobiphenyls. The chromatographic data, including the retention increments, R', are listed in Table 1. As already indicated by the net retention times, the data reveal an increasing R' value with decreasing number of chlorine atoms in the ortho-position of the biphenyl molecule. PCBs with low numbers of chlorine atoms in the ortho-position can more readily assume a planar conformation. Thus, the fullerene stationary phase shows increased affinity with increasing planarity of the PCB molecule.

An increase in the retention of PCB congeners with low degrees of substitution in the ortho-position has also been observed using GC on stationary

mono-*ortho* PCB 159

di-*ortho* PCB 158

tri-*ortho* PCB 132

tetra-*ortho* PCB 155

0   10   20   30   min

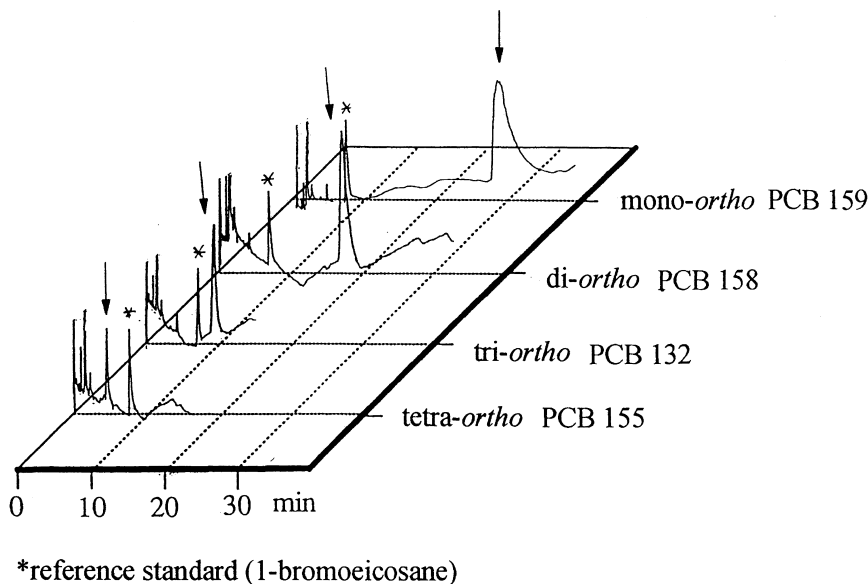*reference standard (1-bromoeicosane)

Fig. 2. Elution order of mono-ortho- to tetra-ortho-substituted hexachlorobiphenyls. Fused-silica capillary column (10 m×0.25 mm I.D.) coated with aminopropyl–polysiloxane-bonded C_{60}; film thickness, 0.25 μm; 190°C, 0.6 bar (gauge) helium, electron-capture detection. *=reference standard (1-bromoeicosane).

phases with polar functional groups, e.g. cyanopropyl groups, phenyl groups or carborane groups [15]. The strong retention of planar PCB congeners could be caused by charge-transfer interactions, as on the fullerene stationary phase, or by dipole interactions [15].

The retention increments, R', were calculated by using the net retention time at the peak maximum. Note that these data are affected by a certain error because of the asymmetric peak shape of the PCBs with low numbers of chlorine atoms in the ortho-position. This could be caused by strong charge transfer interactions. Peak broadening is expected if the rate of equilibration is low [16]. Another reason could be the limited capacity of the fullerene stationary phase. However, this error is assumed to be small and the trend of growing retention increments, R', is unambiguous.

The retention increments, R', have been used for the calculation of $-\Delta_{PCBi/PCB136}(\Delta G)$ according to: $-\Delta_{PCB2/PCB136}(\Delta G)=RT$ ln (R'_{PCBi}/R'_{PCB136}) [13]. $-\Delta_{PCBi/PCB136}(\Delta G)$ represents the difference in the free enthalpy (Gibbs energy) of the association equilibria between the fullerene molecule and the PCB pair PCBi/PCB136 (the first eluted hexachlorobiphenyl). As can be seen in Table 1, the highest free

enthalpy differences occur between the groups with different degrees of substitution in the ortho-position.
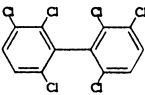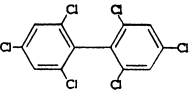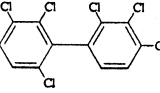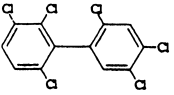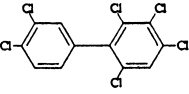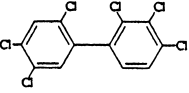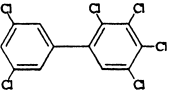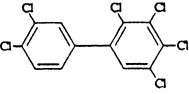
The observed increased interaction of C_{60} with PCB congeners, which was dependent on the planarity of the biphenyl molecule, is in agreement with reports on other carbon-based chromatographic materials in liquid chromatography, e.g. activated carbon [17,18], porous carbon (PGC) [19], or fullerenes [5,6], where non-planar PCB congeners are eluted before planar congeners.

PCB congeners that can assume a planar conformation (no chlorine in the ortho–ortho'-position or mono-ortho-substituted biphenyls), so-called coplanar PCBs, show the same type of toxicity as polychlorinated dibenzo-*p*-dioxins (PCDDs) ([20] and literature cited therein). Therefore, analytical methods for the exact determination of these congeners are very important. Analysis of the coplanar PCBs is difficult because of their relatively low concentration compared to the other congeners of technical mixtures and because of the high number of possible coelutants. Thus, it is mostly necessary to fractionate and enrich the coplanar PCBs from other congeners by liquid chromatography prior to the GC analysis ([21], and literature cited therein).

The polysiloxane-bonded fullerene stationary

Table 1
Chromatographic and thermodynamic data describing the retention behaviour of the investigated PCBs on the fullerene stationary phase

	PCB number [22]	Chemical structure	t' (min)	t'_0 (min)	t'^* (min)	$t'_0{}^*$ (min)	r_{mean}	$r_{0\ mean}$	R'	$-\Delta_{PCBi/PCB136}(\Delta G)$ (kJ/mol)
Tetra-ortho	136		4.367	1.978	6.902	4.804	0.641	0.412	0.56	
			4.373	1.953	6.730	4.736				
	155		3.968	1.491	6.568	4.814	0.599	0.310	0.93	1.88
			4.143	1.543	6.968	4.989				
Tri-ortho	132		8.520	2.993	6.555	4.998	1.315	0.600	1.19	2.90
			8.573	2.966	6.437	4.931				
	149		7.560	2.451	6.727	4.793	1.130	0.507	1.23	3.03
			7.782	2.411	6.850	4.803				
Di-ortho	158		15.465	3.509	6.325	4.676	2.474	0.751	2.29	5.42
			15.465	3.188	6.180	4.238				
	138		15.038	3.348	6.092	4.514	2.499	0.747	2.34	5.50
			15.460	3.116	6.110	4.148				
Mono-ortho	159		25.860	3.664	6.343	4.208	4.183	0.942	3.44	6.99
			25.052	3.518	6.075	4.039				
	156		31.010	4.688	6.027	4.358	5.166	1.081	3.78	7.35
			32.927	4.636	6.348	4.271				

All data were determined at 190°C.
t'=net retention time of the selectand (PCB) on the reactor column containing selector bonded to solvent.
t'_0=net retention time of the selectand (PCB) on the reference column containing pure solvent.
$t^{*'}$=net retention time of the reference compound on the reactor column.
$t'_0{}^*$=net retention time of the reference compound on the reference column.
$r=t'/t'^*$ relative retention on the reactor column.
$r_0=t'_0/t'_0{}^*$ relative retention on the reference column.
R'=retention increment=$(r-r_0)/r_0$.
$-\Delta_{PCBi/PCB136}(\Delta G)=RT\ \ln\ (R'_{PCBi}/R'_{PCB136})$; example: $-\Delta_{132/136}(\Delta G)=2.90$ kJ/mol.

phase described here enables the separation of PCB congeners according to the degree of substitution in the ortho-position. Thus, it may be applied to the selective analysis of the highly toxic coplanar poly- chlorinated biphenyls in GC. Further optimization of the column has to be carried out to improve the peak shapes and the efficiency, e.g. via column deactiva- tion or variation of the fullerene concentration.

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