Elution pattern of planar chlorinated biphenyls and 2,3,7,8-polychlorinated dibenzo-*p*-dioxins/dibenzofurans on chromatographic adsorbents and factors affecting the mechanism of retention
Possibilities of selective separation of both families

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Abstract

A possible retention mechanism for planar chlorinated biphenyls and 2,3,7,8-polychlorinated dibenzo-*p*-dioxins/dibenzofurans on alumina and Florisil columns is proposed. The influence of factors such as the surface activity of the adsorbents as well as the volume, selectivity and polarity of the solvents has been studied. A very similar elution pattern was found for alumina and Florisil. These results suggested that retention of the compounds on the surface of the adsorbent depends on the planarity of the molecule, on the localisation of the π-electron cloud and, less clearly, on the extended π-electron cloud of the congener. The best separation between both families with the highest recoveries of individual polychlorinated biphenyl and polychlorinated dibenzo-*p*-dioxins/dibenzofuran congeners was obtained by using 6 g of Florisil activated at 450°C for 24 h.

Keywords: Adsorbents; Environmental analysis; Solvent effects; Polychlorinated biphenyls; Polychlorinated dibenzo-*p*-dioxins; Polychlorinated dibenzofurans; Organochlorine compounds

1. Introduction

Polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) have been recognised as being highly toxic and ubiquitous environmental contaminants [1,2]. The most toxic congeners of these three organochlorine families are the planar CBs (non-*ortho* substituted) and semiplanar (mono-*ortho* and di-*ortho* substituted) [3], and the PCDDs and PCDFs

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with chlorine in the 2,3,7,8-positions. The determination of these individual congeners in environmental matrices implies sophisticated and tedious multi-stage sample preparation clean-up procedures, or very specific detectors [4,5]. In general, the final clean-up step of the analytical methodology includes the elimination of the similar organic compounds remaining in the extract and the quantitative isolation of the PCBs from the PCDDs/Fs. In this last step it is necessary to attain a good separation between both families, as well as to get good recoveries of all PCB and PCDD/F congeners studied.

Many papers have reviewed the separation of PCDDs/Fs and planar CBs from semi-planar CBs on chromatographic adsorbents such as activated charcoal [6], but some selective retention of the more interesting compounds has been reported for these columns [7]. A more efficient separation of the compounds according to their planarity can be attained in a 2-(1-pyrenyl)ethyldimethylsilycated silica column [7,8], but very high retention of PCDDs/Fs in this phase has been reported [9]. Furthermore, these kinds of adsorbents require the use of HPLC systems. As an alternative to these more sophisticated techniques, efficient separations of the two classes of organochlorine compounds on classic chromatographic adsorbents such as alumina or Florisil have been reported [10,11]. These methods are inexpensive, relatively simple, with sufficient resolution and very adequate for routine work. In spite of their wide use, a general lack of discussion about the possible mechanism of the retention of PCBs and PCDDs/Fs on these adsorbents and factors governing their elution pattern was observed.

This article discusses the elution pattern of planar CBs and 2,3,7,8-PCDDs/Fs on alumina and Florisil classic open liquid chromatography columns. The influence of parameters associated with the adsorbent (amount and surface activity) and with the solvent (volume, polarity and selectivity) on this elution pattern is studied. The retention mechanism of planar CBs on these adsorbents is discussed and the recoveries of individual PCBs, PCDDs and PCDFs in the experiments that allowed the best separation between both families are reported.

2. Experimental method

2.1. Solvents and adsorbents

Particle sizes of different aluminas used in this study are shown in Table 1. Neutral alumina 60G and basic alumina 60 activity (corresponding to Neutral I and Basic I in Table 1, respectively) were purchased from Merck (Darmstadt, Germany). Basic alumina II in Table 1 corresponded to basic alumina AG-10, from Bio-Rad Labs. (Richmond, CA, USA) and alumina III to ICN alumina B-Super I (ICN Biomedicas, Germany). Florisil (150–250 μm) was purchased from Florindin (Berkley Springs, WV, USA).

CH_2Cl_2 and CCl_4 from Merck and n-C_6H_{14} from Promochem (Wesel, Germany) were used as solvents. All solvents were suitable for pesticide residue analysis.

2.2. Standard solutions

2.2.1. PCB standards and standard solutions

The 14 individual PCB congeners studied (Table 5), selected by their abundance and toxicity in environmental samples, were purchased from Ehrenstorfer (Augsburg, Germany).

Two working stock solutions were prepared from individual PCB standards. The first stock solution (A) contained PCBs IUPAC No. 101, 151, 118, 153, 138, 180, 170 and 194 [12] with concentrations between 0.99 and 0.32 μg/ml in n-C_6H_{14}. The second working stock solution (B) contained the

Table 1
Different alumina activation processes

Experiment No.	Type of adsorbent	Particle size (μm)	Activation temperature (°C)		Activation time (h)
1	Neutral I	100–200	50°C (at 4°C/min to)	180°C (at 30°C/min to) (2 h)	250°C (1.5 h)
2	Basic I	100–200	50°C (at 4°C/min to)	180°C (at 30°C/min to) (2 h)	250°C (1.5 h)
3	Basic I	100–200	200		15
4	Basic II	70–230	200		16
5	Basic III	50–200	200		16
6	Basic III	50–200	450		24

eight PCBs of stock solution A at the same concentration plus PCBs IUPAC No. 77, 105, 126, 167, 156 and 169 with concentrations between 1.00 and 0.75 $\mu g/ml$ in n-C_6H_{14}. Both PCB standard solutions were stored at 4°C in borosilicate glass screwtop vials until use.

Stock solution A was used in all alumina experiments except number 6 (Table 3) and Florisil experiment numbers 1, 2, 3 and 4a (Table 4). Stock solution B was used in alumina assay 6 and in the rest of the Florisil experiments (numbers 4b, 5 and 6, Table 4).

A stock solution containing 0.9 ng/μl of Aroclor 1260 in n-C_6H_{14} was used as a technical PCB mixture.

2.2.2. PCDD and PCDF standard and standard solution

The EPA 1613 stock native solution, chemical purity $\geq 98\%$ (Chemsyn Science Labs., KS, USA), consisted of a mixture of 2,3,7,8-substituted congeners from tetra- to octa-CDDs and CDFs, which are the most toxic, at concentrations from 0.4 $\mu g/ml$ to 4.0 $\mu g/ml$. This solution was used as a standard PCDD and PCDF solution.

2.3. Experimental design

All adsorbents were cleaned with CH_2Cl_2 in a Soxhlet apparatus for 6 h and evaporated to dryness at room temperature before activation.

The different activating processes for the aluminas used in each experiment are summarised in Table 1 and denoted with a different number. This same numbering is used in Table 3, where the different assays carried out with the same adsorbent activated

in the same way were denoted with a different letter following the corresponding number. Such a set of experiments made it possible to investigate the influence of parameters such as the amount of adsorbent or the volume, polarity and selectivity of the solvent on the elution pattern of the compounds. The amount of the adsorbent, the column size (as internal diameter, I.D.) and the solvent mixtures used in each experiment are summarized in Table 3.

The different activating processes of Florisil used in each experiment are shown in Table 2. As in the alumina case, they were denoted with a different number and this numbering was maintained in Table 4, in which the different assays are summarised.

2.4. High-resolution gas chromatographic–electron capture detection (HRGC–ECD) analysis

A Model 8600 Perkin-Elmer (Beaconsfield, UK) gas chromatograph equipped with a [63]Ni electron-capture detector was employed for these analyses. The eluates of the different experiments were concentrated and taken up in a working solution containing the PCBs 12 (3,4-dichlorobiphenyl) and 209 (2,2',3,3',4,4',5,5',6,6'-decachlorobiphenyl). These individual congeners were added just before chromatographic injection in order to correct injection errors and detector fluctuations. A 0.5-μl aliquot of this solution was injected in a 60 m DB-5 fused-silica capillary column with 0.25 μm film thickness (J&W Scientific, CA, USA). The column temperature was programmed from 60°C (1 min) to 180°C at 50°C/min, then to 230°C (40 min) at 4°C/min, and finally to 270°C (10 min) at 4°C/min. The injector and detector temperatures were 280°C and 300°C, respectively.

Table 2
Different Florisil activation processes

Experiment No.	Activation temperature (°C)	Activation time (h)	Deactivation
1	650	2	
2	650	2	3% H_2O (w/w)
3	140	16	
4	450	24	
5	130	18	
6	130	16	

Chromatographic data were acquired by the System Gold acquisition data system (Beckman, CA, USA). The identification of the individual PCB, PCDD and PCDF congeners was based on comparison of their retention times with those of stock solution mixtures. The recoveries of the congeners in the different extracts were calculated by comparison of the individual peak area response of each congener with those corresponding to a similar concentration of the stock standard solution.

In all, 14 different experiments were carried out using alumina as adsorbent and 7 using Florisil. All experiments were repeated twice, except for alumina experiment no. 6 and the Florisil essays which were repeated four times to calculate the recoveries of the individual PCBs and PCDDs/Fs (Tables 5 and 6, respectively). In all cases, the relative standard deviations (R.S.D.s) found were lower than 10%.

Triplicate analyses were made with Aroclor 1260. No significant increase in the R.S.D.s were found in these experiments with respect to that found with standard solutions.

3. Results and discussion

3.1. Alumina experiments

The general elution pattern found for the PCBs and the PCDDs/Fs studied in the set of experiments carried out with alumina as the chromatographic adsorbent are shown in Table 3. (Results only refer to the PCBs of the standard solution A.)

Experiments 1, 2, 3 and 4a allowed us to check the influence of the alumina properties in the process. In all cases, the same elution protocol was used.

In experiments 1 and 2 neutral and basic alumina (Merck), respectively, were used. A comparison of both sets of results showed that neutral alumina was useless in the test separation of these compounds. Percentages above 70% of all PCBs and PCDDs/Fs were eluted in the first fraction from the neutral alumina column, whereas in assay 2 the greatest part of the compounds studied were found in every eluted fraction at quantifiable levels. That proved the stronger retention of these pollutants on the surface of the basic alumina.

Comparison of the results obtained in experiments 2 and 3 showed the influence of the surface activity in the elution pattern. The same elution protocol was used in both experiments but the amount of alumina was double in experiment 3 compared to experiment 2. As the percentages of all PCBs and PCDDs/Fs found in the two first fractions of experiment 3 were higher than those found in assay 2, the surface activity of the adsorbent used must be lower in the former assay than in the latter. These results show that when the amount of adsorbent used is large enough, i.e. with respect to the organochlorine levels expected in a sample, the surface activity of the adsorbent is probably a more important factor than the quantity of adsorbent used, keeping in mind that the solvent volumes must be large enough to elute all of the compounds in their corresponding fractions.

Experiments 3 and 4a were carried out with the same quantity of two very similar aluminas, purchased from different companies, and with the same activation process. In both cases, the elution protocol was the same as in previous experiments. Nevertheless, under the experimental conditions, a better separation between PCBs and PCDDs/Fs was achieved when basic alumina II from experiment 4a was used.

Experiments 4a–h, carried out with basic alumina II activated in the same way, made it possible to check the influence of parameters such as the strength, selectivity and quantity of solvent used in the elution pattern. The strength of a solvent depends on its "polarity" (ability to dissolve polar compounds, i.e., nitriles and alcohols). Solvent selectivity, defined by Snyder [13], refers to the ability of a given solvent to selectively "dissolve" one compound as opposed to another with "polarity" (P') not obviously different (one of two polar solvents might preferentially dissolve nitriles and the other alcohols). These two terms must be carefully distinguished when the fractionation of different classes of very similar compounds including a large variety of congeners is required.

In this set of experiments (4a–h), the effect of the successive reduction of the solvent volume used to elute the first fraction is more obvious for PCBs than for PCDDs/Fs because of their lower retention in the adsorbent. The lower the volume of $n\text{-}C_6H_{14}$ the lower the PCB percentages found in fraction 1. It was apparent from the results that volumes of n-

Table 3
Experiments carried out with alumina

Experiment No.[a]	Sub-experiment[b]	Adsorbent (g)[c]	Column I.D. (cm)	Solvent	Distribution[d] PCB[f]	Te	Pe	Hx	Hp	Oct
1		6	1	F[e].1: 40 ml n-C_6H_{14}	****	****	****	****	****	****
				F.2: 50 ml n-C_6H_{14}–CCl_4 (75:25)	–	–	–	–	*	*
				F.3: 50 ml n-C_6H_{14}–Cl_2CH_2 (80:20)	–	–	–	–	–	–
				F.4: 40 ml Cl_2CH_2	–	–	–	–	–	–
2		6	1	F.1: 40 ml n-C_6H_{14}	****	****	***	***	**	**
				F.2: 50 ml n-C_6H_{14}–CCl_4 (75:25)	–	–	**	**	*	*
				F.3: 50 ml n-C_6H_{14}–Cl_2CH_2 (80:20)	*	*	*	*	**	**
				F.4: 40 ml Cl_2CH_2	–	–	*	*	*	*
3		12	1.3	F.1: 40 ml n-C_6H_{14}	****	***	***	***	***	***
				F.2: 50 ml n-C_6H_{14}–CCl_4 (75:25)	–	**	**	**	**	**
				F.3: 50 ml n-C_6H_{14}–Cl_2CH_2 (80:20)	–	–	–	–	*	*
				F.4: 40 ml Cl_2CH_2	–	–	–	–	–	–
4	a	12	1.3	F.1: 40 ml n-C_6H_{14}	****	****	**	*	–	–
				F.2: 50 ml n-C_6H_{14}–CCl_4 (75:25)	*	*	****	****	****	****
				F.3: 50 ml n-C_6H_{14}–Cl_2CH_2 (80:20)	–	–	–	–	–	–
				F.4: 40 ml Cl_2CH_2	–	–	–	–	–	–
	b	6	1	F.1: 30 ml n-C_6H_{14}	****	***	***	**	*	*
				F.2: 25 ml n-C_6H_{14}–Cl_2CH_2 (98:2)	*	*	**	***	***	***
				F.3: 30 ml n-C_6H_{14}–Cl_2CH_2 (80:20)	–	*	*	*	*	*
	c	12	1.3	F.1: 30 ml n-C_6H_{14}	****	***	**	*	–	–
				F.2: 25 ml n-C_6H_{14}–Cl_2CH_2 (98:2)	*	**	***	***	***	***
				F.3: 30 ml n-C_6H_{14}–Cl_2CH_2 (80:20)	*	*	*	*	**	**
	d	6	1	F.1: 25 ml n-C_6H_{14}	***	***	**	**	–	–
				F.2: 25 ml n-C_6H_{14}–CCl_4 (90:10)	**	**	***	***	****	****
				F.3: 30 ml n-C_6H_{14}–Cl_2CH_2 (80:20)	**	**	**	**	**	**
	e	12	1.3	F.1: 25 ml n-C_6H_{14}	***	***	**	–	–	–
				F.2: 25 ml n-C_6H_{14}–CCl_4 (90:10)	–	*	***	****	****	****
				F.3: 30 ml n-C_6H_{14}–Cl_2CH_2 (80:20)	*	*	*	**	**	**
	f	6	1	F.1: 20 ml n-C_6H_{14}	***	**	**	*	–	–
				F.2: 25 ml n-C_6H_{14}–Cl_2CH_2 (96:4)	**	***	***	****	****	****
				F.3: 30 ml n-C_6H_{14}–Cl_2CH_2 (80:20)	–	*	*	*	*	*
	g	12	1.3	F.1: 20 ml n-C_6H_{14}	**	*	*	–	–	–
				F.2: 25 ml n-C_6H_{14}–Cl_2CH_2 (96:4)	***	***	***	****	****	****
				F.3: 30 ml n-C_6H_{14}–Cl_2CH_2 (80:20)	–	*	*	*	*	*
	h	12	1.3	F.1: 10 ml n-C_6H_{14}	–	–	–	–	–	–
				F.2: 20 ml n-C_6H_{14}–CCl_4 (95:5)	*	–	–	–	–	–
				F.3: 40 ml n-C_6H_{14}–Cl_2CH_2 (80:20)	****	****	****	****	****	****
5	a	5	1	F.1: 5 ml n-C_6H_{14}	–	–	–	–	–	–
				F.2: 20 ml n-C_6H_{14}–CCl_4 (90:10)	–	–	–	–	–	–
				F.3: 75 ml n-C_6H_{14}–Cl_2CH_2 (60:40)	****	****	****	****	****	****
				F.4: 20 ml Cl_2CH_2	–	–	–	–	–	–
	b	5	1	F.1: 5 ml n-C_6H_{14}	–	–	–	–	–	–
				F.2: 20 ml n-C_6H_{14}–Cl_2CH_2 (95:5)	****	****	****	***	***	**
				F.3: 75 ml n-C_6H_{14}–Cl_2CH_2 (60:40)	*	*	*	**	**	***
				F.4: 20 ml Cl_2CH_2	–	–	–	–	–	–
6		6	1.3	F.1: 100 ml n-C_6H_{14}	****	–	–	–	–	–
				F.2: 100 ml n-C_6H_{14}	*	–	–	–	–	–
				F.3: 200 ml Cl_2CH_2	–	****	***	****	****	****

[a] Term experiment refers to assays carried out with the same alumina and activated in the same way (see Table 1).
[b] Term sub-experiment refers to different assays carried out with a same alumina, with the same surface activity, but used in different quantity or eluted with different solvents.
[c] Grams of adsorbent used in the experiment.
[d] Different symbols refer to different percentages of compounds found in the fraction: * <10%, ** 25–50%, *** 50–70% and **** >70%.
[e] Fraction.
[f] Results only refer to the PCBs of the standard solution A.

C_6H_{14} smaller than 30 ml might not be sufficient to completely elute fraction 1. (PCB percentages above 70% from experiments 4a–c decreased to the 50–70% range in assays 4d and 4e.) Therefore, in fraction 2 from experiments 4d–h, compounds did not elute in the first fraction because an insufficient volume of n-C_6H_{14} was used and those corresponding to the second fraction were eluted together. This fact must be kept in mind and carefully differentiated from percentage variations due to changes in volume, polarity or selectivity of the solvents used.

The comparison of the PCB and PCDD/F elution patterns found in fractions 2 and 3 from experiments 4b–h carried out with the same quantity of adsorbent showed that CCl_4 was less selective than CH_2Cl_2 for these pollutants. Higher percentages of all the compounds studied were found in fraction 3 when fraction 2 was eluted with the mixture n-C_6H_{14}–CCl_4 (90:10), $P'=0.17$, than when fraction 2 was eluted with the same volume of the mixture n-C_6H_{14}–CH_2Cl_2 (96:4), $P'=0.14$. The former was the case in experiments 4d and 4e, in which PCB percentages were in the 25–50% range and lower than 10%, respectively, were found. The latter was the case in experiments 4f and 4g, in which PCB percentages lower than 10% and no detectable levels, respectively, were found. This fact was even more evident in assays where the polarity of the solvent mixture containing CH_2Cl_2 was lower than those containing CCl_4, as the comparison of the results from experiments 4b,c [n-C_6H_{14}–CH_2Cl_2 (98:2), $P'=0.07$] and 4d,e [n-C_6H_{14}–CCl_4 (90:10), $P'=0.17$] showed. Obviously, an increase in the polarity of the CH_2Cl_2 mixture implied a more efficient elution of the compounds from the column. (See experiments 4c and 4g).

In assay 4h, where warm alumina was used, the 10 ml of n-C_6H_{14} used to elute the first fraction was clearly insufficient to extract the compounds corresponding to this fraction. The low selectivity and polarity of the mixture used as a solvent for fraction 2 [n-C_6H_{14}–CCl_4 (95:5), $P'=0.09$], in addition to its relatively small quantity (20 ml), caused all the compounds to be eluted together in fraction 3.

The results of the experiments 5a and 5b, in which a different commercial basic alumina (ICN) was used, confirmed the previous conclusion about the

different selectivity of the CCl_4 and the CH_2Cl_2 for the compounds studied. The volume and the polarity of the mixtures used as solvents in the second fraction were the same in both essays (20 ml and $P'=0.17$), but whereas important percentages of all the organochlorines were found in the fraction eluted with the CH_2Cl_2 mixture (above 70% for PCBs, tetra- and penta-CDDs/Fs, and 50–70% for hexa- and hepta-CDDs/Fs), no detectable levels of these compounds were found when the CCl_4 mixture was used as solvent.

Experiment 6 provided the best separation between PCBs and PCDDs/Fs with the best recoveries for the different individual congeners. In this assay 6 g of basic alumina ICN was activated at 450°C for 24 h [11], 100 ml of n-C_6H_{14} was used to elute each of the first two fractions, and 200 ml of CH_2Cl_2 to elute the third one. The recoveries of the di-*ortho* and mono-*ortho* CBs in the first fraction ranged from 79% to 106%, and from 1.5% to 9.3% in the second one (Table 5). But none of the PCBs studied were detected in the third fraction.

A higher retention on the adsorbent was observed for the mono-*ortho* CBs and especially, for the non-*ortho* CBs. These congeners were spread among the three fractions. In general, these results agreed with the elution pattern observed by Storr-Hansen et al. [11]. The PCDDs/Fs were exclusively eluted in the third fraction (Table 6) with recoveries above 67%.

These results were confirmed when a mixture containing the Aroclor 1260 plus the 2,3,7,8-PCDDs/Fs studied were eluted through this column. The recovery of the total Aroclor in each fraction was 87% in first one, 25% in the second and below the detection limit in the last one as is shown in Fig. 1A.

3.2. Florisil experiments

The general elution pattern found for the PCBs and the PCDDs/Fs studied in the set of experiments carried out with Florisil as chromatographic adsorbent are shown in Table 4. Symbols and abbreviations are as in Table 3.

Two different PCB standard solutions were used (A in experiments 1, 2, 3 and 4a, and B in assays 4b, 5 and 6) but, in order to simplify the comparison,
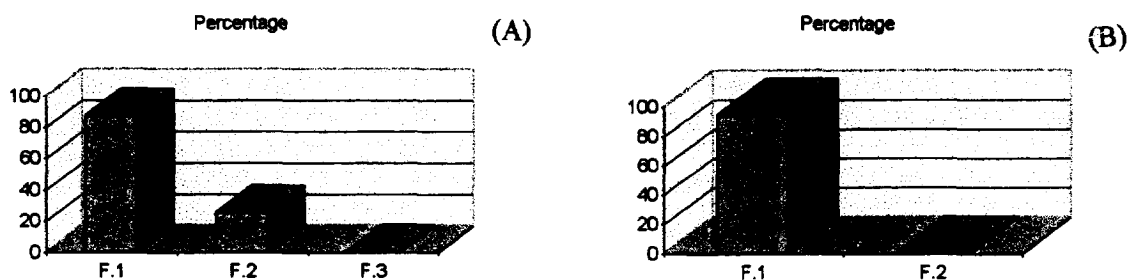
Fig. 1. Recoveries of the Aroclor 1260 in each fraction from alumina experiment No. 6 (A) and Florisil No. 4b (B).

results in Table 4 only refer to the congeners included in the stock solution A.

According to Snyder's considerations about the retention mechanism for aromatic molecules on the surface of this adsorbent [14], a very similar elution pattern to that observed in the case of basic alumina was expected on Florisil. Therefore, the retention of the compounds must increase with the planarity, the linearity and the net electron density of the molecule. Results from alumina experiments showed that, as Snyder suggested [14], the best separation between isomers which exhibited the so called "weak locali-

sation" (i.e., PCBs) occurred with very active aluminas and very weak solvents, such as n-C_6H_{14}. A polar solvent, such as CH_2Cl_2, was able to elute the most retained PCB and PCDD/F congeners. For this reason, except for assay 5, no solvent mixtures were used in the set of experiments carried out with Florisil. An apolar solvent, such as n-C_6H_{14}, was chosen to elute the first fractions and a polar one, such as CH_2Cl_2, to selectively elute PCDDs/Fs in the last fraction. Under these conditions, and except in assay 3, relatively good separations of the bulk of the PCBs from PCDDs/Fs were obtained (Table 4).

Table 4
Experiments carried out with florisil

Experiment No.	Sub-experiment	Adsorbent (g)	Column I.D. (cm) (cm)	Solvent	Distribution					
					PCB[a]	PCDD/F				
						Te	Pe	Hx	Hp	Oct
1		20	2	F.1: 200 ml n-C_6H_{14}	****	–	–	–	*	*
				F.2: 200 ml Cl_2CH_2	*	***	****	****	****	**
2		20	2	F.1: 200 ml n-C_6H_{14}	***	–	–	–	–	–
				F.2: 200 ml Cl_2CH_2	*	**	**	**	***	****
3		20	2	F.1: 200 ml n-C_6H_{14}	***	–	**	**	*	–
				F.2: 200 ml Cl_2CH_2	*	****	***	***	***	**
4	a	6	1.3	F.1: 100 ml n-C_6H_{14}	****	–	–	–	–	–
				F.2: 100 ml n-C_6H_{14}	*	–	–	–	–	–
				F.2: 200 ml Cl_2CH_2	–	****	****	****	****	****
	b	6	1.3	F.1: 150 ml n-C_6H_{14}	****	–	–	–	–	–
				F.2: 250 ml Cl_2CH_2	*	****	****	****	****	****
5		5	1.3	F.1: 20 ml n-C_6H_{14}–Cl_2CH_2 (99:1)	****	*	–	–	–	–
				F.2: 50 ml Cl_2CH_2	*	****	****	****	***	***
6		1	0.5	F.1: 10 ml n-C_6H_{14}	****	–	–	–	–	–
				F.2: 40 ml Cl_2CH_2	*	****	****	****	****	****

Symbols as in Table 3.
[a] Results refers to the PCBs of the standard solution A.

In general, percentages lower than 10% of the more planar CBs or of the lower chlorinated PCDDs/Fs were found outside of their corresponding fraction.

The best separation was obtained using 6 g of Florisil activated at 450°C for 24 h [11], with 100 ml of n-C_6H_{14} as eluent in the first two fractions and CH_2Cl_2 in the third one (experiment 4a). In this assay, the major part of the PCBs was eluted in the first fraction (percentages above 70%). Only percentages below 10% of these compounds were eluted with the second 100 ml of n-C_6H_{14}, and they were not detected in the last fraction, in which the major part of the PCDDs and PCDFs was eluted (percentages above 70%).

The following more favorable elution pattern corresponded to experiments 4b and 6, although that of experiment 4b was only a modification of 4a in order to reduce the number of fractions and the solvent volume. Experiment 6 was the method proposed by Harrad et al. [15] for the separation of PCDDs, PCDFs and PCBs 77, 126 and 169 from *ortho*-chloro substituted CBs. Recoveries and elution patterns achieved in experiments 1, 2, 3 and 5 were not as favorable as in preceding assays.

The comparison of the results from experiments 1 and 2 made it possible to check the influence of the Florisil deactivation with water (3%, w/w) in the elution pattern. Contrary to what was expected and to the results obtained by Storr-Hansen et al. with deactivated basic alumina [11], a similar distribution between fractions was observed in both essays but with lower recoveries, especially for tetra- to hexa-CDDs/Fs, when deactivated Florisil was used as adsorbent.

The recoveries of the individual PCBs included in these experiments are shown in Table 5. In this table, the results of the experiment 4a are not shown because they were similar to that of the assay 4b.

In all experiments, PCBs included in standard solution A (Nos. 101, 118, 138, 151, 153, 170, 180 and 194) had higher recoveries in the first fraction than in the second one. However, differences in retention behaviour depending on the Florisil activity might be observed for individual PCB congeners. In experiment 2, where deactivated Florisil was used, the PCBs with higher recoveries were Nos. 118, 138, 170, 180 and 194 (98, 67, 99, 76 and 102%, respectively). The worst recoveries (34%) corres-

ponded to non-planar CB 151, PCB 153 (45%) and PCB 101 (58%). From these results some features became evident. Among isomers with the same number of total chlorines, retention seemed to increase with the number of *ortho*-chlorines. (The recovery of PCB 101 in the first fraction was lower than that of PCB 118, and that of PCB 151 was lower than those of PCBs 138 and 153.)

Among isomers with the same number of *ortho*-chlorines, in general, retention seemed to decrease with the degree of chlorination (the highest recoveries corresponded to PCBs 170, 180 and 194). Furthermore, among isomers with the same number of total as well as *ortho*-chlorines, the retention of the compounds on the adsorbent seemed to be governed by the substitution pattern. To verify the influence of this factor on retention, recoveries of PCB congeners with a same half-ring structure must be compared. This was the case of PCBs 138 and 153 (2,4,5-substitution on one of their rings) and of the PCBs 170 and 180 (2,3,4,5-substitution). In these two pairs of PCBs, greater recoveries were found when in the other ring the chlorines were together (PCBs 183 and 170, with 2,3,4-substitution) than when these chlorines were spread over the ring (PCBs 153 and 180, with 2,4,5-substitution).

An elution pattern quite similar to that observed with deactivated Florisil was found in experiments carried out with Florisil with relatively low activity, such as experiments 5, 6, and especially 3. The only difference observed in experiments 5 and 6, in which different amounts of adsorbent and solvents were used, was the lower retention of the non-planar CB 151 as opposed to the retention of those with the same number of total chlorines but more planar conformation (PCBs 138 and 153).

A similar behaviour of the non-planar congeners was observed when a highly activated Florisil was used (experiment 1). Furthermore, retention seemed to increase with the degree of chlorination.

All these results show that the elution pattern was strongly dependent upon the surface activity of the adsorbent and that, in accordance with what Storr-Hansen et al. [11] have proposed for alumina, only relatively few very active adsorption sites were removed by deactivation with water while the majority of the more uniform and lower activity sites were not affected.

Table 5
Recoveries of PCB congeners in different experiments (as percentages)

PCB Structure	IUPAC No.	ng[a]	Alumina experiment No. 6			Florisil experiment No.												
						1		2		3		4[b]		5		6	
			F[b].1	F.2	F.3	F.1[c]	F.2	F.1	F.2	F.1	F.2	F.1	F.2	F.1	F.2	F.1	F.2
3,3',4,4'-T₄CB	77	10.2	21	8.5	47	NI[c]	NI	NI	NI	NI	NI	21	77	43	1.9	4.2	48
2,2',4,5,5'-P₅CB	101	8.9	97	2.6	–[d]	89	1.4	58	29	77	25	101	2.8	104	2.5	89	4.1
2,3',4,4'-P₅CB	105	7.5	12	1.1	53	NI	NI	NI	NI	NI	NI	34	82	1.4	32	54	5.4
2,3',4,4',5'-P₅CB	118	9.9	109	3.5	–	99	3.4	98	9.6	102	–	101	2.7	59	4.4	70	1.1
3,3',4,4',5-P₅CB	126	10.0	2.3	1.5	69	NI	NI	NI	NI	NI	NI	4.2	95	1.3	30	0.1	49
2,2',3,4,4',5'-HxCB	138	9.6	79	3.5	–	79	–	62	9.0	67	24	99	2.3	85	5.7	73	4.7
2,2',3,5,5',6-HxCB	151	3.2	105	9.3	–	89	0.8	34	6.9	44	6.4	104	0.2	93	2.9	76	6.6
2,2',4,4',5,5'-HxCB	153	8.9	87	1.9	–	77	2.4	45	8.4	56	16	99	0.7	65	2.2	51	3.7
2,3,3',4,4',5-HxCB	156	8.7	46	41	13	NI	NI	NI	NI	NI	NI	87	3.8	57	9.3	58	0.8
2,3',4,4',5,5'-HxCB	167	9.6	96	3.0	3.7	NI	NI	NI	NI	NI	NI	89	1.0	72	1.3	55	0.8
3,3',4,4',5,5'-HxCB	169	10.0	–	–	73	NI	NI	NI	NI	NI	NI	–	103	–	36	–	59
2,2',3,3',4,4',5-HpCB	170	4.7	106	2.4	–	75	0.7	99	9.5	80	14	103	0.9	91	8.6	92	3.5
2,2',3,4,4',5,5'-HpCB	180	9.4	98	1.5	–	78	–	76	9.6	30	4.2	101	1.3	93	9.3	83	2.3
2,2',3,3',4,4',5,5'-OCB	194	2.3	103	2.6	–	82	–	102	5.8	91	–	106	–	90	0.6	100	0.9

[a] ng=Nanograms of each PCB congener in column head.
[b] F=Fraction.
[c] NI=Congener not included in the experiment.
[d] – = Not detected.

Trends observed were confirmed in experiments 4b, 5 and 6 by the use of the standard solution B containing new PCBs non-*ortho* (PCBs 77, 126 and 169) and mono-*ortho* substituted (PCBs 105, 156 and 167). Retention tended to increase with decreasing number of *ortho*-chlorines. This effect was clearer when increasing the surface activity of the adsorbent, that is, greater in experiment 4b than in assays 5 and 6 (see recoveries of hexa-CBs). Results found for mono-*ortho* penta-CBs confirmed that among isomers with the same number of *ortho*-chlorines as well as total chlorines in each ring, the retention tended to increase when the chlorines were more together. Thereby, PCBs 105 and 118 were eluted in different fractions in experiments 4b and 5, but, possiblyt, the more extended π-electron cloud of PCB 105 contributed to its very different behaviour.

The similarity between the structures of PCBs 156 and 167 might justify their very similar recoveries. Nevertheless, it is difficult to account for the possible retention mechanism for congeners with the same number of *ortho*-chlorines but with a different number of chlorines in each half-ring.

In general, results showed that PCB congeners had an individual retention behaviour on Florisil. However, several factors were involved in their retention, such as the surface activity of the adsorbent, ability of the congener to obtain a planar conformation (number of *ortho*-chlorines), electron density and localisation of the π-electron cloud.

The higher retention of PCDDs/Fs on Florisil (Tables 4 and 6), together with the very similar structure of both families as well as the very similar congener substitution patterns made the discussion less evident than for PCBs. In spite of these facts, it was obvious that there was a high degree of parallelism between the possible retention mechanism for these compounds and that proposed for PCBs (Table 6).

From the point of view of the quantitative separation of the PCBs and PCDDs/Fs (Tables 5 and 6) the best results were obtained with 6 g of Florisil activated at 450°C for 24 h (experiment 4b). The recoveries of the tri-*ortho*, di-*ortho* and mono-*ortho* CBs (except PCB 105) ranged from 87% to 106% in the first fraction and from 0.2% to 106% in the

Table 6
Recoveries of PCDD and PCDF congeners in different experiments

Structure	ng[a]	Recoveries in different experiments as %															
		Alumina experiment No. 6			Florisil experiment No.												
					1		2		3		4b		5		6		
		F.1	F.2	F.3	F.1	F.2	F.1	F.2	F.1	F.2	F.1	F.2	F.1	F.2	F.1	F.2
2,3,7,8-TCDF	1.0	–	–	105	–	63	1.8	60	–	73	–	92	5.4	89	1.5	106
2,3,7,8-TCDD	1.0	–	–	94	–	36	–	48	–	78	–	100	5.3	73	–	108
1,2,3,7,8-PCDF	1.0	–	–	67	–	100	–	51	–	49	–	102	–	79	–	68
2,3,4,7,8-PCDF	1.0	–	–	94	–	82	–	50	15	40	–	104	–	93	–	80
1,2,3,7,8-PCDD	1.0	–	–	86	–	87	–	53	32	75	–	99	–	85	–	76
1,2,3,4,7,8-HxCDF	1.0	–	–	79	–	81	–	51	15	68	–	97	–	86	–	65
1,2,3,6,7,8-HxCDF	1.0	–	–	91	–	72	–	48	16	60	–	96	–	103	–	69
2,3,4,6,7,8-HxCDF	1.0	–	–	98	–	76	–	44	21	61	–	98	–	101	–	74
1,2,3,4,7,8-HxCDD	1.0	–	–	98	–	80	–	44	22	72	–	97	–	78	–	65
1,2,3,6,7,8-HxCDD	1.0	–	–	90	–	69	–	59	29	62	–	99	–	98	–	73
1,2,3,7,8,9-HxCDD	1.0	–	–	96	–	77	–	51	40	70	–	100	NI	NI	NI	NI
1,2,3,7,8,9-HxCDF	1.0	–	–	86	–	71	–	50	43	64	–	100	–	68	–	93
1,2,3,4,6,7,8-HpCDF	1.0	–	–	75	4.1	38	–	45	22	48	–	98	–	53	–	82
1,2,3,4,6,7,8-HpCDD	1.0	–	–	90	4.8	82	–	61	–	61	–	95	–	40	–	88
1,2,3,4,7,8,9-HpCDF	1.0	–	–	102	2.0	101	–	60	–	62	–	102	–	62	–	88
OCDF	2.0	–	–	103	1.8	35	–	76	–	27	–	103	–	69	–	92
OCDD	2.0	–	–	92	2.0	40	–	98	–	24	–	103	NI	NI	NI	NI

Symbols as in Table 5.
[a] Concentration of each PCDD/F congener in column head.

second one. Non-*ortho* CBs were mainly eluted in the second fraction (recoveries of 77%, 95% and 103% for PCBs 77, 126 and 169, respectively). Nevertheless, a non-negligible percentage of PCBs 77 was eluted in the first fraction (21%). The PCDDs/Fs were eluted exclusively in the second fraction (Table 6). Their recoveries ranged from 92% to 104%.

Similar results were found when a mixture containing the Aroclor 1260 plus the 2,3,7,8-PCDDs/Fs studied was eluted through this column. The recovery of the total Aroclor in the first fraction was 94% and was below the detection limit in the second one (Fig. 1B).

In general, the results obtained with Florisil were better than those found in experiment 6 using alumina.

More efficient separation of PCBs from PCDDs/Fs using HPLC with both 2-(1-pyrenyl)ethyldimethylsilylated (PYE) silica and porous graphited carbon (PGC) stationary phases have been recently reported [7,8,15,16]. Nevertheless, the use of classic open columns with Florisil is inexpensive; moreover, it is readily available, ready to use and very adequate for routine work, and it represents an alternative to the more expensive and sophisticated techniques.

There are many papers dealing with the testing of the efficiency of various types of active carbon in this type of separations (e.g., AX-21, PX-21, Carbopack C, Supelco SP-1, Altech SK-4, Carbosphere, etc.) and the suitability of various supports (e.g., polyurethane foam, sand, glass fibres, Chromosorb, Celite, silica gel, etc.) [17–20]. Various types of semi-automated apparatus that include an active carbon column with reversed elution have been introduced [21,22]. Due to the high affinity for coplanar compounds, the use of activated charcoal has some drawbacks, such as high elution-solvent consumption, low recoveries and uncertain reproducibility. Besides it is an expensive adsorbent, it is difficult to obtain a clean blank after extensive cleaning, and it is difficult to optimise the experimental conditions necessary to obtain good recoveries.

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