Separation of tetrachloro-p-dioxin isomers by high-performance liquid chromatography with electron-acceptor and electron-donor stationary phases

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ABSTRACT

An electron-donor and various electron-acceptor (EA) phases were evaluated for the separation of thirteen tetrachlorodibenzo-p-dioxin isomers in both the reversed- and normal-phase modes. It was shown that the selectivity on EA phases can be enhanced to a great extent in the normal-phase mode compared with the selectivity with polar mobile phases. Separation studies showed that the retention mechanism exhibited with non-polar mobile phases is partially masked by solvophobic effects when the same columns are used in the reversed-phase mode.

INTRODUCTION

The synthesis of tetrachlorodibenzo-p-dioxin (TCDD) standards often results in a mixture of isomers [1–4]. The isolation of individual isomers is generally accomplished by high-performance liquid chromatography (HPLC). It has been reported that certain pairs (or triads) re-sisted separation on reversed-phase and silica columns [5].

Therefore, Bamhart and co-workers [5,6] employed an electron-donor phase [1-pyreneethyl-silica gel (PE-SG)] for the separation of TCDD isomers that co-eluted on octadecylsilane (ODS) phases. With the combination of an ODS column and a PE column the separation of 20 of the 22 TCDD isomers was accomplished. The successful separation of polychlorodibenzo-p-dioxin (PCDD) isomers which co-eluted on ODS

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phases and on PE-SG was reported for an elec-tron-acceptor phase (nitrated phenylethyl silica gel) employed in the reversed-phase mode [7,8]. Swerev and Ballschmiter [9] reported the fractio-nation of **PCDDs** on cyanopropyl-, **diphenyl-** and phenyl-silica gel. According to their results, the selectivity of these columns (used in the reversed-phase mode) is mainly governed by the degree of chlorination, but these phases do not seem to be suitable for the separation of in-dividual isomers.

In this study, we examined systematically the separation of thirteen commercially available TCDD isomers on various electron-acceptor (EA) phases with different electron-acceptor strengths and on an electron-donor (ED) phase, and compared the results with the separation of these isomers on an animo-, a cyano- and a phenyl-silica gel column. We were especially interested in mobile phase effects. Therefore, we studied one EA and the ED phase in both the normal- and reversed-phase modes. The reten-tion of PCDD **congeners** on EA and ED columns has hitherto only been studied with polar mobile phases [8].

EXPERIMENTAL

Chemicals

All solvents used were of HPLC grade **(Rath-**bum, Walkerburn, UK). Eleven of the isomers were purchased from Cambridge Isotope Labs, (Wobum, MA, USA) as solutions in **nonane** (50 ± 5 ppm); **1478-, 1236-, 1239-, 1267-, 1378-, 1289-** and **2378-TCDD** were received as indi-vidual isomers. **1237/1238-** and **1368/1379-**TCDD were received as mixtures of two iso-mers. The concentrations of the two isomers in the delivered standard mixtures differed con-siderably. By comparison of the peak height ratios and the retention order on a PONA gas chromatographic capillary column (5.5 μm; 50 m × 0.2 mm I.D.) (Hewlett-Packard, Palo Alto, CA, USA), we succeeded in assigning the peaks of the **1368/1379-TCDD** mixture unambiguously (conditions: HP 5970B mass-selective detector with HP 589OA gas chromatograph; injection mode, splitless; temperature programme, 130°C (0.5 min), increased from 130 to 230°C at **30°C/** min and **from** 230 to 315°C at **1°C/min;** carrier

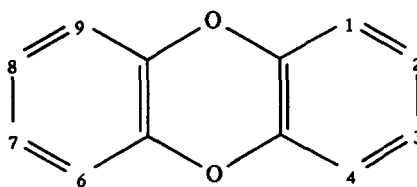
Fig. 1. Numbering of dioxin substituents.

gas, helium). **1237-** and **1238-TCDD** were as-signed according to their shape parameters [10]. **1234-** and **1278-TCDD** were received as crystals from Cambridge Isotope Laboratories. In Fig. 1 the numbering of dioxin substituents is presented according to IUPAC nomenclature.

Apparatus

An HP 1050 liquid chromatograph **(Hewlett-**Packard) with a quaternary gradient pump and autoinjector was used in conjunction with an LDC Spectromonitor III spectrophotometric de-tector. The temperature of the column was controlled by a laboratory-made copper jacket and a water-bath. A PC-based laboratory data system (Hewlett-Packard, Vectra Series) was used to record, store, process and plot the data.

Columns

The following stationary phases were em-ployed: aminopropyl-silica gel (AP-SG); cyano-propyl-silica gel (CP-SG); **pentafluorobenz-**amidopropyl-silica gel (PFB-SG); **3,5-dinitro-**benzamidopropyl-silica gel (DNB-SG); **tetra-**chlorophthalamidopropyl-silica gel (TCP-SG/ **TCP5-SG);**   2,4,7-**trinitrofluorenone-oxime-O-**propyl-silica gel (TNF-SG); phenylpropyl-silica gel (PP-SG); and 1-pyreneethyl-silica gel (**PE-**SG). Structures are shown in Fig. 2.

The EA phases were prepared in our labora-tory according to methods already published: PFB-SG [11], DNB-SG [12], TCP-SG [13] and TNF-SG [14]. The syntheses were performed on a Spherisorb **S10W** [particle diameter (d,) = 10 μm]. In addition, we employed a TCP column (d_p = 5 μm), obtained from **Société Français** Chromato Colonne (**Neuilly-Plaisance ,** France), now available from Silichrom (Pessac, France), prepared according to the method of Felix et *al.* [15,16]. In contrast to the above modified silica
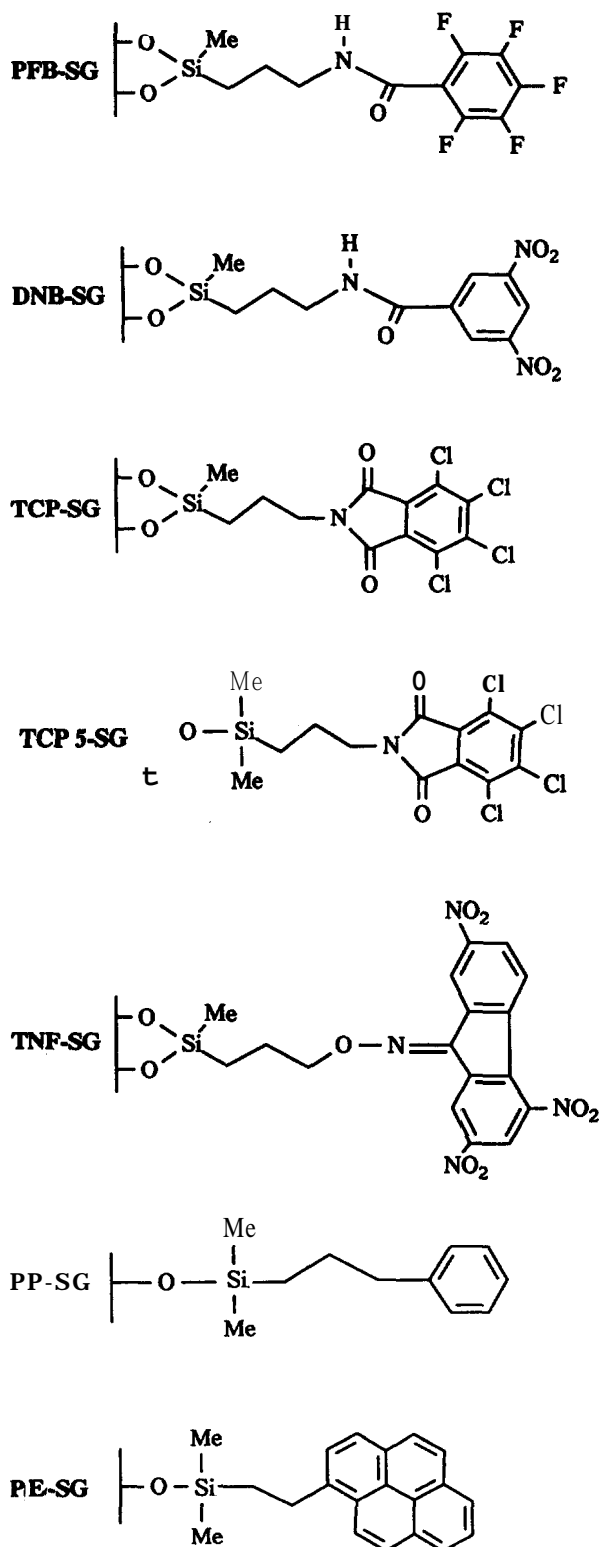
Fig. 2. Structures of the silica gels employed.

gels, the phase of this column was prepared by reaction with a monofunctional silane (*cf.*, Fig. 2). In the following text this phase is abbreviated to **TCP5-SG**.

The AP-SG column was packed with Spherisorb Amino ($d_p = 5 \mu$m) by Cluzeau Info Labo (St.-Foy-la-Grande, France). As a CP-SG column we utilized a Chrompack (Middelburg, Netherlands) column packed with Nucleosil-CN ($d_p = 10 \mu$m). The PP-SG column was prepared in our laboratories according to the method of Thienpont [17] on Spherisorb ($d_p = 5 \mu$m). The EP-SG column (Cosmosil Pye, $d_p = 5 \mu$m) was produced by Nacalai Tesque (Kyoto, Japan).

All columns with the exception of the **DNB**-SG and PP-SG columns (150 mm × 4.6 mm I.D.) had dimensions of 250 mm x 4.6 mm I.D.

Chromatographic conditions

All retention data were obtained under **isocratic** and isothermal (25°C) conditions. The mobile phase was hexane for the EA phases and for AP-SG and CP-SG. TCP-SG was also tested with methanol in the reversed-phase mode. **PP-SG** and PE-SG were employed in the **normal**-phase mode (with hexane) and in the **reversed**-phase mode [methanol-water (80 : 20, v/v)] and pure methanol, respectively). The flow-rate was 1 ml/min in all instances. The UV detector was operated at 235 nm with a response time of 1 s.

Retention times were measured with solutions of the TCDD isomers in **2-propanol** (reversed-phase mode) or hexane (normal-phase mode). The solutions in nonane were diluted with the appropriate solvent. For mixtures the nonane was evaporated before dissolving the standard in **2-propanol** in order to avoid peak broadening or distortion due to solvent effects of the standard solution. The injection volume was $10-30 \mu$l, containing 2-30 ng of each isomer.

Calculation of molecular parameters

Quantum chemical calculations (optimization of the three-dimensional structures; computing of ϵ_{LUMO}, $_{HOMO}$ and dipole moments) were carried out with the semi-empirical method Austin Model 1 **(AM1)** with the software package AMPAC, run on the IBM 3090 in the

CIRCE at **Orsay** (France). **AM1** is a method that is well adapted to large organic molecules [18]. Details of the **optimization** procedure are given elsewhere [10].

RESULTS AND DISCUSSION

EA phases in the normal-phase mode

The EA phases chosen for these studies were characterized with a mixture of polynuclear aromatic hydrocarbons **(PAHs)** by Felix and Bertrand [13]. They classified the PFB-SG as a weak and the DNB-SG as an average EA phase. **TCP-SG** and TNF-SG were classified as strong EA phases.

The capacity factors of thirteen TCDD isomers on the EA columns obtained in the **normal**-phase mode are listed in Table I. The analytes are hardly retained on the PFB-SG column, more retained on the DNB-SG column and well separated on the TCP, **TCP5** and TNF-SG columns with few co-elutions. Fig. 3 shows a chromatogram of the thirteen TCDD isomers

obtained on the TCPS-SG column. The isomers are well separated with high selectivity. Only two co-elutions were observed: **1238- / 1478-TCDD** and **1236- / 1267-TCDD**.

The same results were obtained for the **TCP**-SG and TNF-SG columns. The selectivity on the TNF phase is not enhanced compared with the TCP phases, but with the same mobile phase the retention times are much longer.

The stationary phases employed were designed for the separation of **PAHs** by donor-acceptor complex (DAC) LC. If the formation of weak electron donor-acceptor complexes between the solute and the immobilized ligands of the stationary phase is the dominating retention mechanism, the analytes are separated according to the stability of the **DACs** formed. According to the theory of **DACs** [19], the stability of this class of complexes depends on the electron affinity of the acceptor and the ionization potential of the donor. These quantities can be approximated via Koopman's theorem by **quantum** chemically calculated energies of the lowest unoccupied (ϵ_{LUMO}) and highest occupied (ϵ_{HOMO}) molecular

TABLE I

ENERGY OF HOMO (ϵ_{HOMO}), DIPOLE MOMENTS (μ) AND CAPACITY FACTORS (k') OF TCDDs ON ELECTRON-ACCEPTOR PHASES, CYANOPROPYL-SG (CP-SG) AND AMINOPROPYL-SG (AP-SG) WITH HEXANE AS MOBILE PHASE

TCDD isomer	ϵ_{HOMO} (kcal/mol)	μ (D)	μ (D)[b]	k'						
				PFB-SG	DNB-SG	TCP-SG	TCPS-SG	TNF-SG	CP-SG	AP-SG
1368-	-9.063	0.004'	0.023	0.04	0.37	0.64	1.05	2.46	0.18	0.23
1378-	-9.026	0.860	1.323	0.06	0.41	0.77	1.20	2.89	0.22	0.32
1379-	-9.045	0.744	1.221	0.04	0.37	0.76	1.26	3.06	0.22	0.30
2378-	-8.998	0.021'	0.021	–[d]	0.54	0.93	1.37	3.19	0.26	0.41
1237-	-9.000	1.138	1.710	0.09	0.56	1.10	1.69	4.13	0.28	0.43
1238-	-9.001	1.792	2.668	0.09	0.56	1.10	1.87	4.13	0.32	0.61
1478-	-9.073	1.725	2.480	0.10	0.55	1.07	1.87	4.46	0.32	0.42
1278-	-9.004	1.672	2.467	0.13	0.64	1.23	2.12	4.49	0.37	0.70
1236-	-9.012	2.148	3.095	0.10	0.58	1.44	2.42	5.59	0.36	0.60
1267-	-9.019	0.031'	0.023	0.12	0.77	1.51	2.51	5.94	0.40	0.60
1234-	-8.981	2.515	3.727	0.11	0.58	1.57	2.98	6.60	0.35	0.50
1239-	-9.057	2.772	4.121	0.14	0.79	1.81	3.36	7.65	0.49	0.92
1289-	-9.012	2.802	4.220	0.19	0.90	2.53	4.44	8.79	0.69	1.89

[a] Calculated by AM1.
[b] Calculated by MOPAC (data taken from ref. 26).
[c] Not included in correlation analysis.
[d] Not measured.

orbitals of the acceptor and the donor, respectively [20].

Under the simplifying assumption that the complex formation is caused by the transfer of an electron from the HOMO of the donor to the LUMO of the acceptor, the corresponding stabilization energy *E* of structurally related compounds is given by

$$E = \frac{\text{constant}}{\epsilon_{HOMO} - \epsilon_{LUMO}} \qquad (1)$$

The calculated data for ϵ_{HOMO} are presented in Table I. Compared with ϵ_{HOMO} of strong donors (e.g., PAHs) they are too low for the formation of electron donor-acceptor complexes. We therefore conclude that the retention mechanism of TCDDs on EA phases is not predominantly the formation of DACs.

The immobilized ligands and the TCDDs have large dipole moments owing to the presence of halogen atoms or nitro groups. This suggests that the predominant forces between the solutes and the stationary phase are dipole-dipole interactions (orientation forces).

Kimata et al. [8] studied the retention order of several pairs of PCDD isomers on ODS-SG, PE-SG and an EA phase with methanol as mobile phase. They interpreted the observed retention tendency on the EA phase as the effect of differences in the dipolar character. Isomers with more dipolar character are retained longer than those with less dipolar character. They compared the retention order of isomers produced in the same reaction due to Smiles rearrangement with the magnitudes of the total dipole moment of the solutes.

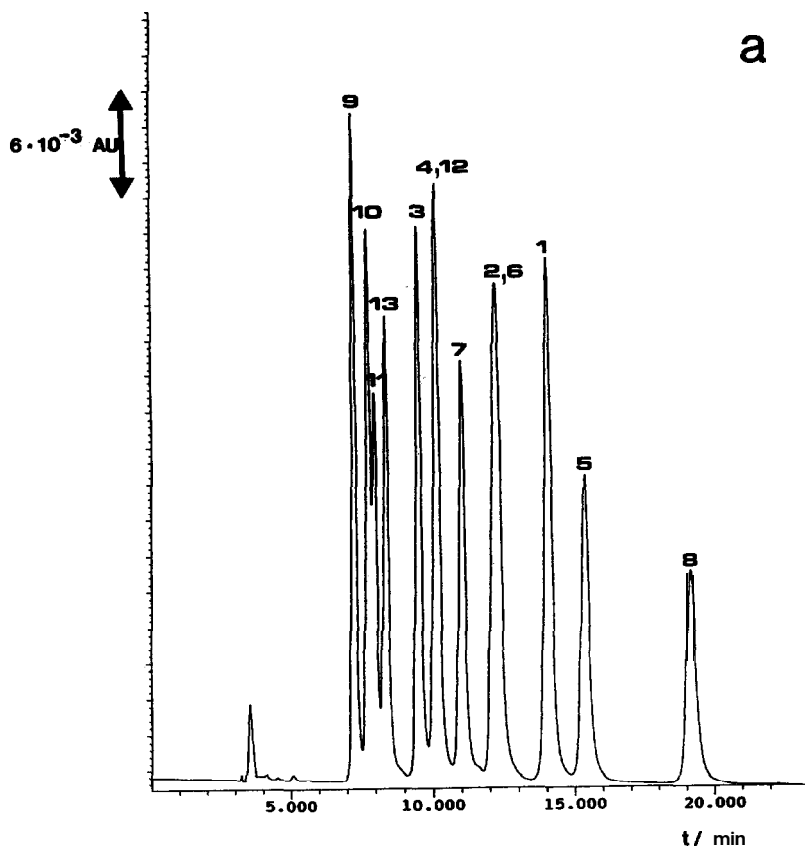The use of the total dipole moment as a descriptor in quantitative structure-retention
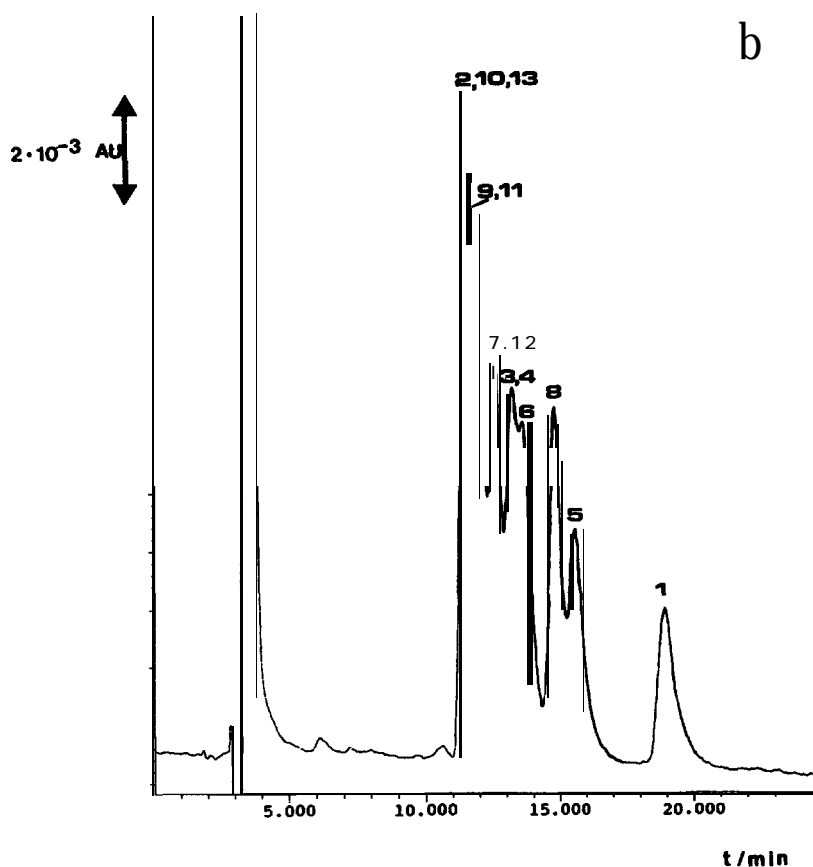

Fig. 3.

b

Fig. 3. Chromatograms of TCDD isomers eluted (a) from TCPS-SG with hexane as mobile phase and (b) from TCP-SG with methanol as mobile phase. Column, 250 mm × 4.6 mm I.D.; temperature, 25°C; detector wavelength, 235 nm; flow-rate, 1 ml/min. Peaks: 1 = 1234-TCDD; 2 = 1236-TCDD; 3 = 1237-TCDD; 4 = 1238-TCDD; 5 = 1239-TCDD; 6 = 1267-TCDD; 7 = 1278-TCDD; 8 = 1289-TCDD; 9 = 1368-TCDD; 10 = 1378-TCDD; 11 = 1379-TCDD; 12 = 1478-TCDD; 13 = 2378-TCDD.

relationship (QSRR) studies is controversially discussed in the literature [21]. Ong and Hites [22] reported that the square of the total dipole moment is a significant descriptor in a prediction equation for the retention of PCDD congeners on a non-polar column in GC. Kaliszan and co-workers [23–25], however, reported that correlation with retention data is better with a submolecular polarity parameter δ (maximum excess electron charge difference for a pair of atoms in the molecule). They stated [24] that if a solute is in specific contact with a stationary phase, not only will the dipole moment formed by the two atoms closest to the interacting surface count, but also the other more distant dipoles.

In the case of TCDD isomers, δ does not vary

greatly. It is given by the excess electric charge difference between the oxygen and its neighbours. For steric reasons the dipoles formed by the oxygen cancel each other, which does not mean that they are inactive in chromatographic interactions.

The differences between the TCDD isomers are given by the substitution pattern. The total dipole moment of the TCDD molecule is related to the symmetry of the substitution pattern. It is maximum if all the partial dipole moments contribute and do not cancel each other. In Table I the dipole moments, μ, calculated by AM1 are presented. They are compared with the values calculated by Koester and Hites [26], who used the MOPAC program. The deviation of the values calculated by different semi-empirical

methods is systematically about 50% of the value calculated by **AM1.** Linear correlation of the values obtained by the different methods shows a squared correlation coefficient $r^2 = 0.997$.

Comparing the capacity factors measured with strong EA phases, of TCDD isomers which differ only in the position of one chlorine sub-stituent underlines the tendency already observed by Kimata et al. [8]. **1234-, 1236-, 1237-** and **1238-TCDD** are eluted in the order given by their dipole moments. At the same time it can be seen that the selectivities of TCP-SG, TCP-SG and TNF-SG, which are basically the same, differ for **1237-** and **1238-TCDD.** The two iso-mers are only separated on TCPS-SG, although they differ considerably in their dipole moments.

The same tendency can also be observed with isomer pairs that differ only in the position of the two benzene rings with respect to each other (prepared in the same reaction, due to Smiles rearrangement). For the **1267-/1289-TCDD** and **1368-/1379-TCDD** pairs, those isomers where the partial dipole moments cancel each other are eluted before those with more aligned partial dipole moments.

Under the simple hypothesis that the inter-action of the solute and the stationary phase can be described by the interaction of the total dipole moment of the analyte, μ, and the dipole moment of the stationary phase, we would expect the following relationship [22]:

$$\ln k' = a + b\mu^2 \qquad (2)$$

Fig. 4 shows the logarithms of capacity factors of TCDD isomers plotted against the square of the dipole moments calculated by **AM1.** It can be clearly seen that analytes that are symmetrical with respect to the centre of gravity (**1267-, 1368-** and **2378-TCDD)** deviate strongly from the gen-eral tendency. Data points for these three iso-mers are given in brackets in Fig. 4. For symme-try reasons their dipole moments are close to zero. Parts of the molecule, however, exhibit large dipole moments and are therefore able to interact with the stationary phase. If these iso-mers are excluded from the regression analysis, a high correlation is observed.

In Table II the regression coefficients and regression parameters (a, *b)* obtained by linear

regression are given. Because of the very short retention times on the PFB-SG column, we did not calculate the regression coefficient for this phase. The need to exclude three isomers from the regression analysis shows that our model is too primitive to describe the retention process fully. Nevertheless, the observed correlation might be helpful in the scope of structure assign-ment [8].

The correlation is much better with the stronger retaining phases (TCPS-SG and **TNF-SG)** than with the phases with smaller retention forces. Comparison of the data for k' in Table I shows that there are some isomers that deviate characteristically from the general trend. These isomers are **1234-, 1236-** and **1379-TCDD,** which elute much earlier from PFB-SG and DNB-SG than expected from the retention order on **TNF-SG** or TCP-SG, respectively.

Comparison with polar bonded phases

In Table I the capacity factors for TCDDs on AP-SG and on CP-SG are also given. The retention order of the tested TCDDs follows the trend of the EA phases. The isomers **1234-, 1236-** and **1379-TCDD** show the same charac-teristic deviation from the retention order as found with PFB-SG and DNB-SG. The selectivi-ty is much better on the very polar AP-SG than on CP-SG.

From this comparison it can be deduced that the retention mechanism on polar bonded phases and on EA phases is basically the same, which supports strongly our assumption that the forma-tion of **DACs** is not involved in the retention of TCDDs on the studied EA phases. Differences in retention characteristics might be due to differences in polarity and also to differences in the geometric parameters of the immobilized ligands on the silica gel. **N-Propyl-2,4,7-trinitro-**fluorenone is about the same size as the TCDDs, so that interactions can take place between the entire molecules. **N-Propyltetrachlorophthal-**amide is smaller than TNF. The retention times on TCP-SG are shorter than those on TNF-SG, but the retention order was not altered.

DNB and PFB and also the aminopropyl and cyanopropyl groups are much shorter than the sorbed analytes. Interactions between the
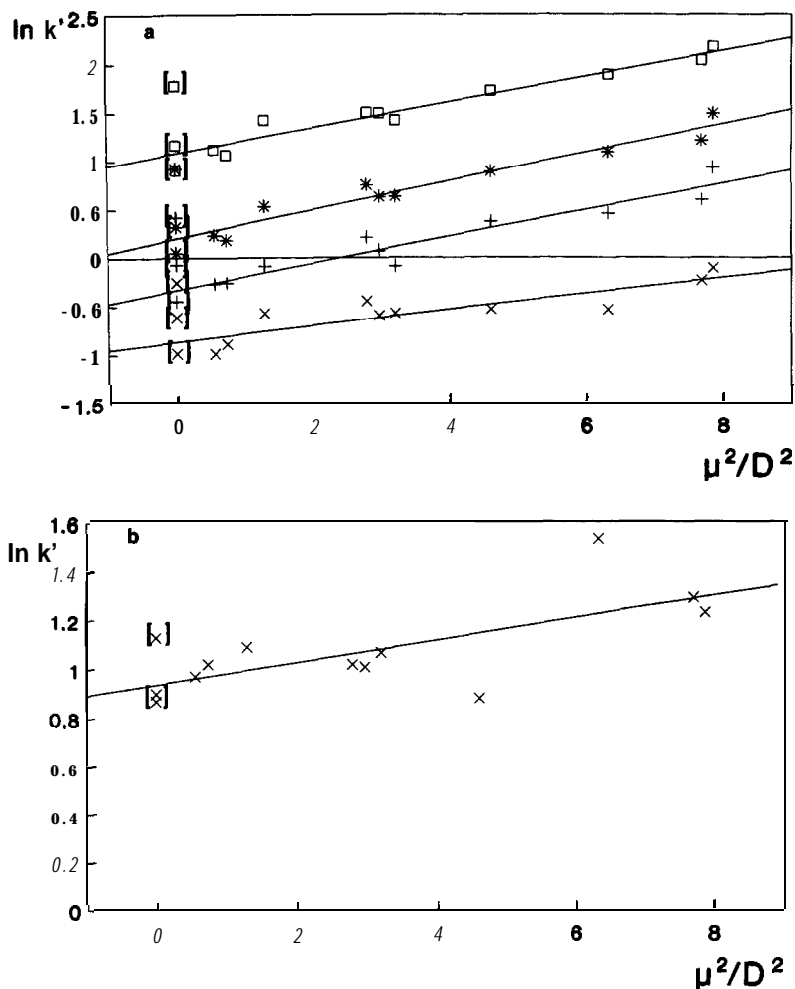
Fig. 4. Logarithms of capacity factory of TCDD isomers, obtained with EA phases, plotted against the square of their dipole moment. Mobile phase: (a) hexane; (b) methanol. Data in brackets were not included in the calculation of the regression line. (a) ✕ = DNB-SG; + = TCP-SG; * = TCPS-SG; □ = TNF-SG. (b) x = TCP-SG.

TABLE II

TABULAR FORM OF THE EQUATIONS EVALUATED BY LINEAR REGRESSION

Silica gel	a	b	R
CP-SG	-1.51	0.11	0.903
AP-SG	-1.18	0.16	0.827
DNB-SG	-0.86	0.083	0.863
TCP-SG	-0.33	0.14	0.952
TCPS-SG	0.20	0.15	0.970
TNF-SG	1.08	0.13	0.974

TABLE III

CAPACITY FACTORS (k') OF TCDDs on TCP-SG IN THE REVERSED-PHASE MODE WITH METHANOL AS MOBILE PHASE

TCDD isomer	k'	TCDD isomer	k'
1368-	2.47	1278-	2.77
137%	2.77	1236-	2.41
1379-	2.63	1267-	3.09
2378-	2.41	1234-	4.63
1237-	2.96	1239-	3.65
1238-	2.92	1289-	3.42
1478-	2.74		

stationary phase ligand and the solute can only take place with parts of the solute. This "shape effect" might explain the observed alterations in the retention order with respect to the retention order on TNF-SG.

TCP-SG in the reversed-phase mode

The TCP-SG column was also tested in the reversed-phase mode with methanol as the mobile phase. The capacity factors of the thirteen TCDD isomers on the column are presented in Table III. In the reversed-phase mode the retention order of the TCDDs is completely altered with respect to the separation in the normal-phase mode. Fig. 3 shows a **chromatogram** of the thirteen TCDD isomers obtained with TCP-SG in the reversed-phase mode in comparison with the chromatogram obtained with the same isomers on **TCP5-SG** with hexane as mobile phase. The alteration in retention order is not simply a reversal. The selectivity is lower compared with the selectivity of the same phase with hexane as the mobile phase. In the normal-phase mode, the selectivity factor α calculated from the first- and the last-eluted peaks is 3.95, whereas α calculated in the same manner after the separation with methanol as mobile phase is only 1.38. All capacity factors are much larger in the reversed-phase mode.

Obviously, in the reversed-phase mode the retention mechanism, which we attribute to dipole-dipole interactions, is partially masked by the separation mechanism of reversed-phase chromatography (separation according to **hydrophobicity** and geometrical parameters in the **analytes)**.

In Fig. 4b the logarithms of capacity factors are plotted against the squares of the dipole moments of the analytes. The correlation (1267-, 1368- and 237%TCDD excluded) is much weaker than in the normal-phase mode. The correlation factor is only 0.65.

For the above-discussed cases of very closely related isomers, the retention order still corresponds to the magnitude of the total dipole moment, with the exception of **1236-TCDD,** which elutes before **1237-** and **1238-TCDD.**

Isomers that could not be separated on an ODS, a PE or an EA column (with methanol as

mobile phase) might be separated on an EA column with non-polar mobile phases owing to the higher selectivity of EA phases in the reversed-phase mode.

PE-SG in the normal-phase mode

The capacity factors of thirteen TCDD isomers on the PE-SG column obtained in the normal-phase mode with hexane as the mobile phase are presented in Table IV The retention order of the analytes is completely different from that on EA phases in the normal-phase mode.

It can be deduced that the retention mechanism on this phase is different from that on the EA phases, which we have suggested to be primarily governed by dipole-dipole interactions. The pyrene immobilized on the column has a very low dipole moment. The dipole moments of **PAHs** are of the order of $\mu = 0.01$ D [22].

PE-SG was designed as an electron-donor phase for DAC LC. The energies of the lowest unoccupied orbital (ϵ_{LUMO}) of the TCDD isomers, presented in Table IV, are very low and suggest that the TCDDs are strong electron acceptors. For comparison, the values calculated by Koester and Hites [26] using the **MNDO** method are also given. They correlate excellently with the values calculated by us ($r^2 = 0.999$).

As already described in a previous section, for a retention mechanism involving the formation of **DACs,** from a theoretical point of view it can be expected that there is a direct but non-linear correlation between ln **k'** of the analytes and ● LUMo. However, a direct correlation of ln **k'** with ϵ_{LUMO} gave unsatisfactory results ($R = -0.05$). In spite of the unobserved correlation of ln **k'** with ● LuMo, which might be explained by an oversimplified theoretical approach not taking steric effects into account, we attribute the retention mechanism on PE-SG in the **normal-**phase mode to the formation of **DACs.** Our assumptions are supported by the following observations. In contrast to PE-SG, we measured no retention of TCDDs on the PP-SG column with hexane as the mobile phase. The phenyl group is a much poorer electron donor than the pyrene group. The retention order of PCDD **congeners** on PE-SG with non-polar

TABLE IV

ENERGY OF LUMO (ϵ_{LUMO}) AND CAPACITY FACTORS OF TCDDs ON PE-SG AND PP-SG

TCDD isomer	ϵ_{LUMO} (kcal / mol)	ϵ_{LUMO} (kcal/mol)[b]	k'		
			PE-SG'	PE-SG[d]	PP-SG
1234-	−0.8505	-1.349	3.19	17.32	6.66
1236-	-0.8280	-1.315	4.18	18.34	5.60
1237-	-0.8744	-1.363	3.90	15.01	4.50
1238-	-0.8731	-1.362	3.63	14.41	4.73
1239-	-0.8205	-1.310	4.22	15.94	4.75
1267-	-0.7925	-1.267	4.04	12.34	4.21
1278-	-0.8524	-1.336	4.01	13.28	4.23
1289-	-0.7892	-1.259	3.78	10.51	4.00
1368-	-0.8350	-1.317	3.42	19.26	6.17
1378-	-0.8743	-1.358	4.02	17.04	4.68
1379-	-0.8408	-1.328	3.63	16.47	5.03
1478-	-0.8116	-1.298	4.78	19.26	5.32
2378-	-0.9095	-1.396	4.74	15.59	3.58

. [a] Calculated by AM1.
[b] Calculated by MOPAC (data taken from ref. 26).
[c] Mobile phase: hexane.
[d] Mobile phase: methanol.
[e] Mobile phase: methanol-water (80: 20, v/v).

mobile phases (hexane-dichloromethane) is mainly governed by the degree of chlorination [27]. Studies with EA phases with different immobilized moieties show that an increasing number of electron-withdrawing substituents enhances the ability to form DAC complexes with electron donors [20].

PE-SG in the reversed-phase mode

In Table IV the capacity factors of the thirteen TCDD isomers on the PE-SG column, obtained in the reversed-phase mode (mobile phase methanol), are compared with those obtained in the normal-phase mode. On changing the mobile phase the retention order was completely altered, as can be seen in Figs. 5 and 6. The alteration is not simply a reversal of the retention order.

The separation of TCDD isomers on the PE-SG column with methanol as the mobile phase was extensively studied by Bamhart and co-workers [5]. They compared the selectivity of a PE-SG column with that of an ODS column and reported large differences, which they attributed

to the contribution of charge-transfer interactions .

Alteration of the retention order with respect to the retention order measured in the normal-phase mode suggests that in the reversed-phase mode the retention mechanism of the normal-phase mode, which we attribute to the formation of DACs, is partially superimposed by other retention forces such as the hydrophobicity of the analytes.

The capacity factors measured with methanol as the mobile phase are much larger than those obtained with hexane. The selectivity of PE-SG towards the TCDDs is enhanced in the reversed-phase mode. Fig. 5b presents a chromatogram of the thirteen TCDD isomers separated on a PE-SG column with methanol as the mobile phase. There are only two co-elutions (1239-/2378-TCDD and 1368-/1478-TCDD).

Comparison with Fig. 3 shows that the selectivity of TCP5-SG towards TCDDs in the normal-phase mode is better than the selectivity on PE-SG. Most of the peaks on TCP5-SG are baseline resolved. However, those isomers which

co-elute from **TCP5-SG** can be easily baseline resolved on PE-SG.

Comparison with PP-SG

The capacity factors of TCDD isomers on PP-SG are given in Table IV in comparison with those on PE-SG. No retention was measured with hexane as the mobile phase.

In the reversed-phase mode the retention order on PP-SG is different from that on PE-SG. PP-SG is not simply a reversed phase. The pair of isomers that cannot be separated with monomeric octadecyl phases (1368-/1379-TCDD) [10] is baseline resolved on PP-SG. Geometric parameters, which greatly influence the separation of **TCDDs** on polymeric octadecyl (ODS) phases, are obviously of no importance with regard to the retention order. **2378-TCDD**, the isomer with the highest length-to-breadth ratio

with respect to the other TCDD isomers [10], is eluted first, which is in contradiction to the theory of shape selectivity on polymeric ODS phases [28].

CONCLUSIONS

The tested DAC phases proved to be very useful for the preparation of pure TCDD standards after synthesis. The selectivities of **TNF**-SG/TCP-SG and PE-SG are complementary. In addition, these phases can be used in both the reversed- and normal-phase modes, exhibiting different selectivities. The highest selectivities were obtained for TNF-, TCP- and **TCP5-SG** in the normal-phase mode. The high capacity factors exhibited with PE-SG and methanol as mobile phase might prevent the application of PE-SG with methanol as mobile phase to PCDD
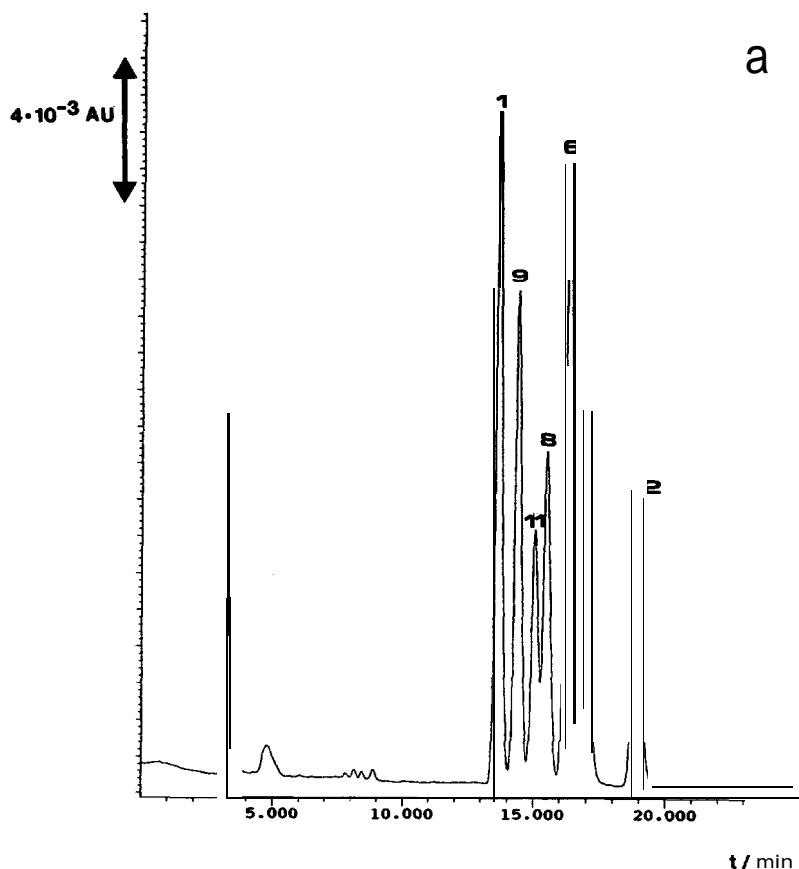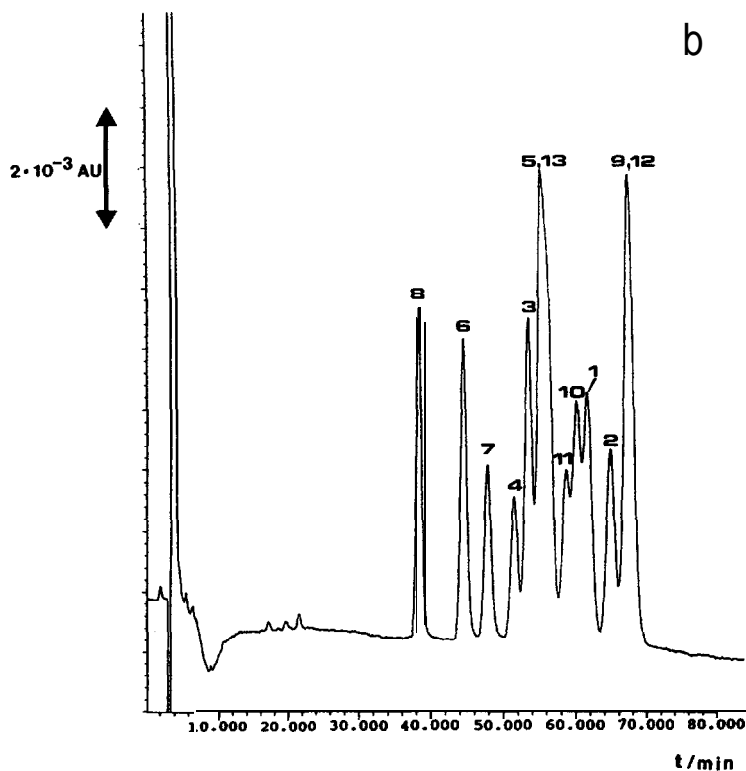

Fig. 5.

Fig. 5. Chromatograms of TCDD isomers eluted from PE-SG in (a) the normal-phase mode with hexane as mobile phase and (b) the reversed-phase mode with methanol as mobile phase. Column, 250 mm x 4.6 mm I.D.; temperature, 25°C; detector wavelength, 235 nm; flow-rate, 1 ml/min. Peak identification as in Fig. 3.
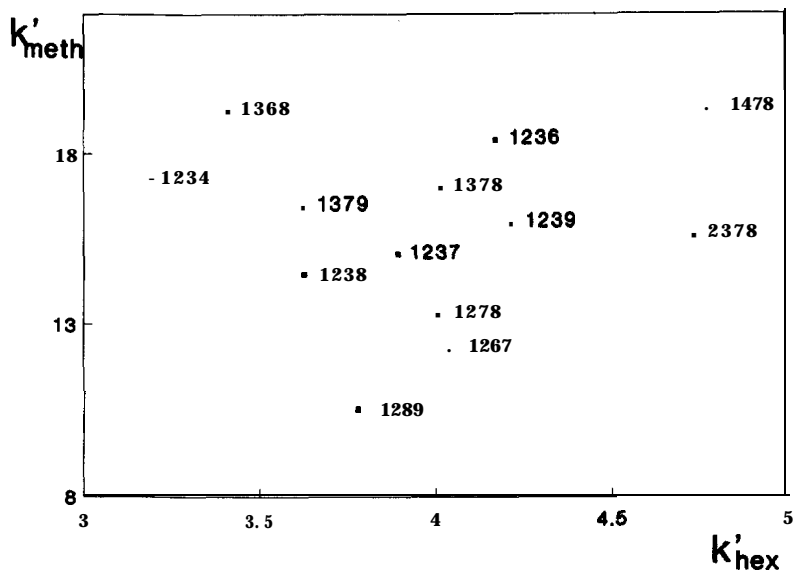

Fig. 6. Plot of capacity factors obtained on PE-SG in the reversed-phase mode (k'_{meth}) against those obtained in the normal-phase mode (k'_{hex}) (experimental parameters as in Fig. 5).

congeners with more than four chlorine sub-
stituents.

Application of non-polar mobile phases avoids
solubility and possibly baseline shift problems
reported by workers who used heart-cutting
techniques employing RP-HPLC on various
stationary phases for the purification and concen-
tration determination of PCDD and PCDF stan-
dard solutions [9].

Another interesting application field of the
studied stationary phases is the clean-up of
samples prior to PCDD and PCDF analysis.
Investigations on this aspect are in progress.

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