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Preparation of nitrophenylethylsilylated silica gel and its chromatographic properties in the separation of polychlorinated dibenzo-*p*-dioxins

Kazuhiro Kimata, Ken Hosoya, Nobuo Tanaka* and Takeo Araki

Kyoto Institute of Technology, Department of Polymer Science and Engineering, Matsugasaki, Sakyo-ku, Kyoto 606 (Japan)

D. G. Patterson, Jr.

Centers for Disease Control, Toxicology Branch, Atlanta, GA 30333 (USA)

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ABSTRACT

Nitrophenylethylsilylated (NPE) silica gel was prepared from phenylethylsilylated silica gel by direct nitration, and its chromatographic properties were compared with those of C_{18} and pyrenylethyl (PYE) bonded phase in reversed-phase high-performance liquid chromatography. The NPE phase showed preferential retention for aromatic compounds, especially for those with dipolar character. In contrast, PYE phase showed preferential retention for symmetrically substituted aromatic compounds. The combination of NPE and PYE phases provided the selectivity required for the separation of isomers of polychlorinated aromatic compounds, namely polychlorodibenzo-*p*-dioxins (PCDDs). The two stationary phases, having aromatic functionalities of opposite nature, also provided the possibility of structural assignment of PCDD isomers based on the chromatographic retention on the two phases.

INTRODUCTION

Reversed-phase high-performance liquid chromatography (RP-HPLC) has been used for the separation of extremely wide ranges of compounds owing to its high performance and the applicability of alkylsilylated silica packing materials (C_{18}). In addition, several packing materials capable of charge-transfer interactions have been reported. This adds more selectivity to RP-HPLC, whereas the most popular silica C_{18} packing materials separate solutes mainly based on their total hydrophobic properties. Although steric discrimination by the alkyl groups in the stationary phase has been observed [1–3], the interaction between the bonded alkyl groups and solutes is believed to be weak [4].

Pyrenylethylsilylated (PYE) silica gel offered much greater selectivity towards molecules with a difference in planarity in aromatic and alicyclic structures [5]. Graphite carbon packing materials have been reported to be even more selective for these compounds based on the strong planarity recognition, charge-transfer interactions and the contribution of dispersion forces [6–9]. These two types of packing materials possess common features in that the planar binding sites having electron-donating and hydrophobic properties contribute to the retention process [9].

When applied to the separation of polychlorinated aromatic compounds, PYE phase permitted the separation of polychloropyrenes [10] and most of the 22 isomers of tetrachlorodibenzo-*p*-dioxins (TCDDs) [11]. Another charge-transfer type of stationary phase having an electron-withdrawing group on an aromatic ring, nitrophenylethylsilylated (NPE) silica gel, permitted the separation of TCDD isomers [12], which was not possible with either C_{18} or PYE phases. Although nitrophenyl-

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bonded phases have been used in RPLC [13] and in many chiral stationary phases, no systematic application of such bonded phases has been reported for the separation of chlorinated aromatic compounds in RP-HPLC.

We report here a simple method for the preparation of NPE phase from readily available phenylethylsilylated (PE) silica gel, and some of the properties of the resulting NPE phase. The combination of the two charge-transfer-type stationary phases, PYE and NPE, and also C_{18}, not only increases the separation capabilities but also provides structural information for isomeric aromatic compounds with multiple substituents, because the NPE and PYE phases possess considerable differences in the dipolar character of the aromatic moieties.

EXPERIMENTAL

Equipment

The HPLC system consisted of an LC-6A pump, a SIL-6A automatic sample injector, an SPD-6A variable-wavelength UV detector, a C-R5A data processor (Shimadzu, Kyoto, Japan) and an RI-8 refractive index detector (Tosoh, Tokyo, Japan). Measurements with chlorinated dioxins were carried out at the Centers for Disease Control (Atlanta, GA, USA) by using a Beckman System Gold HPLC system (Model 126 pump and Model 166 UV detector; Beckman Instruments, San Ramon, CA, USA). The column temperature was maintained at 30°C by using a thermostated water-bath.

Preparation of NPE phase

Concentrated nitric acid (2.5 ml) was added dropwise to 4.0 g of phenylethylsilylated silica gel (particle size 5 μm, prepared from Develosil, Nomura Chemicals, Seto, Japan) suspended in 50 ml of acetic anhydride at 0°C, and the mixture was stirred for 1 h in an ice-bath. The reaction mixture was filtered with a PTFE membrane filter, and the particles were washed with 100 ml of acetic acid. The silica particles were further washed successively with 100 ml each of ethyl acetate, methanol, methanol–water (80:20, v/v), methanol and chloroform before drying. Trimethylsilylation was carried out with this material. Toluene and isopropylbenzene were nitrated under similar conditions in order to examine the reactivity of benzene derivatives.

Structure determination of the NPE phase

The NPE silica (5 g) was dissolved in dilute hydrofluoric acid (1:1, v/v) (30 ml), and the resulting organic portion was chromatographically separated [C_{18} column, methanol–water (70:30, v/v) as eluent]. NMR measurements, were carried out on an XL 200 NMR instrument (Varian, Sunnyvale, CA, USA).

Materials

The NPE prepared as above, PYE (particle size 5 μm, Cosmosil 5-PYE, Nacalai Tesque, Kyoto, Japan) and C_{18} (particle size 5 μm, Cosmosil 5-C_{18}) packing materials were packed into stainless-steel tubes (15 or 10 cm × 4.6 mm I.D.). Polychlorodibenzo-*p*-dioxin (PCDD) mixtures were prepared at the Centers for Disease Control as described previously [14], and 0.1–1.0 μl of toluene solutions (*ca.* 1 mg/ml) were injected. Other chemicals and HPLC-grade solvents were obtained commercially.

RESULTS AND DISCUSSION

Preparation of NPE phase

Direct nitration of PE silica was employed to produce NPE phase. This approach avoids the complicated synthesis of nitrophenylsilanes to be used for the preparation of bonded phases. The precursor PE silica is readily available.

Nitration of monosubstituted benzenes usually results in a mixture of isomeric products. Fig. 1 shows the variation of the composition of the reaction mixture for the nitration of toluene and isopropylbenzene. The reactions proceeded to more than 95% completion in 15 min in both instances. The nitration of toluene with concentrated nitric acid in acetic anhydride gave *o*- and *p*-nitrotoluene in a 65:35 ratio. In contrast, isopropylbenzene mainly resulted in nitration at the *para* position (75%) owing to the steric repulsion by the bulky isopropyl group. The results agreed well with those reported [15].

The nitration of PE silica was carried out for 1 h under the same reaction conditions. The surface coverages of NPE and other bonded phases are listed in Table I. Elemental analysis indicates the presence of one nitro group per phenyl ring on NPE. Up to 25% loss of PE bonded phase owing to the decomposition of the stationary phase under the high-

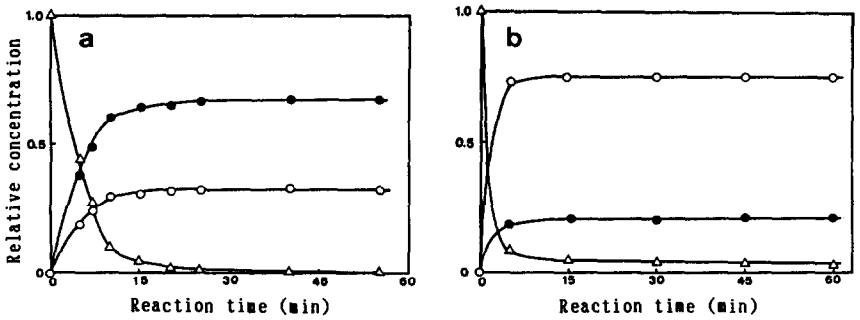


Fig. 1. Nitration of (a) toluene and (b) isopropylbenzene. Vertical axis represents relative concentrations. (△) Starting material; (○) *para*-substituted product; (●) *ortho*-substituted product. Determined by RP-HPLC. Column, C_{18} (15 cm × 4.6. mm I.D.); mobile phase, (a) methanol–water (60:40, v/v); (b) methanol–water (70:30, v/v).

ly acidic reaction condition was noted. Although the nitration afforded good reproducibility (± 1%) in terms of capacity factors (k' values) for hydrocarbons, esters and ketones, for the three preparations of NPE phases from one batch of PE silica gel, poorer reproducibility (± 2.8% in k') was observed for dipolar molecules such as dinitrobenzenes or dinitronaphthalenes. The separation factors between *o*- and *p*-dinitrobenzene were in the range 1.54 ± 0.05 on the seven NPE phases prepared from three different batches of silica gel. The two dinitrobenzenes showed reversal of the elution order between NPE and PE phases, and gave poorer reproducibility than the other compounds.

In order to determine the structure of the bonded nitrophenylethyl group, the NPE silica was subjected to hydrofluoric acid degradation followed by chromatographic separation and subsequent NMR spectroscopy. Fig. 2 shows the chromatogram obtained for the hydrofluoric acid degradation products. The ratio between the areas of peaks A and B was found to be 28:72 based on the response of the refractive index detector.

The NMR spectra obtained for the two fractions indicated that peak B contained a 2-*p*-nitrophenylethyldimethylsilyl group. The steric requirement of the bonded phenylethyl group resulted in selective nitration at the *para* position.

Chromatographic properties of NPE phase

The hydrophobic property of NPE phase, as measured by the separation factor between toluene and benzene, was much smaller than those of C_{18} and PYE and similar to that of PE silica. NPE

TABLE I

SURFACE COVERAGE AND CHROMATOGRAPHIC PROPERTIES OF PACKING MATERIALS

Stationary phase	Elemental Analysis[a]			Surface coverage (μmol/m^2)	Separation factor (k')			
	H (%)	C (%)	N (%)		CH_3[b]	$COOCH_3$[b]	PAH[c]	(C_6H_6)[d]
C_{18}	3.68	19.17	0	3.22	1.96	0.80	6.25	(3.36)
PYE	1.82	18.45	0	2.99	1.83	1.86	24.4	(1.86)
PE	1.72	11.50	0	3.42	1.60	1.20	4.04	(1.47)
NPE	1.44	9.25	1.04[e]	2.56	1.58	1.39	11.5	(0.96)

[a] Prior to trimethylsilylation.
[b] The k' ratios between toluene and benzene, and between methyl benzoate and benzene in methanol–water (60:40, v/v).
[c] The k' ratio between triphenylene and benzene in methanol–water (90:10, v/v).
[d] The k' value of benzene in methanol–water (60:40, v/v).
[e] Corresponds to 1.02 NO_2 group per phenyl group.

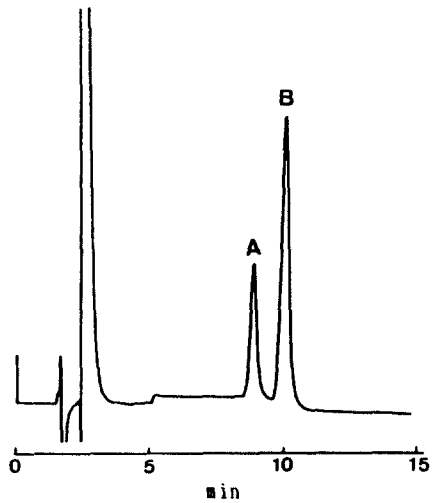


Fig. 2. Separation of hydrofluoric acid degradation products of NPE phase by RP-HPLC. Column, C_{18} (15 cm × 4.6 mm I.D.); mobile phase, methanol–water (70:30, v/v).

phase shows selective retention of polar and polycyclic aromatic compounds compared to PE phase, as shown in Table I. PYE showed the influence of charge-transfer interactions.

Fig. 3 shows the plot of log k' values for benzene derivatives against log P values obtained in a 1-octanol–water liquid–liquid partition system [16]. The C_{18} phase showed good linearity, indicating that the hydrophobic interaction played a major role in the retention process. PYE showed a positive deviation for compounds with either electron-withdrawing or electron-donating substituents. This is similar to the case with carbon packing material [9].

It is interesting to note the similar behaviour of the PYE and NPE phases, both selectively retaining polar compounds compared with non-polar solutes such as alkylbenzenes. There is, however, a slight difference between these two aromatic stationary phases. The NPE phase showed a slightly greater

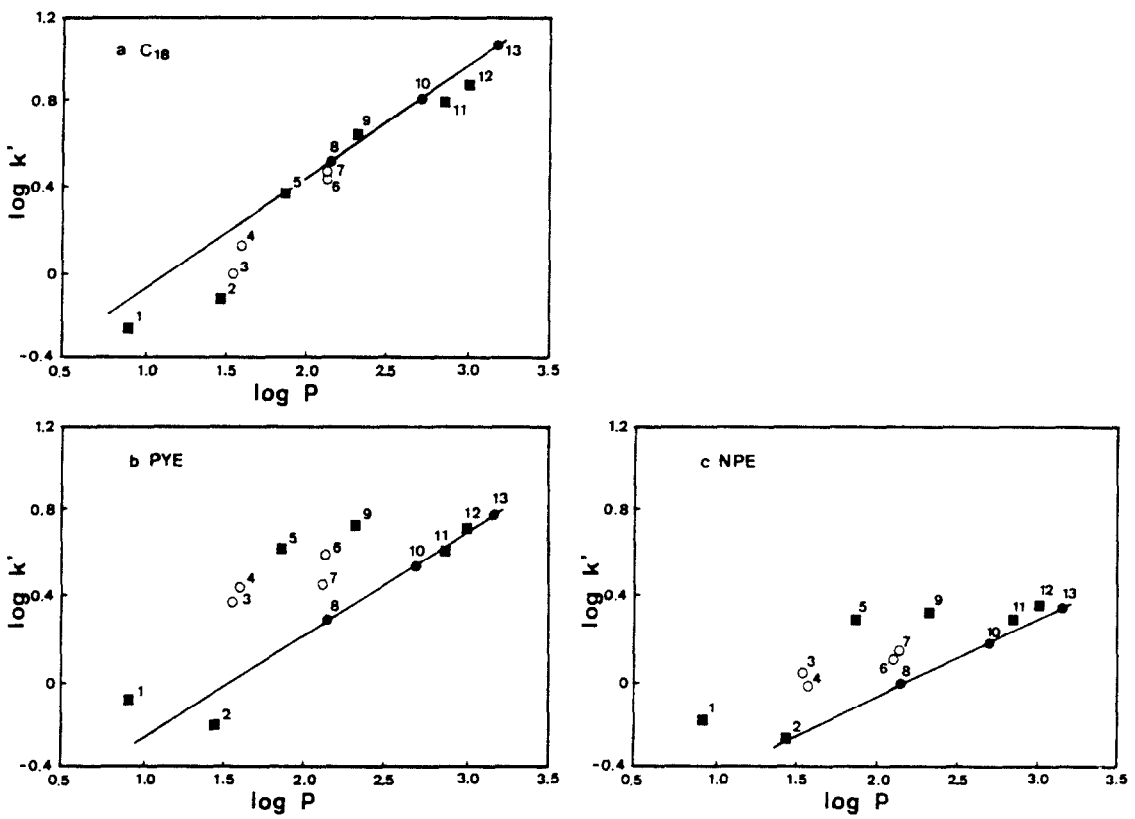


Fig. 3. Plots of log k' values against log P values for monosubstituted benzenes. (1) NH_2; (2) OH; (3) CN; (4) $COCH_3$; (5) NO_2; (6) $COOCH_3$; (7) OCH_3; (8) H; (9) $N(CH_3)_2$; (10) CH_3; (11) Cl; (12) Br; (13) C_2H_5. Mobile phase, methanol–water (60:40, v/v). Column: (a) C_{18}; (b) PYE; (c) NPE. The straight lines were drawn through the plots for (8), (10) and (13).

preference for substituted benzenes with OH, NH_2, $N(CH_3)_2$, Cl, Br and NO_2 groups (squares in Fig. 3) than the PYE phase, which favoured other compounds with electron-withdrawing substituents (open circles in Fig. 3).

The chromatograms in Fig. 4 show a preferential retention of dinitronaphthalenes (peaks 1 and 2) on the charge-transfer-type phases compared with the C_{18} phase. The difference between PYE and NPE is also clearly noticeable. Whereas 1,5-dinitronaphthalene (peak 2) was preferentially retained by the PYE phase, 1,8-dinitronaphthalene (peak 1) was retained longer on the NPE phase. This cannot be explained by consideration of charge-transfer interaction between the aromatic rings.

The results with the NPE phase in Figs. 3 and 4 indicate the presence of strong dipole–dipole interactions. The molecule of 1,8-dinitronaphthalene possesses the two nitro group dipoles aligned for a much greater dipolar interaction with the bonded nitrophenyl group than 1,5-dinitronaphthalene. Similarly, *o*-dinitrobenzene was retained longer

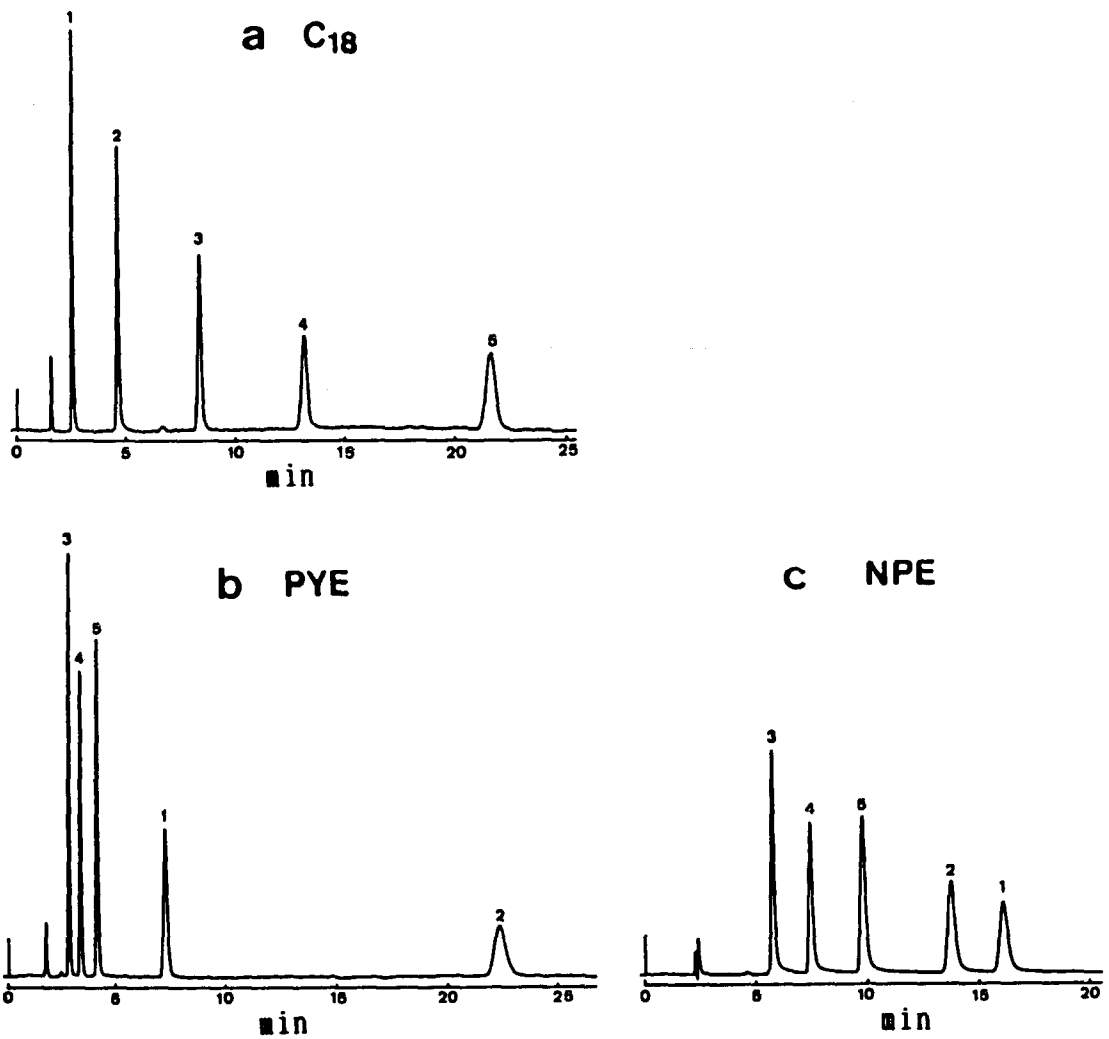


Fig. 4. Separation of naphthalene derivatives. Mobile phase and column: (a) C_{18}, methanol–water (70:30, v/v); (b) PYE, methanol–water (90:10, v/v); (c) NPE, methanol–water (70:30, v/v). Solutes: (1) 1,8-dinitronaphthalene; (2) 1,5-dinitronaphthalene; (3) naphthalene; (4) 1-methylnaphthalene; (5) 1,5-dimethylnapthalene.

than *p*-dinitrobenzene on NPE phase, whereas the elution order was reversed on PYE phase, and on PE phase.

Separation of polychlorinated aromatic compounds

The three stationary phases, NPE, PYE and C_{18}, showed substantial differences in the retention selectivity toward polychlorinated aromatic compounds. As shown in Fig. 5a, the C_{18} phase showed a preferential retention of polychlorobenzenes with chlorine atoms in *meta* positions to each other. PYE phase preferentially retained those with the chlorine atoms located as far apart as possible from each other. In contrast, NPE phase showed the greatest retention for polychlorobenzenes with an *ortho* arrangement of the chlorine groups. This tendency is similar to the example in Fig. 4, where 1,8-dinitronaphthalene with aligned dipoles was preferentially retained by NPE phase. The least hydrophobic 1,2,3,4-tetrachlorobenzene showed the shortest retention among the three tetrachlorobenzenes on C_{18} and PYE and the greatest retention on NPE phase.

The complete reversal of the elution order of tetrachlorobenzenes between NPE and PYE phase suggests the possibility of providing structural information for isomeric polychlorinated aromatic compounds such as PCDDs based on their chromatographic behaviour. In addition, the present combination of the C_{18}, PYE and NPE stationary phases is expected to increase the separation capability for the closely related PCDD isomers.

The separation of all the congeners of PCDDs is extremely difficult, whereas PCDDs having different numbers of chlorine atoms can be separated by high-resolution gas chromatography (HRGC) [17]. Therefore, several groups of TCDDs are currently measured together in environmental analysis [18–20]. The combintaion of RP-HPLC on C_{18} phase and normal-phase HPLC on silica gel distinguished most of the 22 TCDD isomers, but with several pairs remaining unidentified even with the use of

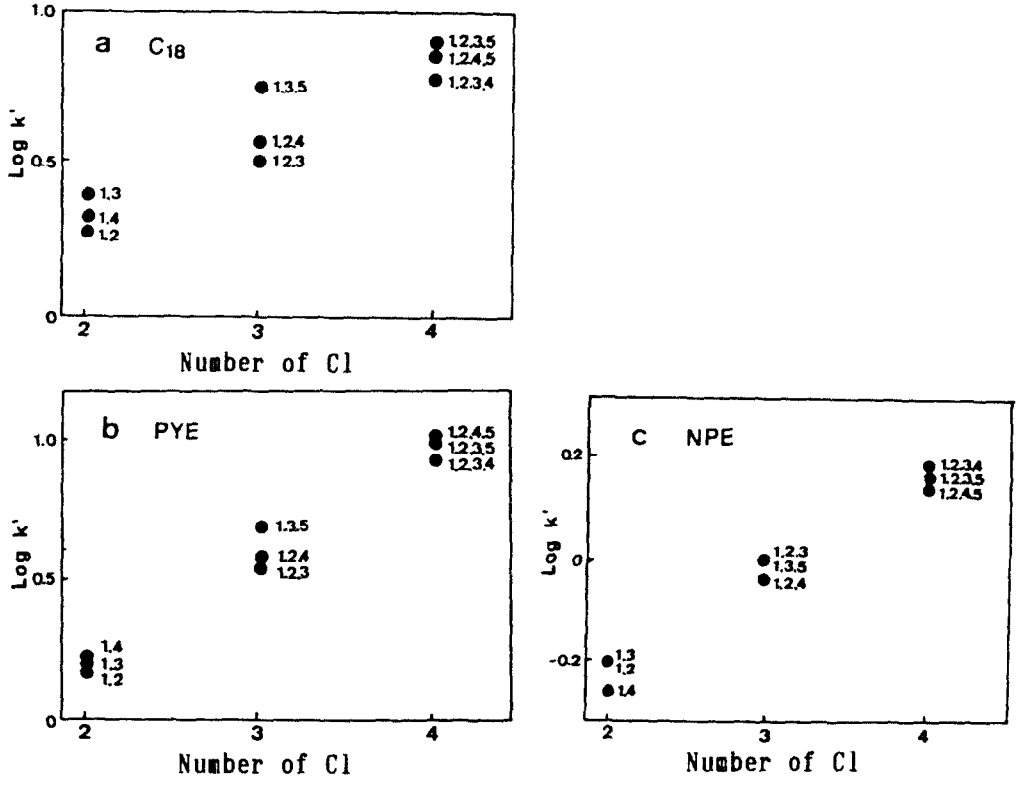
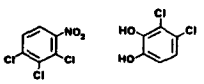
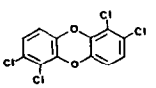
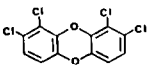


Fig. 5. Retention of polychlorobenzenes. Mobile phase, methanol–water (80:20, v/v). Column: (a) C_{18}; (b) PYE; (c) NPE.

HRGC [21]. In the past, the difficulty with isomer separation led to ambiguous structural assignments, especially when two isomers were present in a mixture at similar concentrations. Some structural assignments have been performed based on Fourier transform IR and NMR spectra by using mixtures [14,22–24]. Many TCDDs in HRGC have been identified by using the photolysis of hexachlorodibenzo-*p*-dioxins (HxCDDs) with known structures [25].

TABLE II

STRUCTURES OF PCDD ISOMERS

Starting materials		PCDD products	
			
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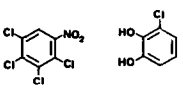		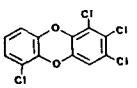	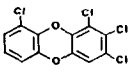
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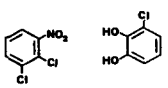		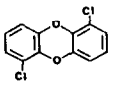	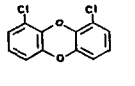
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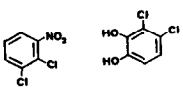		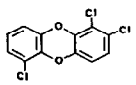	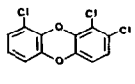
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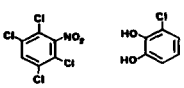		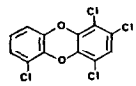	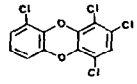
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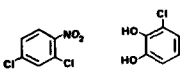		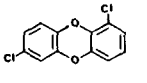	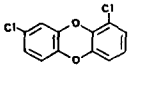
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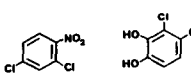
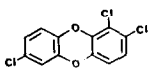
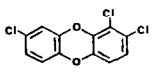
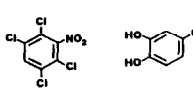
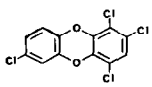
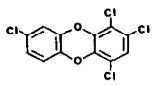
			
		1,2,7-TrCDD	1,2,8-TrCDD
			
		1,2,4,7-TCDD	1,2,4,8-TCDD

The isolation of each PCDD congener is also required in order to examine individual toxicity and teratogenicity. Many PCDDs, however, are necessarily produced as a mixture owing to the Smiles rearrangement during the preparation reaction [26,27]. Table II shows several pairs of PCDD isomers co-produced during preparation that need to be separated. For example, the reaction of 3,4-dichlorocatechol with 2,3,4-trichloronitrobenzene results in a mixture of 1,2,6,7- and 1,2,8,9-TCDD. The separation and identification of 1,2,6,7- and 1,2,8,9-TCDDs and 1,2,3,6- and 1,2,3,9-TCDDs have been reported [28], and these TCDDs were used here to show how the retentions of PCDDs on PYE and NPE phases can be related to their structures.

Figs. 6 and 7 show the separation of synthetic mixtures of 1,2,6,7- and 1,2,8,9-TCDDs and 1,2,3,6- and 1,2,3,9-TCDDs on C_{18}, PYE and NPE phases. The identities of the peaks were provided by comparison of the retention on C_{18} phase [28] and the peak size ratios with the reported values [14]. On C_{18} and PYE phases, the isomers existing in a greater amount with less steric congestion among the chlorine atoms, 1,2,6,7- and 1,2,3,6-TCCD, were retained longer than 1,2,8,9- and 1,2,3,9-TCDD, respectively. In contrast, 1,2,8,9- and 1,2,3,9-TCCD were retained longer than 1,2,6,7- and 1,2,3,6-TCDD, respectively, on the NPE phase. The opposite retention order of these TCDDs on PYE and NPE phases is in agreement with the results for polychlorobenzenes (Fig. 5) and dinitronaphthalenes (Fig. 4).

The retention on C_{18} phase is primarily determined by the hydrophobic property of the solute [4]. As chlorine atoms on an aromatic ring increase the hydrophobic property [16], compounds with isolated chlorine substituents are retained longer than those with sterically congested chlorine atoms owing to the greater hydrophobic surface areas. Hence the proximity between the 1- and 9-chlorine atoms resulted in a smaller retention of 1,2,8,9-TCDD and 1,2,3,9-TCDD than 1,2,6,7-TCDD and 1,2,3,6-TCDD, respectively, on C_{18} phase.

As the greatest retention was observed on PYE phase for the polychlorobenzenes with minimum steric congestion due to the most favourable

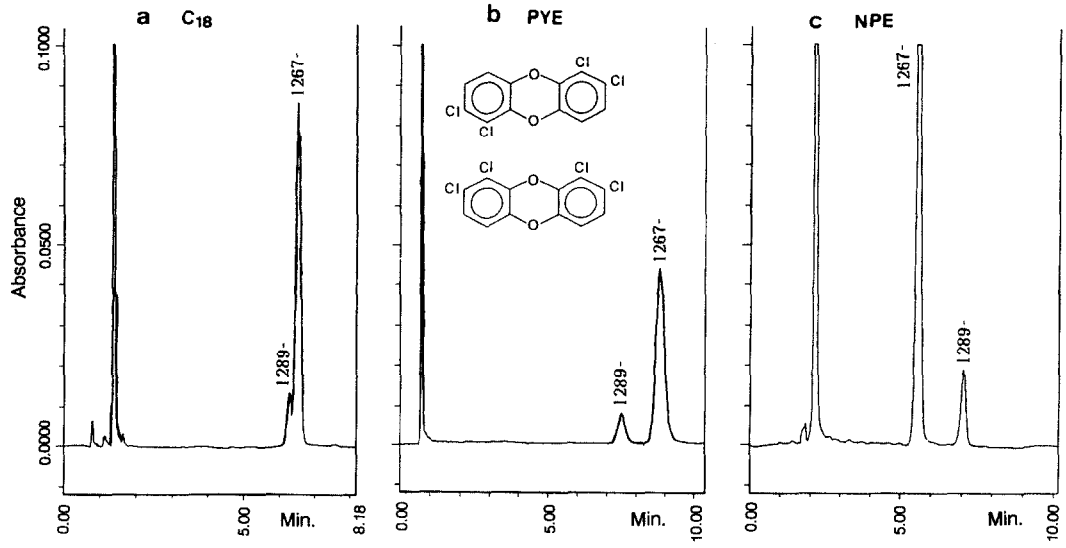


Fig. 6. Separation of 1,2,6,7- and 1,2,8,9-TCDDs. Mobile phase and column: (a) C_{18}, 15 cm, methanol–water (90:10, v/v) at 2 ml/min; (b) PYE, 10 cm, methanol at 2 ml/min; (c) NPE, 15 cm, methanol–water (90:10, v/v) at 1 ml/min.

charge-transfer interactions, the longer retention times for 1,2,6,7-TCDD and 1,2,3,6-TCDD than 1,2,8,9-TCDD and 1,2,3,9-TCDD, respectively, are readily understandable. In contrast, the TCDDs with the greater steric congestion among the chlorine atoms, existing as minor components in reaction mixtures, are retained longer than the more symmetrically substituted TCDDs by NPE phase. This is presumably due the more aligned dipoles in 1,2,8,9- or 1,2,3,9-TCDD than in 1,2,6,7- or 1,2,3,6-TCDD, respectively, as with dinitronaphthalenes and polychlorobenzenes.

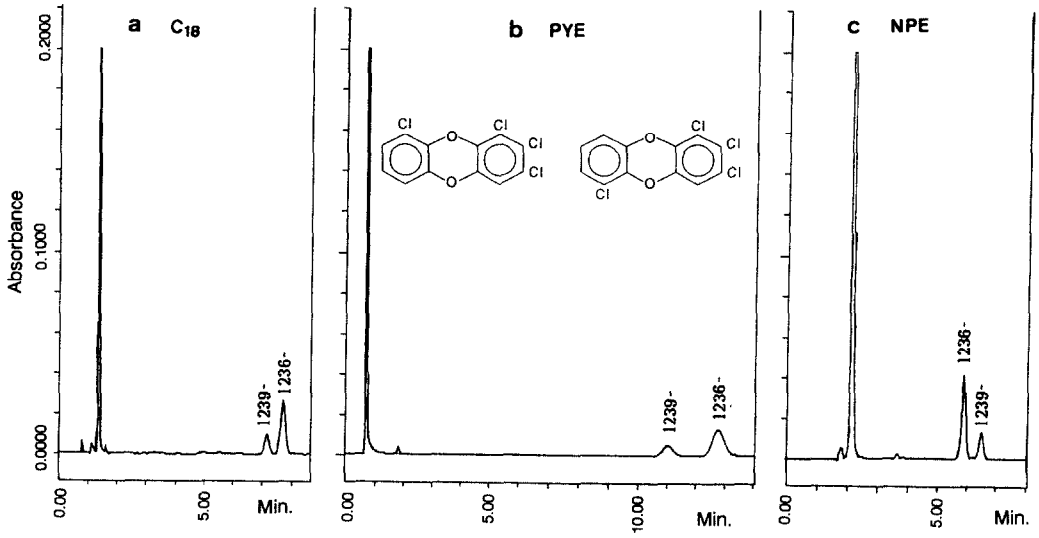


Fig. 7. Separation of 1,2,3,6- and 1,2,3,9-TCDDs. Conditions as in Fig. 6.

Possibility of structural assignment for PCDDs based on retention on NPE and PYE phases

Difficulties in separation are usually encountered for PCDDs with product ratios close to unity, which also made the spectroscopic structural assignment more difficult when dealing with a mixture. The results showing the clear reversal of the elution order on PYE and NPE phases indicate the possibility of structural assignment for such PCDD isomers that have been providing difficulties. The following reports the first attempts at the application of the chromatographic system with a combination of NPE and PYE phases to the structural assignment of PCDDs.

The separation and identification of two pairs of TCDD isomers, 1,2,4,6- and 1,2,4,9-TCDDs and 1,2,4,7- and 1,2,4,8-TCDDs, are particularly interesting, because these TCDDs have not been separated by either HRGC or RP-HPLC on C_{18} phase. Normal-phase chromatography on silica gel separated these TCDD isomers, but identification was not achieved [21,28]. We previously reported the separation of 1,2,4,6- and 1,2,4,9-TCDDs using NPE [12] and 1,2,4,7- and 1,2,4,8-TCDDs using PYE [11], but could not provide definite structure assignments. In this study, we included 1,6- and 1,9-dichlorodibenzo-*p*-dioxins (DCDDs) and 1,2,6- and 1,2,9-trichlorodibenzo-*p*-dioxins (TrCDDs) with 1,2,4,6- and 1,2,4,9-TCDDs, and 1,7- and 1,8-DCDDs and 1,2,7- and 1,2,8-TrCDDs with 1,2,4,7- and 1,2,4,8-TCDDs for the following reasons. First, these DCDD and TrCDD isomers are assumed to be easier to separate than the TCDDs, because of the greater difference in dipolar character. Second, the chromatographic behaviour of the DCDDs and the TrCDDs would give structural information for the TCDDs by taking into account the effect of additional chlorine atoms. Third, the structural assignment of DCDDs and TrCDDs is also important.

Fig. 8 shows the chromatograms of 1,6- and 1,9-DCDDs, 1,2,6- and 1,2,9-TrCDDs and 1,2,4,6- and 1,2,4,9-TCDDs on C_{18}, PYE and NPE phase. The C_{18} phase showed the greater retentions for the DCDD and TrCDD isomers present in greater amounts, which are assumed to be the isomers possessing sterically less congested structures. PYE phase showed a preference for PCDDs with more symmetrical chlorine substitution, generally giving a similar retention order to C_{18} phase. NPE phase also gave easy separations of 1,6- and 1,9-DCDDs and 1,2,6- and 1,2,9-TrCDDs. The elution orders,

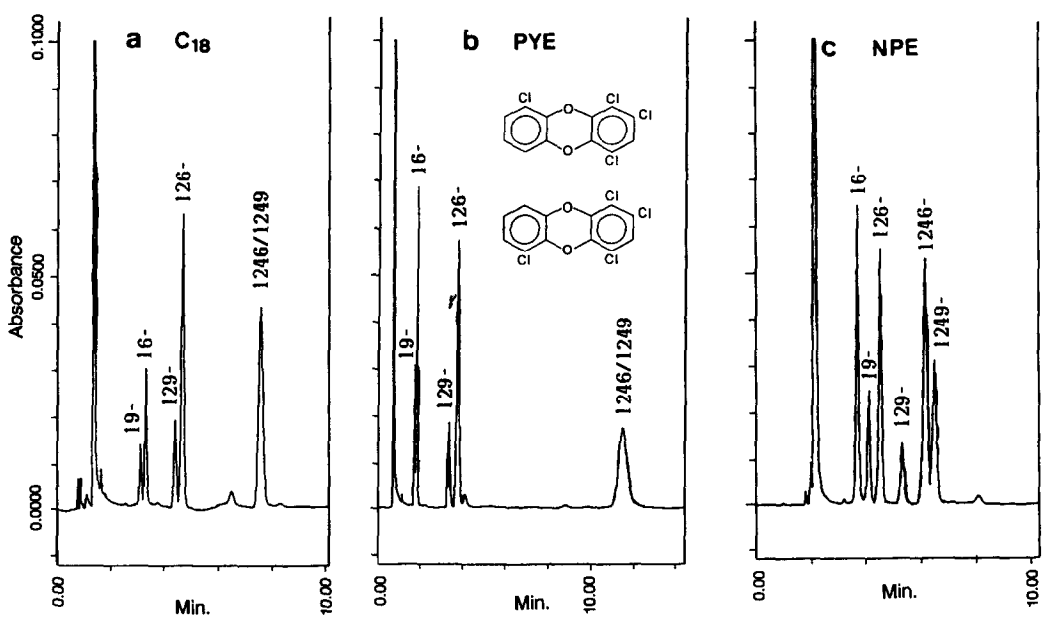


Fig. 8. Separation of 1,6- and 1,9-DCDDs, 1,2,6- and 1,2,9-TrCDDs and 1,2,4,6- and 1,2,4,9-TCDDs. Conditions as in Fig. 6.

however, are reversed from those on C_{18} or PYE phase. The results are understandable on the basis of the interaction of the more aligned dipoles of 1,9-DCDD and 1,2,9-TrCDD with NPE phase compared with 1,6-DCDD and 1,2,6-TrCDD, respectively.

Based on these considerations, the late-eluting peaks on C_{18} and PYE with the greater size are tentatively assigned to 1,6-DCDD and 1,2,6-TrCDD, as shown in Fig. 8. The present assignment is consistent with the results with 1,2,6,7- and 1,2,8,9 - TCDDs and 1,2,3,6- and 1,2,3,9-TCDDs.

Although 1,2,4,6- and 1,2,4,9-TCDDs have not been separated by using either C_{18} or PYE phase, NPE phase permitted the easy separation of these TCDDs. The chromatographic behaviour of the three pairs of isomers are expected to follow a similar tendency with the successive addition of chlorine atoms first at the 2-position and then at the 4-position to the original 1,6- and 1,9-DCDD skeleton. Thus the second peak on NPE existing in a smaller amount is tentatively assigned to 1,2,4,9-TCDD, which agrees with its more dipolar character than 1,2,4,6-TCDD (Table III) [29]. This assignment,

TABLE III

RETENTION OF PCDDs IN RP-HPLC AND STRUCTURE ASSIGNMENTS

PCDD mixture	k' value (proportion, %)			Dipole moment[e] (D)	Heat of formation[e] (kcal/mol)
	C_{18}[b]	PYE[c]	NPE[d]		
1,2,8,9-/	6.39 (11)	10.4 (12)	2.64 (12)	4.220	−40.488
1,2,6,7-	6.66 (89)	12.4 (88)	1.91 (88)	0.023	−40.913
	[α = 1.04][a]	[α = 1.19]	[α = 0.72]		
1,2,3,9-/	8.08 (25)	16.1 (32)	2.53 (25)	4.121	−40.012
1,2,3,6-	8.72 (75)	18.8 (68)	2.20 (75)	3.095	−40.324
	[α = 1.08]	[α = 1.17]	[α = 0.87]		
1,9-/	2.74 (29)	1.67 (28)	1.17 (29)		
1,6-	2.96 (71)	1.86 (72)	0.96 (71)		
	[α = 1.08]	[α = 1.11]	[α = 0.82]		
1,2,9-/	4.21 (22)	4.18 (22)	1.78 (22)		
1,2,6-	4.53 (78)	4.81 (78)	1.38 (78)		
	[α = 1.08]	[α = 1.15]	[α = 0.78]		
1,2,4,9-/	7.95[f]	16.6[f]	2.37 (38)	2.922	−40.436
1,2,4,6-			2.19 (62)	2.178	−40.458
			[α = 0.92]		
1,8-/	3.53[f]	1.78[f]	3.33 (37)		
1,7-			2.90 (63)		
			[α = 0.87]		
1,2,8-/	5.20[f]	4.28[f]	5.43 (27)		
1,2,7-			4.60 (73)		
			[α = 0.85]		
1,2,4,8-/	9.21[f]	14.6 (43)	7.94 (41)	1.607	−42.533
1,2,4,7-		15.3 (57)	7.67 (59)	0.623	−42.552
		[α = 1.05]	[α = 0.96]		

[a] α = k' ratio between the peak of larger size and the peak of smaller size.
[b] Mobile phase: methanol–water (90:10, v/v), flow-rate 2.0 ml/min.
[c] Mobile phase: methanol, flow-rate 2.0 ml/min.
[d] Mobile phase: methanol–water (90:10, v/v), except for 1,7- and 1,8-DCDDs, 1,2,7- and 1,2,8-TrCDDs and 1,2,4,7- and 1,2,4,8-TCDDs, which were chromatographed using methanol–water (80:20, v/v), flow-rate 1.0 ml/min.
[e] Ref. 29.
[f] Insufficient separation was observed.

however, contradicts the previous one [14] based on NMR and Fourier transform IR spectra of the mixture.

So far we have been using the different peaks sizes in each PCDD pair only for tracing the elution order. However, the relationship between retention order and relative peak size is clearly noticeable in Figs. 6–8. In each pair of PCDDs co-produced during preparation, the isomer present in excess was generally retained longer on C_{18} and PYE phases.

The results are explainable as follows, assuming that thermodynamic equilibrium is reached during the preparation reaction which involves the rapid Smiles rearrangement [26,27]. Sterically less hindered species should be preferentially produced owing to the greater thermodynamic stability via a common spirocyclic intermediate. Strong 1,9-nonbonded (repulsive) interaction via ether oxygen is suggested for dioxins having chlorine atoms at 1- and 9-positions that deform ether linkages [30]. The heats of formation of such TCDDs were accordingly smaller than those of TCDDs with less steric congestion [29], as shown in Table III.

Thus 1,2,6,7-TCDD and 1,2,3,6-TCDD were produced more than 1,2,8,9-TCDD and 1,2,3,9-TCDD, respectively. Similarly, 1,6-DCDD is expected to be produced more than 1,9-DCDD, and 1,2,6-TrCDD more than 1,2,9-TrCDD. It would be of interest to examine the difference between the present results and those with HxCDDs [26] with respect to the reaction conditions and the work-up procedure. Spectroscopic and X-ray crystal analysis will be required to confirm the assignments, although the structural assignment with proton NMR may be increasingly difficult for DCDDs and TrCDDs because of coupling.

Fig. 9 gives another example showing the possibility of the combined use of NPE and PYE phases in establishing the structures of TCDDs. The structure assignments of 1,2,4,7- and 1,2,4,8-TCDDs based on NMR and IR spectrometry were contradictory, even after the separation of the isomers on PYE phase [14,22,23].

Mixtures of 1,7- and 1,8-DCDDs and 1,2,7- and 1,2,8-TrCDDs were readily separated with NPE phase, in spite of the difficulties in separation on C_{18} and PYE phases. Again, the compounds present in smaller amounts were retained longer on NPE phase, and tentatively assigned to 1,8-DCDD and 1,2,8-TrCDD as in Fig. 9c, because they presumably possess the more aligned dipoles and the greater steric congestion in each pair.

Although the separation between 1,2,4,7- and 1,2,4,8-TCDDs on NPE phase is only partial, the elution order on NPE was clearly reversed from that on PYE phase. Taking into account the retention order and the peak size ratios of 1,7- and 1,8-DCDDs and 1,2,7- and 1,2,8-TrCDDs, the TCDD isomer present in the greater amount, or the first peak on NPE phase, can be tentatively assigned to 1,2,4,7-TCDD, which supports the previous structural assignment based on NMR [23]. In Fig. 9, it is noticeable that the steric discriminations with 1,7- and 1,8-DCDD skeletons in terms of the product

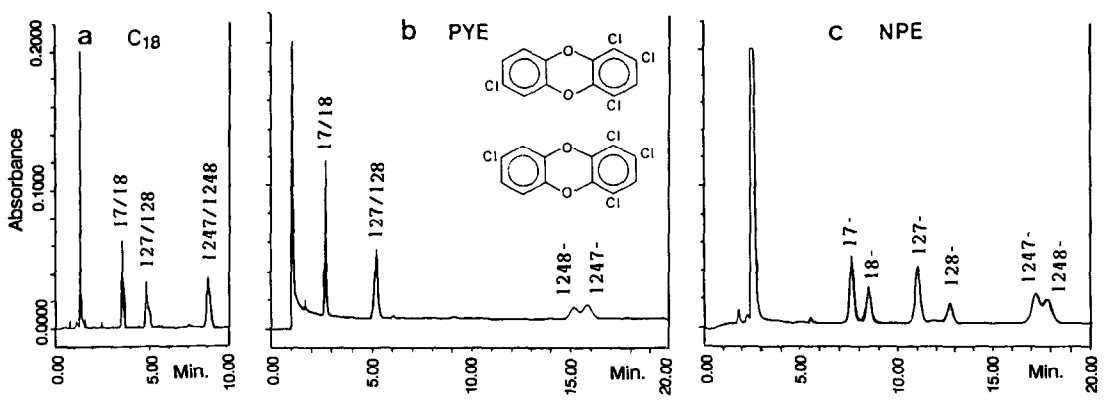


Fig. 9. Separation of 1,7- and 1,8-DCDDs, 1,2,7- and 1,2,8-TrCDDs and 1,2,4,7- and 1,2,4,8-TCDDs. Mobile phase and column: (a) C_{18}, 15 cm, methanol–water (90:10, v/v) at 2 ml/min; (b) PYE, 15 cm, methanol at 2 ml/min; (c) NPE, 15 cm, methanol–water (80:20, v/v) at 1 ml/min.

ratios and chromatographic separation were enhanced by the addition of a chlorine atom at the 2-position and reduced by a chlorine atom at the 4-position, as occurred with those with 1,6- and 1,9-DCDD skeletons in Fig. 8.

The interpretation of Figs. 8 and 9 is consistent with the results with 1,2,6,7- and 1,2,8,9-TCDDs and 1,2,3,6- and 1,2,3,9-TCDDs in terms of retention on NPE and PYE phases, which is related to the dipolar character, and the peak size ratios, related to the thermodynamic stability or the steric congestion. The present interpretation of the retention tendency on NPE phase based on the effect of the more aligned dipoles on one isomer seems to agree with the relative magnitude of dipole moments calculated for these TCDDs (Table III) [29].

The present results clearly indicate the utility of PYE and NPE phases, and the need for further studies. The identification of each congener, based on isolation and structure determination, is essential for assessing environmental safety with respect to the chlorinated aromatic compounds. The chromatographic method, not frequently used for structure determinations, is very straightforward in the present instance, and will be effective for isomers having differences in dipolar character, including PCDDs, chloronaphthalenes and chlorobiphenylenes, especially when spectroscopic methods are associated with difficulties.

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