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Review

Stationary phase effects in reversed-phase liquid chromatography

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

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

ABSTRACT

Selectivity of reversed-phase packing materials was discussed based on the solute–stationary phase interaction. Solute retention on a silica C_{18} phase is primarily determined by the hydrophobicity of a solute, whereas the long alkyl groups result in preferential retention of rigid, planar solutes over non-planar, bulky ones. An organic solvent imbibed in the stationary phase also contributes to the retention. Thus an electron donor, such as tetrahydrofuran, tends to give longer retention for acidic compounds or electron acceptors.

The presence of micropores in polymer gel packing materials results in the preferential retention of solutes with rigid, compact structures, and the presence of dipolar groups (ester and ether linkages) contributes to the preferential retention of dipolar and/or aromatic compounds based on dipole–dipole or dipole–π interactions. Attractive interaction between the graphite carbon surface and solutes, presumably based on the dispersion force, results in selective retention of planar compounds compared with non-planar ones. Attractive interactions provided by electron–donor–acceptor bonded phases are also shown to be very effective for structural recognition.

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* Corresponding author.

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1. INTRODUCTION

A retention mechanism universally operative in RPLC is hydrophobic interaction between a solute (analyte) and a stationary phase in the presence of an aqueous mobile phase. The simplest model for the retention mechanism involves the intermolecular association between the hydrophobic moieties, one in a solute and the other in a stationary phase [1]. A similar interpretation has been provided by taking into account the solvation of these components [2,3]. It is possible to explain most observations in RPLC based on these mechanisms, especially when the results were obtained for compounds with a similar skeleton under a limited range of conditions. Good correlations have been observed between log k' values in RPLC under such conditions and log P values in 1-octanol–water two-phase systems [4]. It is well known that a methylene group in a molecular structure contributes to the proportional increase in k' values as in the distribution coefficients in liquid–liquid partitioning, with free-energy changes associated with the transfer of one methylene group from water to the organic phase of 820–880 cal/mol in liquid–liquid partitioning [5] and *ca.* 810 cal/mol in RPLC with a C_{18} phase and water [6] (1 cal = 4.184 J). These results imply the partitioning mechanism in a primitive sense to be operative between an aqueous mobile phase and stationary phase alkyl groups, the latter not contributing to the selectivity but merely controlling k' values via phase ratios.

There are, however, numerous results that clearly indicate the contribution of additional effects of the stationary phase to solute retention and selectivity. Here secondary retention processes caused by the participation of silanols and metal impurities on the silica support are omitted. Details of these topics have been presented elsewhere [7–9]. The following discussion is limited to the contribution of essential constituents of a stationary phase, *viz.*, alkyl groups and solvent molecules existing in the stationary phase under elution conditions. The effects of these factors on retention are not very large with the alkyl-type bonded phases, but are of greater importance for at least two reasons. First, only a minor change in selectivity is needed to effect a separation owing to the high efficiency of RP-HPLC, and second, hydrophobic interaction alone is inadequate as a means of varying the selectivity; changes in the composition of the alkyl-bonded phase and in the organic solvent can provide additional selectivity effects, as discussed below.

Although many mechanisms have been proposed to account for retention process in RPLC, most of them are based on or can be applied to the retention behavior of a limited range of solute and mobile phase compositions. It is desirable to have a unified understanding of the retention process that should be able to explain every retention behavior including that provided by the stationary phase effects.

In fact, important information can be gained from the stationary phase effects in terms of the retention mechanism, which indicates an active role for the stationary phase. The participation of not only the bonded moieties but also the organic solvents residing in the stationary phase is important to effect separation in practice. This subject is related to the question of what the actual constituents of the stationary phase are.

In the case of an electron donor- or acceptor-bonded silica phase, the stationary phase effect can dominate the retention and separation. Characteristic selectivities of polymer gel and graphite carbon packings are also provided by the stationary phase whose effects are so large that they are readily observable.

The major topics of this review will be as follows: (i) effect of alkyl chain length of silica bonded phases, (ii) effect of organic solvents in mobile phase and (iii) effect of major structural features of polymer gel, graphite carbon and donor–acceptor-bonded silica phases on selectivity based on steric factors (planarity and bulkiness) and polar groups (hydrophilic–hydrophobic properties and dipolar character). We shall consider results obtained mainly in our laboratory, indicating various effects of the stationary phase structure to be taken into account in elucidating the retention mechanisms in RPLC.

2. ALKYL-BONDED SILICA PHASE

2.1. Effect of alkyl chain length of bonded phase on selectivity

We can begin with a simple model of reversed-phase retention as a means of predicting the effect of alkyl chain length on retention. Let us assume that the primary factors in determining retention are (a) unfavorable interaction between the hydrophobic portion of a solute and aqueous solvent and (b) association of a solute with the individual alkyl chains so as to reduce the hydrophobic surface area, which are referred to as hydrophobic [5,10] or solvophobic [1] interactions. Then the absolute retention will be dependent on the phase ratio determined by the alkyl chain length and the surface density, but the selectivity will not be affected by solute structures in terms of shape and polar properties.

Early studies discussed the linear relationships between k' values and the surface coverage and the alkyl chain length of the stationary phase, or phase ratios, assuming that the retention is determined by the area of contact between the hydrophobic moiety of the solute and the stationary phase [1]. A linear relationship can hold for a narrow range of solutes. This argument, however, does not take into account how the alkyl groups are arranged and solvated in the stationary phase. The k' values are not necessarily proportional to the phase ratios determined by the length of alkyl chains and surface coverages. In other words, the selectivities based on steric and polar characteristics of solutes were clearly affected by these factors beyond the predictions based on the phase ratios.

Table 1 gives the k' values obtained for several polar and non-polar compounds on C_8 and C_{18} stationary phases. The more hydrophobic solutes were eluted with 80% methanol, whereas the more hydrophilic solutes were eluted with 20% methanol. Any difference in selectivity between the two stationary phases in the same mobile phase must be attributed to the difference in the alkyl chain length of the stationary phase. With 80% methanol, the C_{18} phase showed up to 2–3 times longer retentions than the C_8 phase for the hydrophobic solutes. With 20% methanol, how-

TABLE 1

RETENTION OF POLAR AND NON-POLAR COMPOUNDS ON SILICA C_8 AND C_{18} PHASES

Mobile phase	Solute	k'	
		C_{18}	C_8
80% CH_3OH	Pyrene	9.0	2.9
	n-C_6H_{14}	6.7	3.3
	n-$C_{12}H_{25}OH$	9.4	5.3
20% CH_3OH	*n*-C_3H_7OH	1.2 (4.2)[a]	1.1 (4.2)[a]
	n-C_4H_9OH	4.2 (9.9)[a]	3.7 (9.4)[a]
	trans-1,4-Cyclohexanedimethanol	4.2 (9.8)[a]	3.3 (8.8)[a]
25% CH_3CN in 0.2 *M* H_3PO_4	Somatostatin	3.6	3.3

[a] Values in parentheses are retention times in minutes at a flow-rate of 1 ml/min with a 15 cm × 4.6 mm I.D. column.

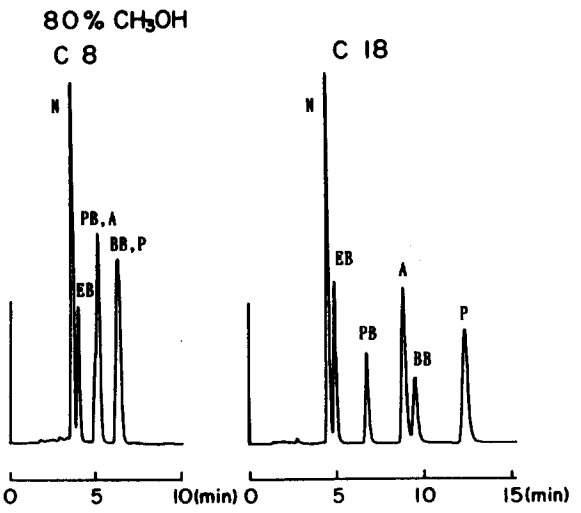

Fig. 1. Elution of alkylbenzenes and PAHs on C_8 and C_{18} phases with 80% methanol. Solutes: naphthalene (N), anthracene (A), pyrene (P), ethylbenzene (EB), propylbenzene (PB) and butylbenzene (BB).

ever, the C_8 phase, although having a much lower carbon content (*ca.* 11%) than C_{18} (*ca.* 20%), showed comparable retention times for the polar compounds. The retention times with 20% methanol are given together with k' values in Table 1 to emphasize the preferential retention of the more hydrophilic solutes on C_8.

The k' values include the differences both in the phase ratios (which would favor C_{18} by a factor of about two, as seen with the hydrocarbons) and in the partition coefficients (which must be much greater with C_8 than C_{18} for the hydrophilic solutes). The results indicate the preferential retention of polar, hydrophilic solutes by the C_8 phase compared with the C_{18} phase. In contrast, as shown in Fig. 1 and Table 1, the C_{18} stationary phase preferentially retains rigid, planar polynuclear aromatic hydrocarbons (PAHs) *vs.* *n*-alkanes or non-planar aromatic compounds with rotational freedom of phenyl groups (such as *o*-terphenyl and triphenylmethane, shown as Ar–Ar) relative to the C_8 stationary phase. These simple examples show that the mechanism assuming the retention to be determined by the sole contribution of the mobile phase effect (solvophobic effect), or by the amount of hydrophobic groups in the stationary phase (phase ratios), cannot explain every result.

2.2. Steric selectivity of stationary phase

In the following discussion involving Figs. 2–7, we shall attempt to justify a simple picture of the role of the stationary phase in affecting retention as a function of molecular shape. *n*-Alkane solutes are flexible molecules that can readily penetrate into the bonded phase, whereas PAHs are more rigid and planar and Ar–Ar even bulkier, having greater difficulty in penetration. A C_{18} stationary phase is known to be relatively tangled, especially for mobile phases with low organic solvent contents. As organic solvent content increases, the alkyl chains sorb organic solvents, swell and become more ordered in their conformation. As a result, PAHs can more readily penetrate the stationary phase and their retention relative to the more bulky and/or flexible solutes increases.

In Fig. 2, the k' values of aromatic and aliphatic hydrocarbons on silica-based C_{18} are

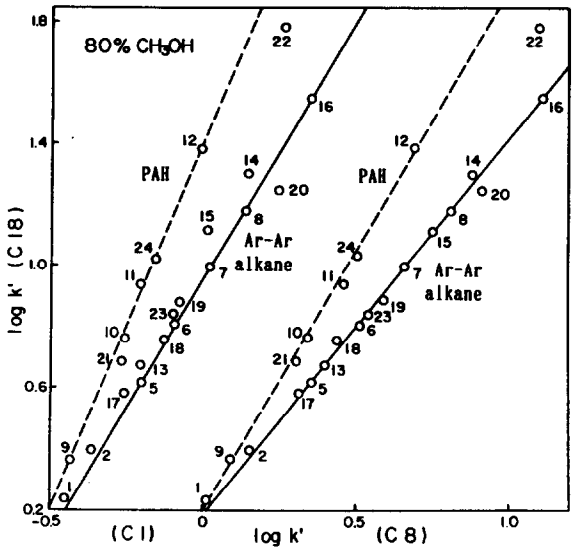

Fig. 2. Plots of log k' values on C_{18} phase against log k' values on C_1 and C_8 phases in 80% methanol [11]. Compounds: 1 = toluene; 2 = ethylbenzene; 3 = propylbenzene; 4 = butylbenzene; 5 = pentane; 6 = hexane; 7 = heptane; 8 = octane; 9 = naphthalene; 10 = anthracene; 11 = pyrene; 12 = 3,4-benzpyrene; 13 = cyclohexane; 14 = *trans*-decalin; 15 = adamantane; 16 = decane; 17 = diphenylmethane; 18 = 1,2-diphenylethane; 19 = triphenylmethane; 20 = tetraphenylethylene; 21 = fluorene; 22 = bidiphenyleneethylene; 23 = *o*-terphenyl; 24 = triphenylene. The solid line was drawn based on *n*-alkanes and the dashed line on PAHs.

plotted against those on C_8 and C_1 phases [11]. (All stationary phases were prepared from monochlorosilanes.) Solid lines were drawn based on *n*-alkanes (Nos. 5–8), and the dashed lines indicate the location of the PAHs (Nos. 9–12). PAHs consistently gave larger log k' values than the other hydrocarbons on the C_{18} phase compared with the C_1 or C_8 phase.

The comparison of the chromatographic behaviors of the three pairs of compounds 17 and 21, 20 and 22, and 23 and 24 (Fig. 3) is instructive. Compounds 21, 22 and 24 have the same number of carbon atoms and double bonds as 17, 20 and 23, respectively. The only difference is that the multiple linkages between the phenyl rings in 21, 22 and 24 increase the rigidity and planarity of the molecules. The steric repulsion between the phenyl rings in 17, 20 and 23 makes them much bulkier than 21, 22 and 24, respectively. The planar compounds showed much longer retention than non-planar compounds on the C_{18} phase. The differences, however, were much smaller on the C_1 and C_8 phases. The ordered alkyl groups on the C_{18} phase seem to be responsible for the selectivity.

As we shall show next, the results in Figs. 4–6 indicate that at least part of the stationary phase effects also include the contribution of the conformational change of bonded alkylsilyl groups caused by the change in mobile phase composition [11]. When the mobile phase was changed from 60% to 80% methanol with the C_1 phase, all aromatic compounds showed a much greater decrease in retention than saturated hydrocarbons, regardless of planarity, as shown in Fig. 4. The difference in behavior between PAHs and Ar–Ar is not so large with the C_8 phase, as shown in Fig. 5. The negative deviation of aromatic compounds in these plots (Figs. 4 and 5) can be attributed to the increased solvation of these compounds, which is expected to be more dominant in mobile phases with a higher methanol content.

The effect of the organic solvent content on selectivity among aromatic hydrocarbons is considerably different on the C_{18} phase, as shown in Fig. 6. Planar aromatic hydrocarbons, namely

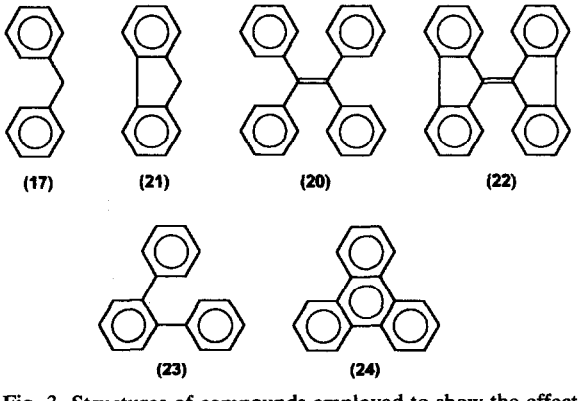

Fig. 3. Structures of compounds employed to show the effect of planarity.

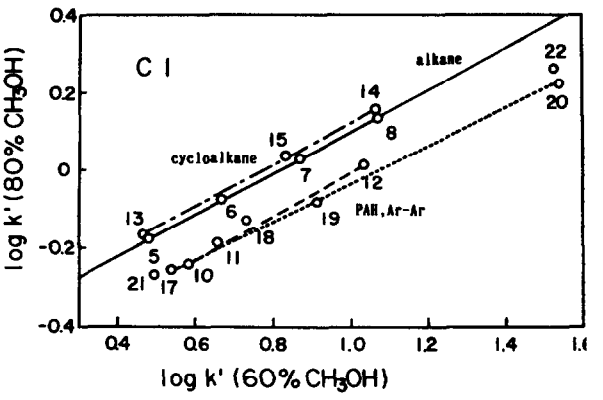

Fig. 4. Plots of log k' values on C_1 phase in 80% methanol against log k' values in 60% methanol [11]. Compounds as in Fig. 2. The solid line was drawn based on *n*-alkanes, the dashed line on PAHs, the dotted line on Ar–Ar and the dashed-dotted line on cycloalkanes.

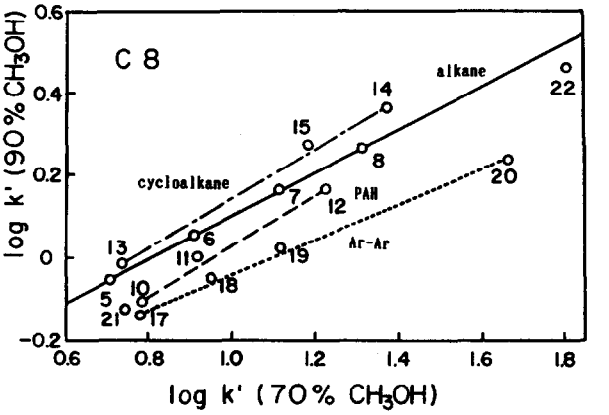

Fig. 5. Plots of log k' values on C_8 phase in 90% methanol against log k' values in 70% methanol [11]. Compounds as in Fig. 2. The solid line was drawn based on *n*-alkanes, the dashed line on PAHs, the dotted line on Ar–Ar and the dashed-dotted line on cycloalkanes.

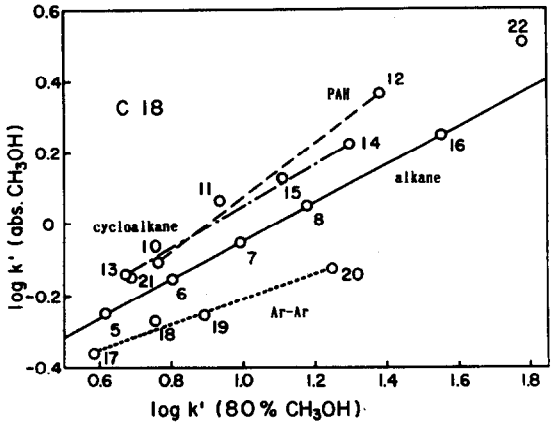

Fig. 6. Plots of log k' values on C_{18} phase in 100% methanol against log k' values in 80% methanol [11]. Compounds as in Fig. 2. The solid line was drawn based on *n*-alkanes, the dashed line on PAHs, the dotted line on Ar–Ar and the dashed-dotted line on cycloalkanes.

PAHs, showed a large increase in relative retention compared with *n*-alkanes with increase in methanol content, whereas non-planar aromatic compounds (Ar–Ar) showed a lower deviation as on the C_1 and C_8 phases. The plots for acetonitrile mobile phases showed a similar tendency.

One can see the similarity between the plots in Figs. 2 and 6 with respect to the behavior of the two types of aromatic compounds, taking into account the effect of the organic solvent content in Fig. 6, which should have produced a lower deviation for all aromatic compounds, as observed for C_1 and C_8 phases in Figs. 4 and 5. In other words, the increase in the methanol content from 80% to 100% on the C_{18} phase gave results as if the alkyl chains in the stationary phase had been lengthened, differentiating rigid planar aromatic compounds (PAHs) from bulky non-planar compounds (Ar–Ar). Solvation of alkyl chains with organic solvents is known to increase the ordering of anchored alkyl chains, as observed by Fourier transform IR spectroscopy [12]. The preference towards the more planar solutes was emphasized with the C_{18} phase having higher surface coverages.

An aggregated structure of octadecyl chains was suggested for the C_{18} phase with an aqueous mobile phase [13]. It is reasonable to assume that the alkyl chains are more extended in mobile phases of higher organic solvent content, which could produce results similar to the increase in the alkyl chain length. In other words, as the C_{18} phase is more swollen at higher organic solvent contents, it is more ordered to favor PAHs relative to Ar–Ar. This effect is much smaller with C_8 and negligible with C_1 phases.

Following our earlier work [11], the steric discrimination of planar from non-planar compounds was also reported with the polymeric C_{18} phase compared with a monomeric C_{18} phase [14–20]. The results were interpreted as follows. The alkyl groups on polymeric C_{18} phases provide binding sites having alkyl chains close to each other, and hence are more ordered than those on a monomeric phase, providing "slots" for the selective retention of planar molecules [14–20]. A reduced mobility of alkyl groups in such sites of a polymeric C_{18} phase has been reported by using NMR spectrometry [21]. The mechanism for this to occur has been well documented in a series of papers by Wise and co-workers [14–18] and by Jinno and co-workers [19–21], and will not be discussed further here.

2.3. Effect of organic solvent

It has also been demonstrated that the steric selectivity and the difference based on the chain length are affected by the mobile phase organic solvent [22,23]. Fig. 7 shows the plots between log k' values with methanol and acetonitrile mobile phases on the three stationary phases [11]. On the C_1 phase all the plots were very close to the solid line drawn for *n*-alkanes. As shown in Fig. 7b, the two types of compounds showed different behavior in the two mobile phases on the C_{18} phase regardless of rigidity or planarity which caused the large differences in Figs. 2 and 6. The results with the C_8 phase are between those for the C_1 and C_{18} phases.

It is notable that the three stationary phases gave different selectivities between aromatic and saturated compounds in methanol and acetonitrile. The results preclude the possibility that the difference in selectivity was caused by the difference between aromatic and saturated compounds in the mobile phase interaction, because no such difference was seen with the C_1 phase. The

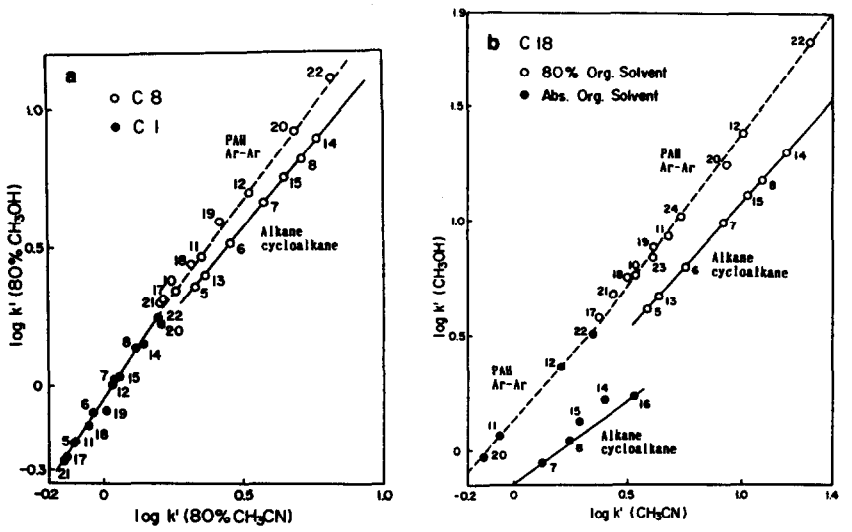

Fig. 7. Plots of log k' values in methanol mobile phase against log k' values in acetonitrile mobile phase [11]. (a) 80% organic solvent; ○ = C_8, ● = C_1; (b) C_{18}, ○ = 80% organic solvent, ● = absolute organic solvent. Compounds as in Fig. 2. The solid line was drawn based on *n*-alkanes and the dashed line indicates the location of aromatic compounds.

difference could not be due to the steric effect either, as planarity of the solutes did not influence the results. It should be explained based on the difference in solvation of solutes in the stationary phase between C_1 and the longer alkyl-bonded phases.

It should be emphasized that the effect of solute–solvent interactions on the C_{18} phase was found to be much larger than on the C_1 phase, and even larger with higher organic solvent contents (Fig. 7b). These results imply that the hydrocarbon solute molecules in the stationary phase can realize the orderedness of bonded chains determined by the chain length (Fig. 2), surface density and mobile phase composition (Fig. 6), and simultaneously associate with the solvent molecules in the C_{18} phase (Fig. 7). The effects are much smaller with C_8 and negligible with C_1 phase (Figs. 4–7). The results suggest a mechanism based on partitioning of solutes between the mobile phase and the effective stationary phase, namely alkyl chains associated with solvent molecules. However, the effective stationary phase should not be taken as a simple mixed solvent, as C_1 or C_8 and C_{18} phases showed considerable differences in their response to the change in the mobile phase. Insensitivity of the C_8 phase toward the change in mobile phase implies that the chain overlap is much less than with the C_{18} phase in this range of mobile phase, as would be the case with the C_1 phase.

2.4. Effect of organic solvent in stationary phase on polar group selectivity

It has been reported that the organic solvents were enriched in the alkyl stationary phase [24,25]. The following example can show more clearly the effect of imbibed solvent molecules on the retention of solutes having polar groups.

Fig. 8 shows plots of log k' values for benzene derivatives in tetrahydrofuran (THF)–water (25:75) against log k' values in methanol–water (50:50) [23]. A straight line was drawn through the plot for benzene parallel to the plots for *n*-alkanols to illustrate the deviation of other plots from the prediction of the simple hydrophobic retention process. The slope of the line is close to unity, indicating the similar contribution of the hydrophobic property at these compositions.

The results indicate notable difference in polar group selectivity between THF–water and

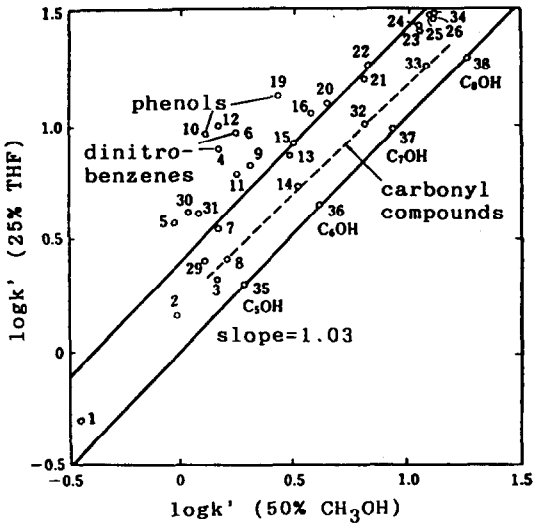

Fig. 8. Plots of log k' in THF–water (25:75) against log k' in methanol–water (50:50) with C_8 phase [23]. Compounds: 1 = benzamide; 2 = benzyl alcohol; 3 = 2-phenylethanol; 4 = *p*-dinitrobenzene; 5 = phenol; 6 = *m*-dinitrobenzene; 7 = benzonitrile; 8 = acetophenone; 9 = nitrobenzene; 10 = *p*-nitrophenol; 11 = *p*-cresol; 12 = *m*-nitrophenol; 13 = anisole; 14 = methyl benzoate; 15 = benzene; 16 = *o*-nitrotoluene; 17 = *p*-nitrotoluene; 18 = *p*-nitrochlorobenzene; 19 = *p*-chlorophenol; 20 = *m*-nitrotoluene; 21 = toluene; 22 = chlorobenzene; 23 = naphthalene; 24 = *o*-xylene; 25 = ethylbenzene; 26 = *p*-xylene; 27 = *m*-chlorotoluene; 28 = *m*-xylene; 29 = benzaldehyde; 30 = *m*-nitrobenzaldehyde; 31 = *p*-nitrobenzaldehyde; 32 = ethyl benzoate; 33 = isopropyl benzoate; 34 = *p*-dichlorobenzene; 35 = 1-pentanol; 36 = 1-hexanol; 37 = 1-heptanol; 38 = 1-octanol.

methanol–water systems, which is greater than that between acetonitrile–water and methanol–water. Thus THF and methanol would constitute an interesting pair to be used with water in a ternary mobile phase for the control of the separation of substances with different functional groups.

The k' values for these benzene derivatives in 50% methanol showed a good correlation with log P values, but not with the k' values in THF–water. The retention in THF *vs.* methanol decreases in the order phenols ≈ nitro compounds > hydrocarbons, chlorobenzenes > esters ≈ alcohols. Alcohols, especially alkanols, were preferentially retained in methanol–water compared with the THF or acetonitrile systems. The difference between phenols and alcohols can be explained by the difference in their ability to stabilize the partial negative charge upon hydrogen bonding as indicated by their pK_a values.

Fig. 9 shows the separation of four substances with different functional groups with methanol and THF mobile phases. It can be seen that the elution order is exactly reversed with the two systems. This example clearly illustrates the significant role that polar group selectivity can play as the organic modifier is changed. It has also been shown that the selectivity change can be realized with the addition of small amounts of interacting solvents to a mobile phase [23].

The results indicate that the compounds containing acidic functions, phenolic OH or a π-acidic benzene ring, are preferentially retained in mobile phases containing THF which can serve as a base, and those with basic dipolar carbonyl groups are disfavored in mobile phases containing basic dipolar THF or acetonitrile. The results indicate the contribution of solute–solvent interactions in the stationary phase to the retention and selectivity. Such a contribution would be needed to stabilize polar functionality in the non-polar stationary phase. It is well known that stationary phases with medium alkyl chain lengths (C_4) or a half-coverage C_{18} phase show maximum retention for peptides [26], especially for those with high molecular masses. This is understandable, because the presence of hy-

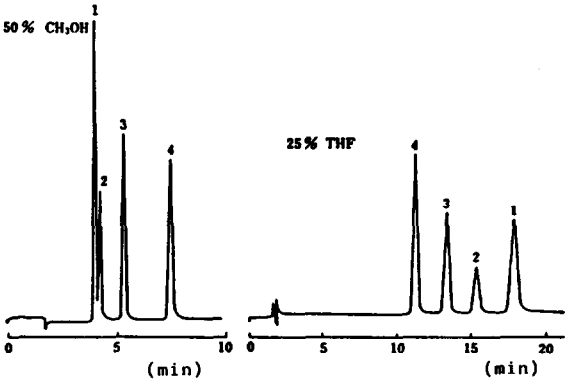

Fig. 9. Chromatograms illustrating the difference in functional group selectivity caused by organic solvents with C_8 phase [23]. Peaks: 1 = *p*-nitrophenol; 2 = *p*-dinitrobenzene; 3 = nitrobenzene; 4 = methyl benzoate.

drophilic or ionic species in the hydrophobic environment is energetically unfavorable.

In summary, the stationary phase effects are compatible with a mechanism based on the partitioning of solutes between the mobile phase and the effective stationary phase, anchored alkyl chains associated with solvent molecules. The simple solvated alkyl chains or simple mixed solvents, however, are not an adequate description of the alkyl-bonded silica stationary phase. The same mobile phase composition results in a difference in selectivity for different stationary phases. The difference in alkyl chain length or in surface coverage can be envisioned to produce the difference in steric requirement and also hydrophobic–hydrophilic properties, which in turn determine the chromatographic properties. In this sense, the properties of the C_{18} phase are primarily determined by the extent of surface coverage with alkyl groups.

3. SELECTIVITY OF POLYMER-BASED PACKINGS

3.1. Biporous structure of polymer gels

Although several high-efficiency polymer gel packings are available, it has been reported that their selectivities are difficult to understand, or are considerably different from those of silica-based phases. This arises from the stationary phase effects. The pore structures of polymer gels are different from those of silica, as seen in Figs. 10 and 11 [27–29]. The pore size distributions show the presence of micropores in addition to macro- or meso pores in all the polymer gels tested, regardless of the type of repeating units (Fig. 10).

Molecular mass–elution volume curves obtained for polymer gels are always associated with the second plateau in a molecular mass range below 500, corresponding to the micropores, whereas such micropores are not present in ordinary silica particles. This indicates the biporous structure of cross-linked polymer gels, macroporous particles being composed of microporous materials. Lightly cross-linked polymer chains on the surface of solid cores of polymer gels might be responsible for the microporosity [30]. As we shall show next, the micropores play a major role in determining selectivity via the size-exclusion effect that represent the characteristic selectivity of all the polymer gels [27–29,31].

3.2. Steric selectivity of polymer gels

It has been shown recently that characteristic steric selectivity of polymer gels is in fact the result of the size-exclusion effect of the micropores, and that the micropore structure, and hence the selectivity between bulky, flexible and rigid, compact solutes, can be controlled by the choice of the diluents in suspension polymerization [32] or in multi-step swelling polymerization [33].

Fig. 12 shows the difference in selectivity of two polymer gel packings prepared from methyl methacrylate and ethylene dimethacrylate in two diluents, cyclohexanol and 2-octanol [32]. In spite of the similarity in meso–macro pore sizes, the two gels showed different selectivities for hydrocarbons with different planarity and size. This is due to the smaller micropores of the polymer gel prepared in 2-octanol [32]. Similar results were obtained with aliphatic compounds.

Fig. 13 shows typical plots indicating the difference in selectivity in terms of the shape of solute molecules between alkyl-bonded silica and polymer gel packings, and also among the three polymer gel packings (Table 2) which must be related to the chemical and three-dimensional structure of these polymer gels [29]. A group of compounds having similar structure behave similarly.

Shodex DE-613 poly(alkyl methacrylate) gel, having short alkyl groups, showed a clear preference for aromatic compounds having more than one phenyl group compared with alkylbenzenes, and for cycloalkanes compared with linear alkanes, whereas esterified Asahipak ODP-50 poly(vinyl alcohol) gel (PVA) showed a clear preference for planar PAHs compared with bulky aromatic compounds. The general preference by polymer gels decreases in the order PAHs > polyphenylalkanes (Ar–Ar) > alkylbenzenes > cycloalkanes > linear alkanes. Such a preference was observed with all the polymer-based stationary phases regardless of

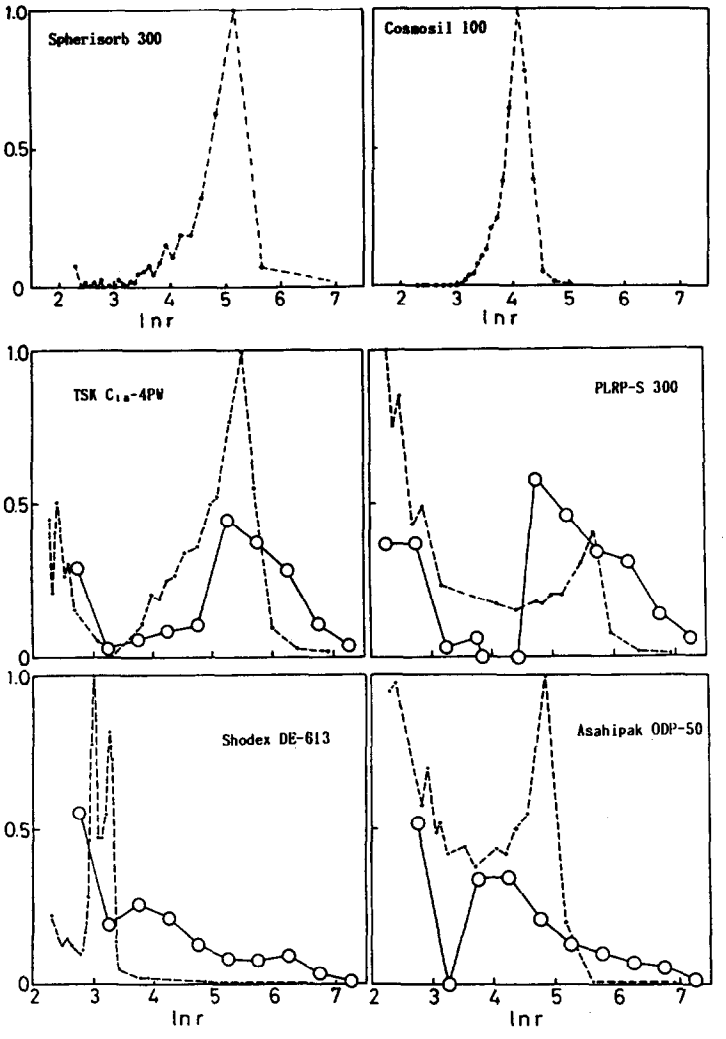

Fig. 10. Pore size measurement of silica particles (Cosmosil and Spherisorb-300) and polymer particles (Table 2) by nitrogen adsorption (dashed lines) and size-exclusion chromatography (solid lines) [29]. The vertical axis corresponds to the fraction of pore volume, normalized in the case of inverse size-exclusion chromatography, and the abscissa corresponds to the logarithm of pore radius (Å).

the alkyl chain length or the size of the macropores. Shodex DE-613 short alkyl-bonded polymer gel showed a greater selectivity for different types of hydrocarbons than did Asahipak ODP-50 and TSK C_{18}-4PW C_{18}-bonded types because the effect of polymer network structure is predominant with Shodex DE-613 owing to the smaller extent of hydrophobic interaction [28,32].

Polymer gels with alkyl backbones, DE-613 and TSK C_{18}-4PW, showed preferential retention of aromatic compounds in spite of their saturated structures, possibly owing to the dipole–π interactions involving the ester groups [34]. The preference toward rigid, compact compounds over bulky, flexible compounds was also seen with saturated compounds having no functional groups such as cyclohexane and adamantane,

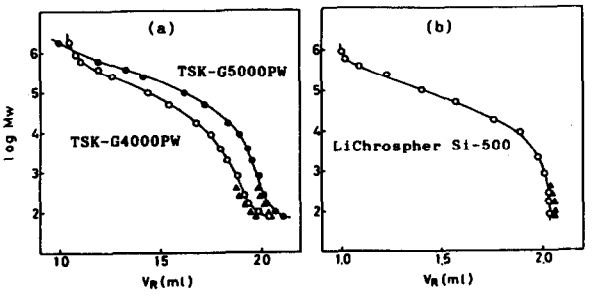

Fig. 11. Elution of (○, ●) polystyrene standards, styrene oligomers and alkylbenzenes and (△, ▲) alkanes on polymer gel (TSK G4000PW and G5000PW, 50 cm × 7.6 mm I.D.) columns and on a silica (LiChrospher Si-500, 15 cm × 4.6 mm I.D.) column. Mobile phase: THF.

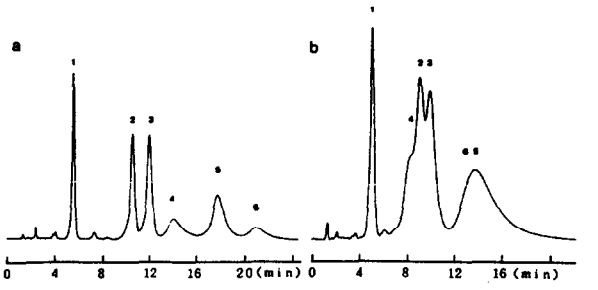

Fig. 12. Chromatograms of aromatic hydrocarbons on poly(methyl methacrylate) gel prepared in (a) cyclohexanol and (b) 2-octanol [32]. Peaks: 1 = benzene; 2 = butylbenzene; 3 = diphenylmethane; 4 = triptycene; 5 = pyrene; 6 = triphenylmethane. Mobile phase: acetonitrile–water (60:40). Flow-rate: 0.8 ml/min.

which are expected to undergo minimum specific interactions with the stationary phase except hydrophobic and steric interactions. Therefore the shape selectivity associated with polymer gels can be attributed, at least in part, to the structural matching between the solute and the rigid polymer network structure, or the micropores.

The C_{18}-type polymer packings, TSK C_{18}-4PW, alkylated poly(hydroxyalkyl acrylate or methacrylate) and Asahipak ODP-50, esterified poly(vinyl alcohol), showed a greater retention of alkyl compounds than did DE-613, having short alkyl groups. The preference for compact solutes compared with bulky solutes increases in the order silica C_{18} < DE-613 < C_{18}-4PW < ODP-50 < PLRP-S 300. The results indicate a greater population of the relatively large micropores of the DE-613 than ODP-50 or PLRP-S,

TABLE 2

LIST OF POLYMER GEL, GRAPHITIZED CARBON AND DONOR–ACCEPTOR-BONDED SILICA PACKING MATERIALS

Description	Stationary phase	Supplier
Polymer gel		
Shodex DE-613	Poly(alkyl methacrylate)	Showa Denko
TSK C_{18}-4PW	Alkylated poly(hydroxyalkyl acrylate or methacrylate)	Tosoh
Asahipak ODP-50	Octadecanoate of poly(vinyl alcohol)	Asahi Chemical
PLRP-S 300	Poly(styrene-divinylbenzene)	Polymer Labs.
Graphite carbon		
Hypercarb	Spherical graphite prepared by template method	Shandon
Carbonex	Spherical graphite prepared from pitch	Tonen
Silica bonded phase		
PYE	2-(1-Pyrenyl)ethyldimethylsilylated	Nacalai Tesque
NPE	2-(Nitrophenyl)ethyldimethylsilylated	Nacalai Tesque
NPO	3-(*p*-Nitrophenoxy)propyldimethylsilylated	Nacalai Tesque

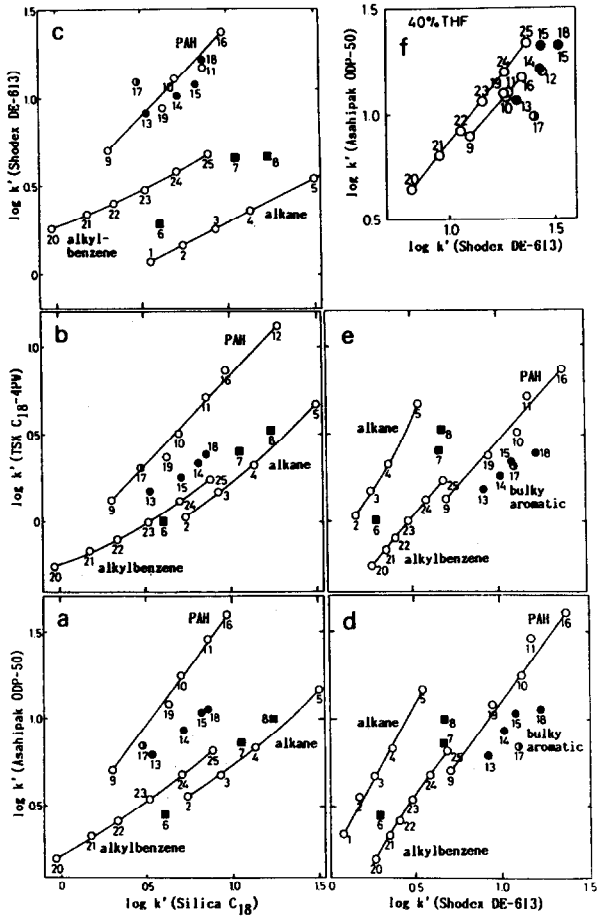

Fig. 13. Comparison of retention selectivity between silica C_{18} and polymer gel columns [29]. Mobile phase: 80% methanol unless indicated otherwise. Compounds: solid circles (●) indicate aromatic compounds with phenyl groups having rotational freedom, or bulky compounds, and solid squares (■) indicate cycloalkanes: 1 = pentane; 2 = hexane; 3 = heptane; 4 = octane; 5 = decane; 6 = cyclohexane; 7 = adamantane; 8 = *trans*-decalin; 9 = naphthalene; 10 = anthracene; 11 = pyrene; 12 = benz[*a*]pyrene; 13 = dephenylmethane; 14 = 1,2-diphenylethane; 15 = *o*-terphenyl; 16 = triphenylene; 17 = triptycene; 18 = triphenylmethane; 19 = fluorene; 20 = benzene; 21 = toluene; 22 = ethylbenzene; 23 = propylbenzene; 24 = butylbenzene; 25 = amylbenzene.

probably caused by the type of diluent used in their preparation.

3.3. *Effect of mobile phase on steric selectivity*

The structural selectivity was much reduced in 40% THF, as shown in Fig. 13f as compared with Fig. 13d. The mobile phase effects are understandable in terms of the combination of the facts that microscopic swelling occurs in polymer gels due to better solvation in THF, which would favor the retention of bulky compounds, and that THF selectively binds to the micropores which otherwise would preferentially bind rigid, compact compounds. A size-exclusion effect based on polymer network structure seems to be operative with these gels, as mentioned above, which may lead to slower diffusion or lower column performance for particular aromatic compounds.

When the column performance was examined in methanol–water (△ in Fig. 14), an increase in reduced plate height, (h) was seen with increasing k' values for PAHs (dashed line through 9–12, 16), and more so for bulky aromatic compounds (15, 17, 18), whereas consistent h values were obtained for alkanes and alkylbenzenes, as shown in Fig. 14. The results are understandable in terms of the restricted diffusion of bulky solutes in the polymer network structure.

In 40% THF (● in Fig. 14), however, evidently different features of column performance were seen between the two groups of polymer-based packing materials. DE-613 and C_{18}-4PW, alkyl ester-type gels, showed consistently good performance for all the hydrocarbons tested, as did silica C_{18}, although the h values were slightly higher than those on silica C_{18}. Conversely, ODP-50 and PLRP-S 300 showed increases in h values with increasing k' values, only for planar PAHs in THF–water (shown by the dashed line in Fig. 14c and d) and in acetonitrile–water. In the same mobile phases, these packing materials showed excellent performance for more bulky compounds (see the plots for compounds 15, 17 and 18 in 40% THF).

The difference in the effect of solute structure on column efficiency can be explained on the basis of the differences in the structures of the polymer gels. DE-613 and C_{18}-4PW are alkyl-type gels, containing no aromatic functionality, whereas PLRP-S 300 and ODP-50 contain aromatic groups in monomer and/or cross-linking reagents. These functionalities can provide interactions with aromatic compounds, especially

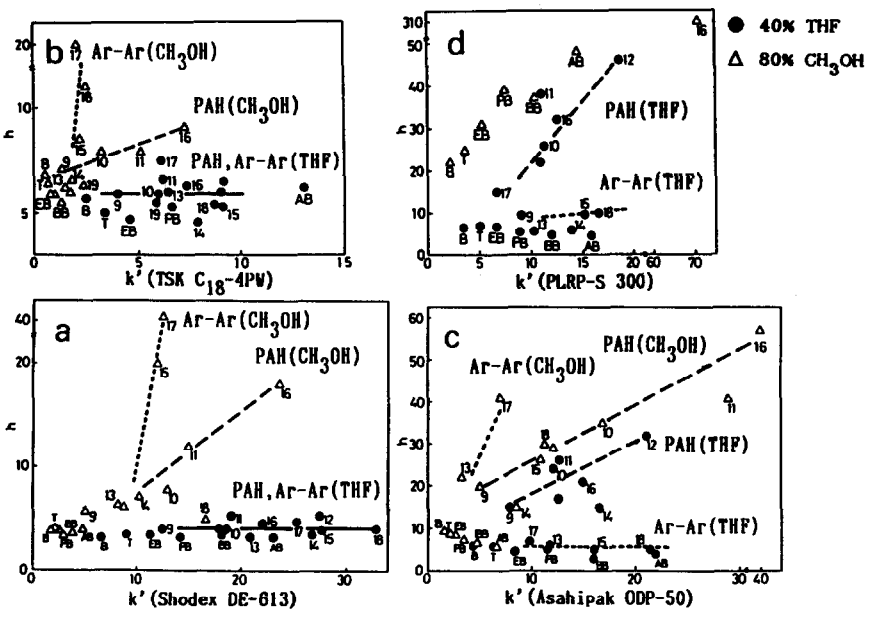

Fig. 14. Plots of reduced plate height, h, against retention, k', of hydrocarbons on polymer gel columns in (△) 80% methanol and (●) 40% THF [29]. Compounds as in Fig. 13. The dashed line indicates the location of PAHs and the dotted line Ar–Ar.

when the solutes are planar. The fact that the retention of bulky compounds on ODP-50 and PLRP-S 300 is relatively weaker than those of PAHs compared with DE-613 and C_{18}-4PW in similar mobile phases indicates that the former possess tighter network structures than the latter. With better solvation in THF and more so in acetonitrile with a closer solubility parameter to the PVA backbone, only PAH showed slightly higher h values, whereas excellent efficiencies were observed for the other solutes with ODP-50.

3.4. *Effect of biporous structure on chromatographic properties*

Fig. 15 shows the chromatograms obtained with four polymer gel packings for polypeptides (molecular mass 12 000–66 000) and a small molecule, 1-naphthalenemethanol. Polymer-based packings showed excellent performance for polypeptides including bovine serum albumin (BSA), owing to the favorable macropore structure [35,36]. Although the retention times for polypeptides are comparable on all the packings, those for 1-naphthalenemethanol are considerably different. Whereas the retention and separation of large molecules indicate an abundance of macropores, the retention of a small molecule

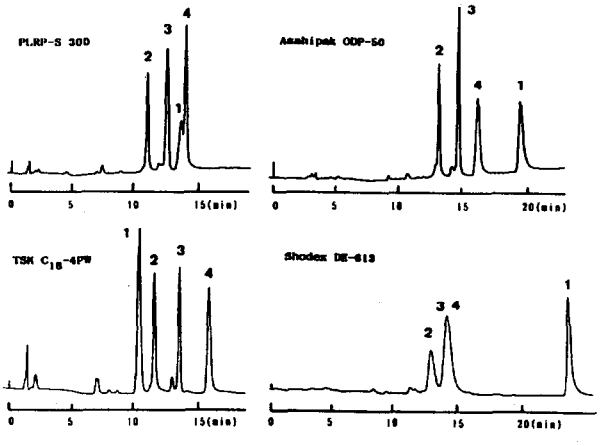

Fig. 15. Elution of polypeptides with polymer gel columns [35]. Peaks: 1 = 1-naphthalenemethanol; 2 = cytochrome c (molecular mass 12 000); 3 = lysozyme (molecular mass 14 000); 4 = bovine serum albumin (molecular mass 66 000). Linear gradient of acetonitrile from 20% to 60% in 20 min in the presence of 0.1% trifluoroacetic acid.

indicates an abundance of micropores in each packing. The chromatograms in Fig. 15 show that large molecules such as polypeptides are chromatographed by using macropores, and the small molecules are chromatographed by using micropores of polymer gels. Such a difference cannot be seen with silica-based phases [35].

3.5. Effect of polymer structure on polar group selectivity

In addition to the size-exclusion effect of micropores, some polymer gels possess another factor, the presence of dipolar carboxylate groups in methacrylate and in esterified poly(vinyl alcohol) gels, to show unique selectivity. These polymer gels in methanol show a somewhat similar selectivity to cyanoalkyl-bonded phases on silica or to alkyl-bonded phases in dipolar solvents [37]. Preferential retention of aromatic compounds, especially π-acids is notable. A stronger mobile phase for aromatic compounds, with *ca.* 10% more methanol, and a weaker mobile phase for aliphatic compounds, with *ca.* 10% less methanol, are recommended with polymer gels in comparison with an ordinary silica C_{18} phase.

The results obtained with polymer gels suggest the wide applicability of these packings in RPLC in wider mobile phase conditions than for silica C_{18} [38], although the full scope of application is yet to be explored. A basic understanding of polymer gel structures and the resultant selectivity will help one with the selection of separation conditions.

Silica- and polymer-based packings are frequently compared with each other, and they should complement each other in the separation of small molecules, because silica-based packings are not as chemically stable as polymer gels, and polymer gels are generally less efficient. One area of RPLC where silica- and polymer-based packings can be in serious competition will be in the separation of polypeptides, where micropores do not play a role, as reported for a comparison between packings based on wide-pore silicas and those based on polymer gels [35]. Silica C_{18} phases prepared from some wide-pore silicas showed poorer performance than short alkyl-bonded phases owing to the smaller pore sizes, which are much less stable than C_{18} in trifluoroacetic acid (TFA) solution. Polymer gels provided equal or better recoveries and performance and much greater stability in TFA solution compared with C_{18} phases. The easy control of pore size in both macro- and micropore size range is an additional advantage of polymer-based packings [39].

4. GRAPHITIZED CARBON PACKING MATERIAL

A number of stationary phases, including donor–acceptor-bonded silica or carbon packings, capable of more positive interactions with analytes than silica C_{18} and polymer packings, involving charge-transfer, dipole–π, dipole–dipole and steric interactions, are available for the separation of compounds with structural similarity [40–50]. Graphitized carbon is one of the extremes as a stationary phase for RPLC, possessing rigid, planar surfaces and functions capable of dispersion and charge-transfer interactions [43–50], as studied with unsaturated compounds. The packing has been reported to be useful for the separation of solutes with closely related structures, including stereoisomers. Here it will be shown that the dominant factor in the retention process on graphitized carbon is the dispersion force, as studied with saturated hydrocarbons, which are free from other electronic interactions.

4.1. Hydrophobic adsorption mechanism

Table 3 indicates that the retention increase caused by one methylene group, a measure of the hydrophobic property of the stationary phase, is always greater on the carbon phase than on alkyl- or aryl-bonded silica phases. The free-energy change associated with the transfer of one methylene group from water to the stationary phase was about -900 cal/mol with the carbon phase compared with -810 cal/mol on silica C_{18} [6]. The difference in methylene-binding energy between C_{18} and carbon phases was even greater at a higher methanol concentration. The free energy change associated with the transfer of one methylene group from water

TABLE 3

HYDROPHOBIC PROPERTIES OF PACKING MATERIALS [6]

Values given are $\alpha(CH_2)$, calculated from the retention times of alkanols $C_nH_{2n+1}OH$ ($n = 2$–5), except in 80% methanol, where alkylbenzenes (ethylbenzene to amylbenzene) were used.

Stationary phase	Methanol (%)				
	0	10	30	50	80
C_{18}	3.84	3.62	2.94	2.25	1.54
PYE [a]	3.27	3.15	2.81	2.13	1.45
Carbon I [b]	4.50	4.09	3.67	2.83	2.10
Carbon II [c]	4.53	4.49	3.74	2.87	2.11

[a] 2-(1-Pyrenyl)ethyldimethylsilylated silica [41]. See Table 2.
[b] Hypercarb (Shandon). See Table 2.
[c] Carbonex (Tonen). See Table 2.

to organic liquid phase was − 820 to 850 cal/mol for polar solutes and − 884 cal/mol for alkane–water partitioning [5].

These results are striking, if the rigid planar graphite surfaces are assumed to be the binding sites for alkyl groups. Some solvent-like behavior of C_{18} stationary phases is expected to reduce the contact between the hydrophobic C–H surface of a solute and water as in aqueous–organic liquid–liquid partition systems, which lowers the free energy of the system, although smaller in magnitude. On the other hand, the rigid carbon surface would not be able to surround the alkyl chain of the solute completely. The results suggest the presence of positive interactions between the stationary phase and the solute, namely dispersion forces, as suggested by previous workers [51], even for C–H groups [52]. In this sense, the retention process can be described as hydrophobic adsorption, as opposed to hydrophobic partitioning with C_{18}, where the analyte–stationary phase interaction is not very positive.

Supporting a mechanism including dispersion forces, any molecular mass increase in solutes, be it in hydrophilic or dipolar moieties, tends to cause a retention increase [6] in comparison with other chromatographic systems. Then the close proximity of the molecular surface of a solute and a stationary phase made possible by mutual steric compatibility should be a critical factor for retention to be favorable, leading to pronounced steric selectivity.

4.2. Steric selectivity of carbon packing

The properties of C_{18}, polymer and carbon packings are well illustrated in the separation of cycloalkanes. The use of alkanes and cycloalkanes is appropriate to investigate the steric selectivity of various types of stationary phases, because only dispersion force are expected to play a role in their interactions with the stationary phase and solvents. The retention on a carbon phase seems to be determined by how much contact is possible between a solute and carbon surface.

As shown in Table 4, the separation factors, $k'_{n\text{-hexane}}/k'_{\text{cyclohexane}}$ and $k'_{\text{decalin}}/k'_{\text{adamantane}}$, were very large on a carbon phase compared with silica C_{18}. This is exactly what is expected from the contribution of dispersion forces to retention, because a greater dispersion interaction is expected for more planar solutes with rigid planar graphite surfaces than with flexible C_{18}.

The carbon atoms in *n*-hexane can assume a completely planar arrangement whereas those in cyclohexane cannot adopt a stable conformation. Also, decalin can have more points of contact with a flat surface than adamantane at a similar molecular mass. A slightly greater separation factor between decalin and adamantane on 2-(1-pyrenyl)ethylsilylated silica (PYE in Table 2) than C_{18} is an indication of the selective retention of the more planar hydrocarbon in each pair, as with the carbon phase, taking into

TABLE 4

STERIC SELECTIVITY FOR CYCLOALKANES [6,32]

Mobile phase: 80% methanol.

Stationary phase	k'		α [a]	k'		α [b]
	Hexane	Cyclohexane		*trans*-Decalin	Adamantane	
C_{18}	5.24	3.73	1.40	15.9	10.3	1.54
PYE [c]	0.99	0.82	1.21	3.47	2.24	1.55
Carbon I [c]	0.97	0.32	3.0	3.55	0.79	4.49
Carbon II [c]	0.52	0.17	3.0	2.20	0.45	4.86
PMMA (CHN) [d]	0.95	1.31	0.72	3.07	3.01	1.02
PMMA (OCT) [e]	1.49	1.75	0.85	4.11	2.38	1.72

[a] Separation factor, $k'_{hexane}/k'_{cyclohexane}$.
[b] Separation factor, $k'_{trans\text{-}decalin}/k'_{adamantane}$.
[c] See Table 2.
[d] Poly(methyl methacrylate) gel prepared in cyclohexanol [32].
[e] Poly(methyl methacrylate) gel prepared in 2-octanol [32].

account the smaller hydrophobic selectivity of PYE than C_{18}.

As mentioned earlier, polymer gels showed a selective retention of more rigid, compact compounds, cycloalkanes over linear alkanes. The results on a carbon phase are different from those on the polymer-based phase and on C_{18}. Previous workers discussed the retention tendency of xylenes based on the points of contact with the carbon surface [43,46]. The results with cycloalkanes, which are electronically most inert, support their conclusion of the importance of matching up atoms in the surface and the solute molecules.

4.3. Selectivity difference among carbon, PYE and C_{18} phases

Dimethylcyclohexanes do not possess functional groups to undergo interactions except hydrophobic and dispersion forces. The more planar compounds with two equatorial methyl groups, *trans*-1,2-, *cis*-1,3- and *trans*-1,4-isomers, were preferentially retained by all the stationary phases [6,53]. As shown in Table 5, the PYE phase resulted in a similar resolution in a much shorter time than C_{18}, and this tendency was further emphasized on the carbon phase.

For cyclohexanediols, especially for 1,3- and 1,4-isomers, the variations in k' values and separation factors clearly indicate the preference for the more planar isomer by carbon and PYE phases. These examples show the similarity between carbon and PYE phases in that structural matching plays a role in retention between a solute and the hydrophobic binding sites with a planar structure. The situation is different for dipolar solutes.

The C_{18} phase gave little separation for isomers of 1,2-cyclohexanedicarboxylic acid dialkyl esters, whereas PYE and the carbon phase provided much better separations. A preference on carbon, however, was shown toward the *trans* isomers with two equatorial ester groups with a more planar structure, in contrast to the results with the PYE phase which favored *cis* isomers possibly owing to the increased dipole–π interaction. Note that the effect of solute planarity was pronounced with the carbon packing for all the 1,4-disubstituted cyclohexanes in Table 5.

The contrast between the separation factors for 1,4-diols (much greater α on C_{18} than on carbon) and those for 1,4-diesters and 1,4-di-

TABLE 5

SELECTIVITY FOR DISUBSTITUTED CYCLOHEXANES [6,53]

Mobile phase: 70% methanol.

Solute	Separation factor between *cis* and *trans* isomers (k') [a]			
	C_{18}	PYE [b]	Carbon I [b]	Carbon II [b]
1,2-$(CH_3)_2$	1.05 (*c*; 23.7)	1.07 (*c*; 4.39)	1.51 (*c*; 2.11)	1.45 (*c*; 1.49)
1,3-$(CH_3)_2$	1.12 (*t*; 23.8)	1.16 (*t*; 4.38)	1.49 (*t*; 2.44)	1.58 (*t*; 1.67)
1,4-$(CH_3)_2$	1.13 (*c*; 23.7)	1.13 (*c*; 4.21)	1.67 (*c*; 2.22)	1.58 (*c*; 1.57)
1.2-$(COOEt)_2$	1.01 (*t*; 2.79)	1.19 (*t*; 3.41)	1.11 (*c*; 3.43)	1.03 (*c*; 2.08)
1,2-(COOPr-*n*)$_2$	1.01 (*t*; 6.98)	1.19 (*t*; 6.81)	1.32 (*c*; 10.3)	1.10 (*c*; 6.70)
1,4-$(COOEt)_2$	1.10 (*c*; 2.40)	1.06 (*c*; 3.82)	4.88 (*c*; 1.81)	4.83 (*c*; 1.12)
1,4-(COOBu-*n*)$_2$	1.28 (*c*; 15.5)	1.14 (*c*; 18.0)	5.87 (*c*; 2.49)[c]	–
1,2-$(OH)_2$ [d]	1.07 (*t*; 6.62)	1.09 (*t*; 4.20)	1.10 (*t*; 2.37)	–
1,3-$(OH)_2$ [d]	1.03 (*c*; 2.32)	1.14 (*t*; 1.67)	1.25 (*t*; 1.76)	–
1,4-$(OH)_2$ [d]	5.42 (*t*; 0.46)	3.39 (*t*; 0.61)	1.05 (*t*; 1.70)	–

[a] k' of the first peak indicated by *t* or *c*, which represents *trans* or *cis* isomer, respectively.
[b] See Table 2.
[c] Mobile phase: 90% methanol.
[d] Mobile phase: 5% methanol.

methyl derivatives (much greater α on carbon than on C_{18}) is striking. The results can be explained by taking into account the contribution of structural planarity of the solute (*trans* isomers being more planar than *cis* isomers), and hydrophobic properties (*trans*-diesters and -dimethyl derivatives being more hydrophobic than *cis* isomers, whereas *trans*-diols are less hydrophobic than *cis*-diols owing to the greater exposure of the hydroxyl groups). Thus an increased selectivity was observed with carbon for 1,4-dimethylcyclohexanes and 1,4-diesters, where selectivity based on planarity was added to the hydrophobic selectivity. In contrast, *trans*-1,4-diols with a much smaller hydrophobicity resulted in increased retention on carbon based on structural planarity, leading to much smaller separation factors than on C_{18}. It is clear that graphitized carbon is selective for a pair with different structural planarities which cannot be separated based on hydrophobic properties.

In summary, a graphitized carbon packing showed retention characteristics based on the major contribution of dispersion forces, and hence steric factors of solutes. The solute–stationary phase interactions with the carbon phase are much greater than on any silica-based packing materials, resulting in the preferential retention of planar compounds. The results clearly show the utility of carbon and PYE phases having rigid, planar interacting surfaces to provide the steric selectivity for the separation of compounds with similar hydrophobicities which are difficult to separate with a C_{18} phase, although the mechanism of separation is not necessarily common in some instances. Silica-based PYE phase showed properties intermediate between those of the carbon and alkyl-type silica-based stationary phases, and also provided a better column efficiency than the carbon packing.

The steric selectivity and excellent chemical stability of the carbon phase under extreme pH values are obvious advantages. One can obtain a much better separation in a much shorter time in favorable instances [6,43–50]. Stronger eluents with dipolar properties such as THF and acetonitrile or a proton donor should be used with the carbon phase to avoid peak tailing and/or long retention times for late-eluting substances.

5. DONOR–ACCEPTOR-TYPE BONDED STATIONARY PHASE

A number of charge-transfer-type packing materials have been utilized in LC. They include a donor type, naphthalene- or pyrene bonded, as well as an acceptor type, nitroaromatic group bonded. The latter is believed to serve as an electron acceptor, and is frequently employed with chiral stationary phases. As charge-transfer-type stationary phases have already been reviewed [40], with ample examples showing evidence for them working as charge-transfer phases, they will not be discussed here. Rather it is shown here that a nitroaromatic stationary phase undergoes very effective dipole–dipole interactions to discriminate very closely related aromatic compounds, which was not possible with any other stationary phases in RPLC [54–56]. In fact, PYE and nitroaromatic-bonded phases showed a reversed elution order for all the polychlorodibenzo-*p*-dioxin (PCDD) isomer pairs co-produced during syntheses, which permitted the structural identification of the isomers.

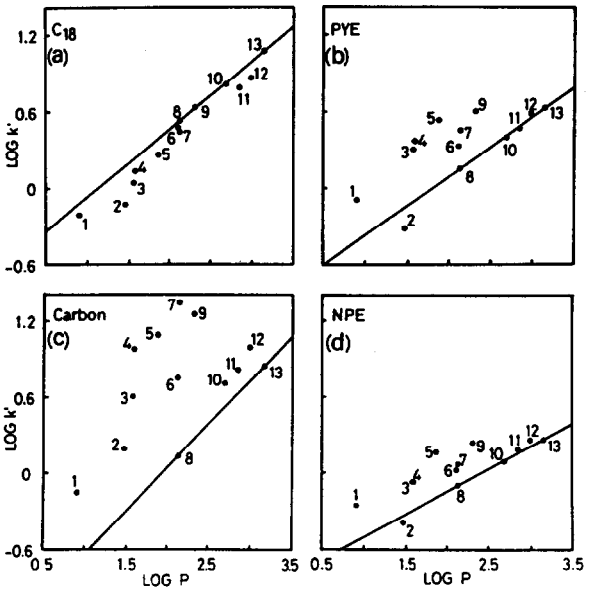

Fig. 16. Plots of log k' values against log P values for monosubstituted benzenes in 60% methanol [6]. Stationary phase: (a) C_{18}; (b) PYE; (c) carbon; (d) NPE. Substituents: 1 = NH_2; 2 = OH; 3 = CN; 4 = $COCH_3$; 5 = NO_2; 6 = OCH_3; 7 = $COOCH_3$; 8 = H; 9 = $N(CH_3)_2$; 10 = CH_3; 11 = Cl; 12 = Br; 13 = C_2H_5. The straight lines were drawn through the plots for 8, 10, and 13.

5.1. Dipole–dipole interaction with nitroaromatic bonded phase

In Fig. 16, log k' values of monosubstituted benzenes on carbon and silica-based phases are plotted against log P values [57] obtained from 1-octanol–water partitioning. A straight line was drawn through the plots for benzene, toluene and ethylbenzene, which indicates the contribution of hydrophobic interactions on every phase.

In contrast to the good linearity observed with silica C_{18} phase, indicating the major contribution to be hydrophobic interaction on this stationary phase, all the plots in Fig. 16c were found to be above the straight line, indicating the preferential retention of those with higher molecular mass, particularly for those with electron-withdrawing substituents (Nos. 4, 5, 7). The results agree with a retention mechanism including contributions of dispersion forces, charge-transfer interaction and dipole–π interaction. The correlation between the magnitude of dispersion forces and molecular volume has been documented [58].

In spite of the opposite electronic properties expected, not much difference was found between PYE and NPE stationary phases for monosubstituted benzene derivatives, except a slightly greater preference for dipolar molecules with Cl, Br, and NO_2 groups with NPE than PYE, as shown in Fig. 16. Clear differences were found, however, when these two stationary phases were compared for disubstituted benzene derivatives with electron-withdrawing substituents.

As shown in Fig. 17, PYE showed a preferential retention of *p*-dichloro- and *p*-dinitrobenzene, presumably owing to the more efficient electron removal from the benzene ring with the two electron-withdrawing groups at the *para* position. In contrast, NPE showed a preferential retention of *ortho*-disubstituted derivatives. This can be explained based on the more efficient dipole–dipole interaction between the aligned

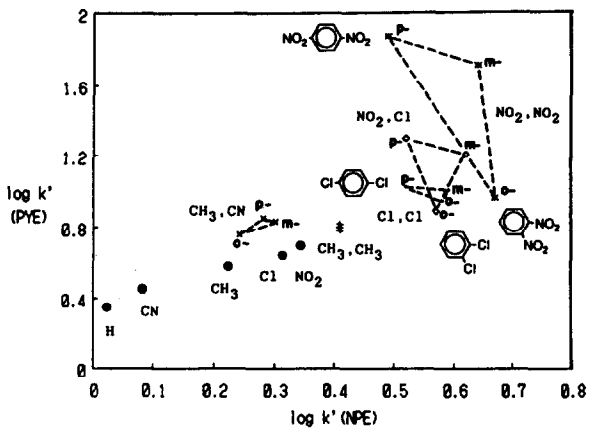

Fig. 17. Plots of log k' values on PYE phase against log k' values on NPE phase in 60% methanol. Compounds: mono- and disubstituted benzenes.

dipoles at the *ortho* positions and the NPE phase.

The chromatograms in Fig. 18 indicate a preferential retention of dinitronaphthalenes (peaks 1 and 2) on the donor–acceptor phases compared with the C_{18} phase [55]. The difference between PYE and NPE is also clearly noticeable. Whereas 1,5-dinitronaphthalene (peak 2) was preferentially retained with the PYE phase, 1,8-dinitronaphthalene (peak 1) was retained longer on the NPE phase. The results can be explained by considering charge-transfer interactions on PYE and dipole–dipole interactions on NPE. 1,8-Dinitronaphthalene possesses a much greater dipolar property than the 1,5-isomer with the two nitro group dipoles aligned, as was the case with *ortho*-disubstituted benzenes. In contrast, 1,5-dinitronaphthalene would undergo more effective charge-transfer interactions with the more electron-deficient naphthalene ring.

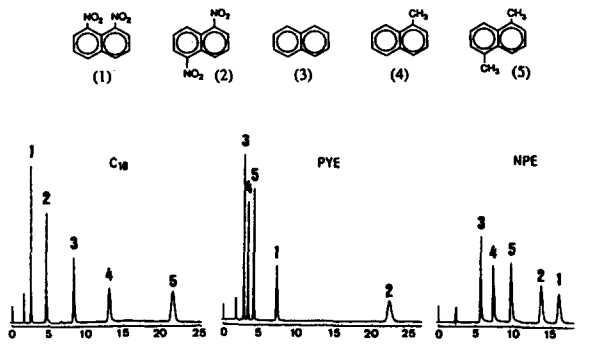

Fig. 18. Separation of naphthalene derivatives [55]. Mobile phase and column: (a) C_{18}, 70% methanol; (b) PYE, 90% methanol; (c) NPE, 70% methanol. Peaks: 1 = 1,8-dinitronaphthalene; 2 = 1,5-dinitronaphthalene; 3 = naphthalene; 4 = 1-methylnaphthalene; 5 = 1,5-dimethylnaphthalene.

5.2. Separation of polychlorinated aromatic compounds

The PYE and NPE stationary phases showed large differences in the retention selectivity toward polychlorinated aromatic compounds. The PYE phase preferentially retained those with the chlorine atoms located as far apart as possible from each other, *viz.*, 1,4-dichloro-, 1,3,5-trichloro- and 1,2,4,5-tetrachlorobenzene. In contrast, the NPE phase showed the greatest retention for polychlorobenzenes with an *ortho* arrangement of the chlorine groups, *viz.*, 1,2-di-, 1,2,3-tri- and 1,2,3,4-tetrachlorobenzene. The least hydrophobic compound, 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 [55].

These results suggest the possibility of providing structural information for isomeric polychlorinated aromatic compounds such as PCDDs based on their chromatographic behavior. This possibility has been realized in the isolation and structural identification of each PCDD congener from mixtures produced by the Smiles rearrangement during the preparation reaction [59,60].

Fig. 19 shows the separation of synthetic mixtures of 1,2,3,6- and 1,2,3,9-TCDDs on C_{18}, PYE and NPE phases. (The identity of the peaks was provided by comparison of the retentions on C_{18} phase [61].) On C_{18} and PYE phases, the isomer existing in a greater amount with less steric congestion among the chlorine atoms, 1,2,3,6-TCDD, was retained longer than 1,2,3,9-TCDD. In contrast, 1,2,3,9-TCDD was retained longer than 1,2,3,6-TCDD on the NPE phase. The opposite retention order of these TCDDs on PYE and NPE phases is in agreement with the results in Fig. 17.

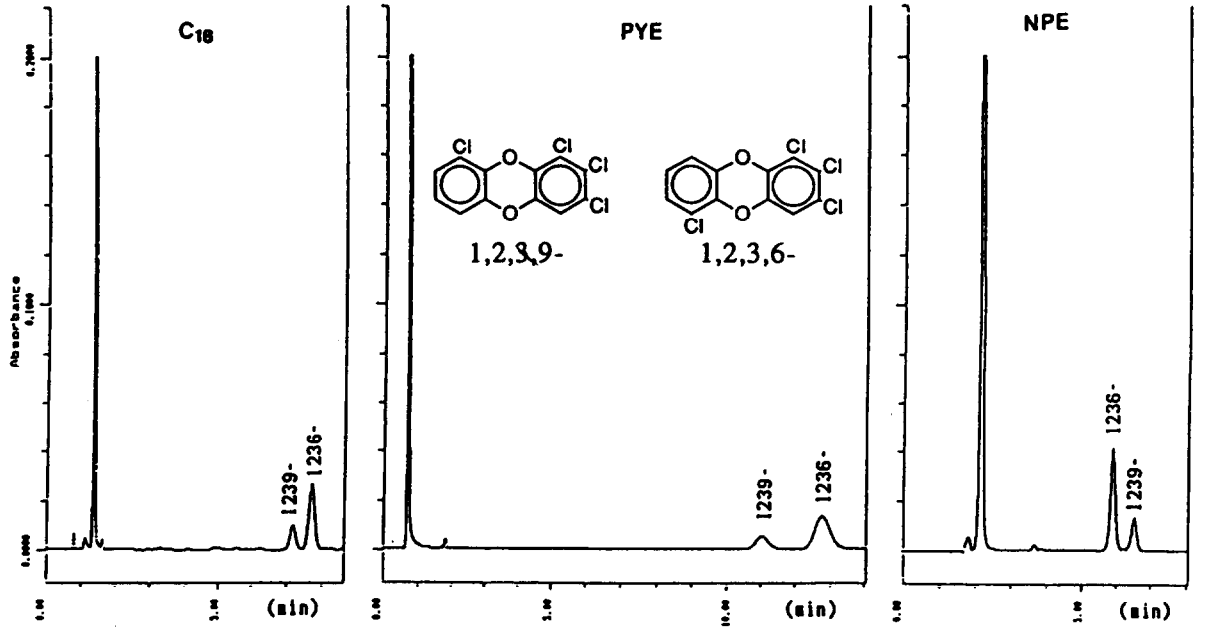

Fig. 19. Separation of 1,2,3,6- and 1,2,3,9-TCDDs [55]. Mobile phase, column and flow-rate: (a) 15-cm C_{18}, 90% methanol, 2 ml/min; (b) 10-cm PYE, methanol, 2 ml/min; (c) 15-cm NPE, 90% methanol, 1 ml/min.

The retention on the C_{18} phase can be explained by the hydrophobic property of the solute. As chlorine atoms on an aromatic ring increase the hydrophobic property [57], compounds with isolated chlorine substituents are retained longer than those with sterically congested chlorine atoms owing to the greater hydrophobic surface areas. Thus the proximity between 1- and 9-chlorine atoms resulted in a smaller retention of 1,2,3,9-TCDD than 1,2,3,6-TCDD on C_{18} phase, the latter giving the larger peak owing to the higher thermodynamic stability than the former.

As the greatest retention was observed on the PYE phase for the polychlorobenzenes with minimum steric congestion, the longer retention time for 1,2,3,6-TCDD than 1,2,3,9-TCDD is readily understandable based on the most favorable charge-transfer interactions. 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 the NPE phase. This is presumably due the more aligned dipoles in 1,2,3,9-TCDD than in 1,2,3,6-TCDD. A similar reversal of elution order on the PYE and NPE phases was obtained for all the PCDD pairs that are separable with the three stationary phases.

5.3. Structural assignment based on retention on NPE and PYE phases

Fig. 20 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 phases. The separation of these TCDDs by RPLC or high resolution gas chromatography (HRGC) has not been reported. Although not confirmed by other means, the earliest eluting larger peaks in the separation of 1,6- and 1,9-DCDD and 1,2,6- and 1,2,9-TrCDD on NPE or the second peaks on PYE and C_{18} phases should be 1,6-DCDD and 1,2,6-TrCDD, as predicted from a consideration of the retention order for 1,2,3,6- and 1,2,3,9-TCDD. The retention order is consistent with the retention mechanism on each phase.

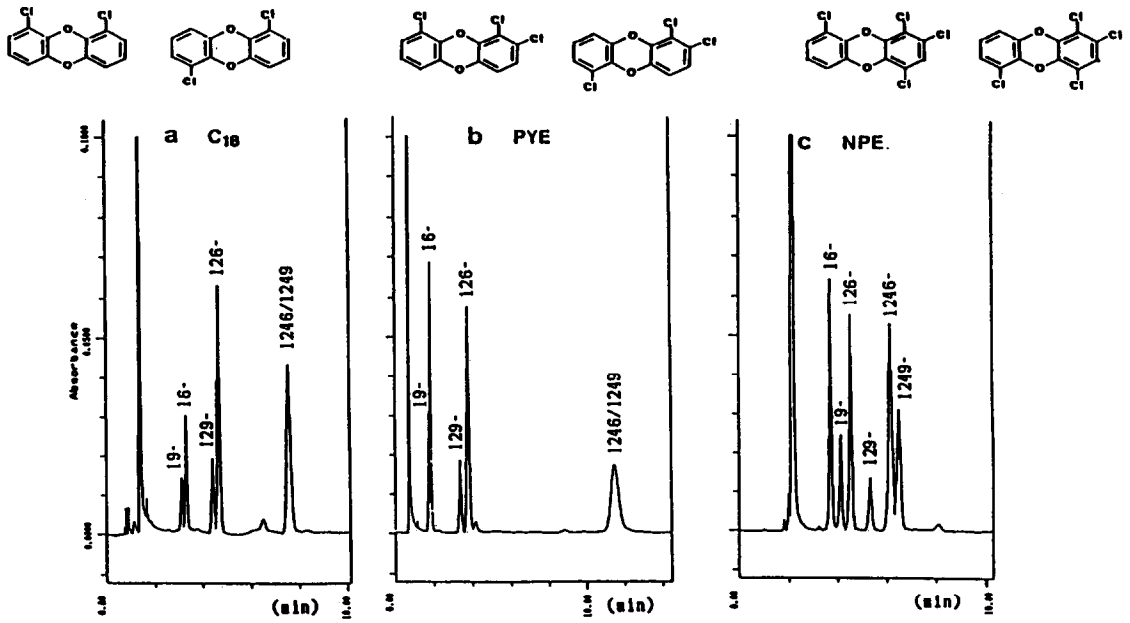

Fig. 20. Separation of 1,6- and 1,9-DCDDs, 1,2,6- and 1,2,9-TrCDDs and 1,2,4,6- and 1,2,4,9-TCDDs [55]. Conditions as in Fig. 19.

Substitution at the 4-position of 1,2,6- and 1,2,9-TrCDD should not reverse the retention order, although it actually prevents separation on C_{18} and PYE phases. Therefore, prediction of the elution order and peak size for this pair on the chromatograms on NPE gives the 1,2,4,6- assignment to the first and larger peak.

All six pairs of PCDDs previously unassigned and unseparated by RPLC and HRGC were similarly separated and assigned by using these nitroaromatic stationary phases. 3-(*p*-Nitrophenoxy)-propyl-bonded silica (NPO) [56] provided a greater selectivity than NPE, having *ca.* 70% of the nitro groups at the *para* position to the phenyl–silicon linkage [55]. The predictions based on the elution order on NPE and NPO agreed with the calculated dipole moments of PCDDs, indicating that the dipole–dipole interaction is actually dominant on these stationary phases. The interactions with NPE or NPO phase, however, seem to occur with the central part of the dioxin structure, not with the whole molecular structure. The chlorine atoms at the lateral positions had less effect than those at the vicinity of the two ether linkages.

In order to maximize the selectivity of these charge-transfer and dipolar stationary phases, alcoholic solvents (methanol or ethanol in combination with dichloromethane or water) should be used rather than acetonitrile or THF, which interact with the stationary phase. These simple donor–acceptor phases showed high selectivity, and have been shown to be useful for the separation and identification of closely related compounds, PCDDs [55,56] and fullerenes [62]. The chromatographic structure determination, although not frequently used, is very straightforward in these instances. The detailed understanding of the molecular interactions between analytes and stationary phases is essential for such an application.

6. CONCLUSIONS

The underlying mechanisms with all the packings in RPLC include hydrophobic and solute–solvent interactions, resulting in increased retention of compounds with greater C–H (hydrophobic) surface area, and smaller retention for compounds containing polar (hydrophilic) func-

tionality. In addition to these universal interactions seen with the use of any packing materials, (i) polymer gel packings show the size-exclusion effect even for low-molecular-mass compounds, which results in the preferential retention of rigid compact aromatic solutes; polymer packings containing ester or ether functionalities show preferential retention of dipolar and/or aromatic compounds based on dipole–π and dipole–dipole interactions; (ii) electron donor–acceptor-type bonded silica phases show the selectivity that can be explained in terms of charge-transfer, dipole–π and dipole–dipole interactions between stationary phase and solutes, and (iii) the carbon packing shows a dominant contribution of dispersion forces, leading to the preferential retention of planar molecules which may be termed as hydrophobic adsorption.

The silica C_{18} phase normally undergoes none of these positive interactions except for the weak steric effect based on the ordered structure of long alkyl chains. The simple understanding of the retention process, the partitioning of solutes between the mobile phase and the bonded alkyl groups containing organic solvents extracted from the mobile phase, can explain the effects of mobile phase composition and stationary phase structure. The alkyl chain length and the surface density primarily determine the phase ratio between the mobile and stationary phases in a column, orderedness of alkyl chains as well as the solvent content (or polarity) of the stationary phase, resulting in different selectivity on stationary phases with different surface coverage or alkyl chain length.

7. ACKNOWLEDGEMENTS

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