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EFFECT OF STATIONARY PHASE STRUCTURE ON RETENTION AND SELECTIVITY IN REVERSED-PHASE LIQUID CHROMATOGRAPHY

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SUMMARY

The effect of the structure of the stationary phase on retention and selectivity in reversed-phase liquid chromatography was studied, using chemically bonded stationary phases on silica gel. Nine stationary phases with various size, rigidity and degree of unsaturation were prepared, including alkyl, aryl, aralkyl and alicyclic structures. In addition to the solvophobic interaction and the solvation of the solutes in the mobile phase, stationary phase effects such as steric recognition and π–π interaction between solutes and the stationary phase, and the effect of solvent molecules bound to the stationary phase, were found to be important in determining retention in reversed-phase liquid chromatography. Planar solutes were preferentially retained by the stationary phases of planar structure and rejected by the non-planar stationary phases. Extended octadecyl groups and large aromatic rings in the stationary phase contributed to the preferential retention of planar solutes. The aromatic stationary phases showed greater retention for aromatic and polar solutes and lesser retention for saturated hydrocarbons than the saturated stationary phases. Retention and selectivity on stationary phases with aryl or aralkyl functionality were found to be more sensitive to solvent changes than on saturated stationary phases. The results suggest the possibility of controlling the magnitude of the stationary phase effects by selecting a stationary phase structure that can enhance the separation capability of reversed-phase liquid chromatography. The versatility of stationary phases with large aromatic groups was shown, and the complementary use of stationary phases of widely different nature was suggested to provide maximum selectivity.

INTRODUCTION

In a previous paper[1], we reported the effect of the alkyl chain length of the stationary phase in reversed-phase liquid chromatography (RPLC). We showed that the appearance of the chain-length effect was strongly influenced by the structure of the solutes and the type of organic solvent used in the mobile phase, both of which affect the extent of interaction associated with the stationary phase. The results implied that it was possible to vary the magnitude of these stationary phase effects by selecting the structure of the hydrocarbon moiety of the stationary phase. This is expected to increase the versatility of RPLC. The examination of the retention characteristics of stationary phases with various structures may lead to new stationary phases with greater separation capability, and at the same time provide information on the retention mechanism in RPLC.

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There have been some reports in this respect in the past. Little *et al.*[2] tried to increase the selectivity of the stationary phase by using a very long alkyl chain in the stationary phase. Attempts to control selectivity in RPLC by the use of various stationary phase structures have been popular[3–5], but the role of the stationary phase in RPLC is not yet fully understood.

Although RPLC is more widely used than normal-phase chromatography and improvements are still being made, the former has generally been considered to be less selective than the latter with respect to changes in retention due to steric factors associated with the solutes and changes in the type of solvents in the mobile phase. The major factor in retention in RPLC is the solvophobic interaction[6], which has less polar and steric selectivity compared with polar adsorption in normal-phase chromatography.

The present practice in RPLC is that a preliminary attempt at separation on a C_{18} stationary phase with a certain composition of the aqueous mobile phase is followed by a change in the eluent composition in order to adjust k' values and the separation. The use of tetrahydrofuran and solvents other than methanol or acetonitrile was shown to be useful[7–9]. If the desired separation is not obtained, one can try other stationary phases, such as C_8 or phenyl, which are commercially available. Several manufacturers offer instruments that can accomodate up to three or four solvents and carry out systematic development of the mobile phase[10].

A few points can be made on this approach of the development of the chromatographic system. First, this approach assumes the superiority of the C_{18} phase. Second, the selection of the alternative stationary phase is largely based on personal experience. Third, the search for suitable solvents with a single column can be time consuming, and some organic solvents for high-performance liquid chromatography (HPLC) are very expensive.

Another possible approach of attaining separations is to employ well characterized columns with widely different nature and to use simpler mobile phases and less time to develop the system. Thorough understanding of the interrelation between the solute structure, stationary phase structure and the retention seems to be very valuable in this regard, and the development of a stationary phase superior to the C_{18} phase, or a good alternative to the C_{18} phase, will greatly enhance the versatility of RPLC.

EXPERIMENTAL

Equipment

An HPLC system was constructed with an LC-3A pumping system (Shimadzu, Kyoto, Japan), a 7125 valve loop injector (Rheodyne, Berkeley, CA, U.S.A.) and M440 UV and R401 refractive index detectors (Waters Assoc., Milford, MA, U.S.A.). A thermostated bath was used to maintain the column temperature at 30 ± 0.1°C.

Materials

Spherical silica gel (Develosil; Nomura Chem., Seto, Japan) of average particle diameter 5 μm (surface area *ca.* 330 m^2/g) was used. Silylating reagents were either purchased from Petrarch System (Levittown, PA, U.S.A.) or prepared from corresponding olefins by standard methods. Chemical bonding reactions including end capping and column packing were carried out as described previously[7]. Mobile phases were made up by volume from LC-grade solvents (Nakarai Chem., Kyoto, Japan) and distilled water. Absolute methanol and absolute acetonitrile were obtained by standard methods[1].

Sample substances were either purchased or prepared by standard methods. Some polar compounds, including *n*-alkyl alcohols, *n*-alkyl carboxylic acid methyl esters and aromatic nitro compounds, were used in addition to hydrocarbons with various structures as follows: toluene (1), ethylbenzene (2), *n*-propylbenzene (3), *n*-butylbenzene (4), *n*-pentane (5), *n*-hexane (6), *n*-heptane (7), *n*-octane (8), naphthalene (9), anthracene (10), pyrene (11), 3,4-benzpyrene (12), cyclohexane (13), *trans*-decahydronaphthalene (14), adamantane (15), *n*-decane (16), diphenylmethane (17), 1,2-diphenylethane (18), triphenylmethane (19), tetraphenylethylene (20), fluorene (21), bidiphenyleneethylene (22), *o*-terphenyl (23), triphenylene (24), triptycene (25), tetrahydronaphthalene (26), *n*-dodecane (27), *n*-tetradecane (28) and *n*-hexadecane (29). Samples were made up in the mobile phase in most instances.

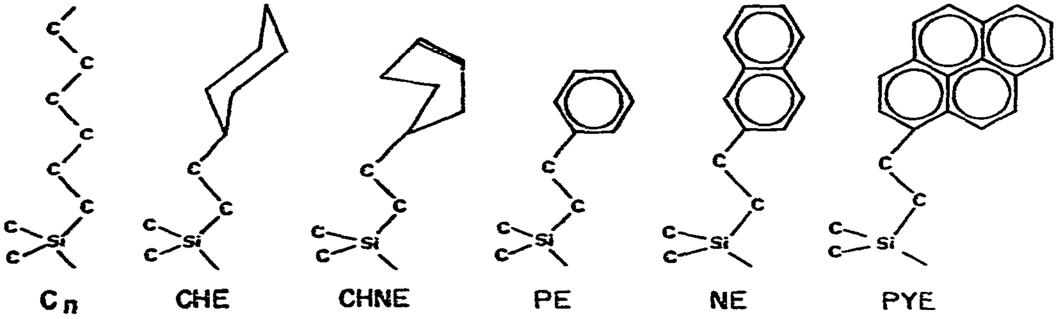
Chromatographic measurement

Chromatographic runs were carried out in duplicate. The reproducibility between the runs was better than ± 0.5%. The void volume (t_0) was obtained by the injection of water and glycerine. The outer column dead volumes in the connecting tubing and detectors were corrected.

RESULTS AND DISCUSSION

The structures of the organic silyl groups of the stationary phases differing in size, rigidity, planarity and degree of unsaturation are shown in Scheme 1. They include trimethylsilyl (C_1), *n*-octyldimethylsilyl (C_8), *n*-octadecyldimethylsilyl (C_{18}), 2-cyclohexylethyldimethylsilyl (CHE), 2-(3-cyclohexenyl)ethyldimethylsilyl (CHNE), phenyldimethylsilyl (Ph), 2-phenylethyldimethylsilyl (PE), 2-(2-naphthyl)ethyldimethylsilyl (NE) and 2-(3-pyrenyl)ethyldimethylsilyl (PYE). As shown in Table I, surface coverages are considered to be maximum for each bonded phase[11]. The stationary phases were treated with trimethylsilylating reagents to reduce the number of residual silanols. Maximum coverages are desirable when comparing the retention characteristics of the stationary phases.

The four types of hydrocarbons which visualized the difference in chainlength of the stationary phases[1] were used to study the nine stationary phases in 80% methanol. The solutes included *n*-alkanes, planar polynuclear aromatic hydrocarbons (PAHs), aromatic compounds that cannot adopt a planar structure owing to steric repulsion within the molecule, and alicyclic compounds. The three pairs of solutes,



Scheme 1

TABLE I

SURFACE COVERAGES OF THE STATIONARY PHASES

Parameter	*Stationary phase*								
	C_1	*C_8*	*C_{18}*	*CHE*	*CHNE*	*Ph*	*PE*	*NE*	*PYE*
Carbon content (%)*	5.3	12.2	20.8	11.7	12.0	9.8	13.1	15.5	20.0
Surface coverage (μmol/m^2)	4.9	3.7	3.6	3.5	3.6	3.6	4.0	3.5	3.4

* From elemental analysis, prior to trimethylsilylation, except for the C_1 phase.

17 and 21, 20 and 22, and 23 and 24, have the same number of carbon atoms and π-electrons. Steric repulsion between the phenyl rings in 17, 20 and 23, makes these molecules non-planar. This type of analysis is expected to provide clues to the problems of why the C_{18} phase preferentially retains planar hydrocarbons[1] compared to the C_1 or C_8 phases and how the planarity, rigidity and degree of unsaturation of the stationary phase affects retention and selectivity in RPLC. The results are shown in the form of log *k′ versus* log *k′* plots in Fig. 1–3.

Fig. 1 shows a comparison of four stationary phases with eight carbon atoms in the major hydrocarbon part. Although straight lines are expected only for *n*-alkanes, the groups of solutes are located very close to straight lines, and it is convenient to show the retention characteristics of each stationary phase in this way. The solutes used to construct these lines were *n*-alkanes from C_5 to C_{10}, PAHs from naphthalene (9) to benzpyrene (12), alicyclic compounds from cyclohexane (13) to adamantane (15) and the non-planar aromatic compounds diphenylmethane (17), triphenylmethane (19), tetraphenylethylene (20) and *o*-terphenyl (23).

In these log *k′ versus* log *k′* plots, the *n*-alkanes can be taken as the solutes with a minimum degree of specific interactions with the stationary phase. When a

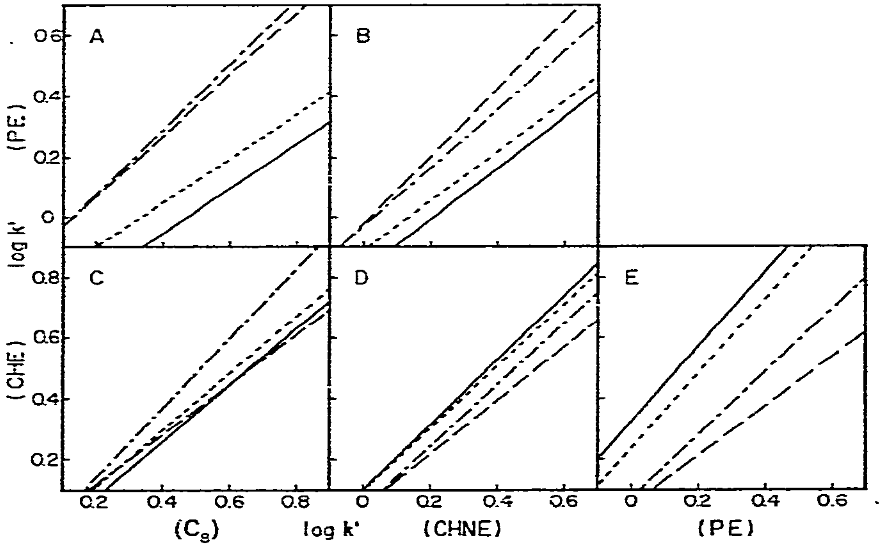


Fig. 1. Plots of log *k′* values on CHE and PE phases against log *k′* values on C_8, CHNE and PE phases in 80% methanol. ———, *n*-Alkanes; – – –, PAHs; –·–·–, non-planar aromatics; -----, alicyclics.

group of solutes behaved differently from *n*-alkanes in the plots, the solutes were preferentially retained by the stationary phase whose axis is closer to the line compared to the line for *n*-alkanes. When the straight lines were well separated in the plots, there is a difference in selectivity between the two stationary phases toward the different type of compounds. In other words, if two solutes belonging to different groups gave similar k' values or no separation on one stationary phase, they can be easily separated on the other stationary phase with the same mobile phase. Thus a pair of columns which give the most scattered plot can provide maximum selectivity difference. The difference in selectivity is large between aromatic and aliphatic stationary phases, and also significant between straight chain and cyclic stationary phases as shown.

In Fig. 1C, two saturated stationary phases, CHE and C_8, are compared. The difference in the retention behaviour is relatively minor between these two phases, but the non-planar aromatic compounds showed significantly larger retentions compared with other types of hydrocarbons on the cyclic CHE phase than the C_8 phase. This suggests that the non-planar aromatic compounds were either favoured by the CHE phase or disfavoured by the C_8 phase compared with planar PAHs and saturated hydrocarbons. The results shown later indicate there seem to be no positive effects by the CHE phase. The apparent preference of non-planar aromatic compounds by the CHE phase was caused by the fact that the straight chain C_8 phase disfavors these hydrocarbons. The results obtained by Řehák and Smolková[5] can also be explained by similar shape compatibility.

Fig. 1D, the plot between the CHE phase and the CHNE phase, shows the influence of a double bond in the stationary phase structure. As can be seen, only one double bond significantly affected the retention, favouring the aromatic compounds, particularly the planar PAHs. The introduction of a double bond increases the planarity and rigidity of six-membered ring while increasing the extent of electronic interactions. In the comparison of the two stationary phases having six-membered rings, in Fig. 1E, the PE phase showed preferential retention for aromatic compounds, as expected. Similarly, the PE phase showed a larger retention for aromatic compounds than saturated compounds when compared to the C_8 phase, as shown in Fig. 1A. The two phases did not differentiate the aromatic compounds based on their planarity. Among the four stationary phases examined in Fig. 1, the largest difference was seen between the CHE and PE phases with respect to the retention of the four types of the hydrocarbon solutes.

In Fig. 2, the C_1 phase, which had been thought to have unusual characteristics as an RPLC stationary phase, was compared with three other phases. Fig. 2A suggests that the two alkyl phases, C_1 and C_8, are very similar with respect to the retention of these hydrocarbons. The difference between the two was close to that between the Ph and the PE phase. Fig. 2B indicates that the CHE phase favours non-planar aromatic compounds and disfavours planar PAHs in comparison with the shortest C_1 phase. In other words, the CHE phase, which cannot take a planar structure, rejects planar hydrocarbons even in comparison with the C_1 phase. This emphasizes the importance of the planar structure of the stationary phase to show preferential retention of planar solutes. The plot between the C_1 and the Ph phase is similar to that between the C_8 and the PE phase in Fig. 1A. This implies that the stationary phases having one phenyl ring did not show much preference toward planar PAHs over non-planar aromatic compounds. A phenyl ring seems to be insufficient to show much planarity.

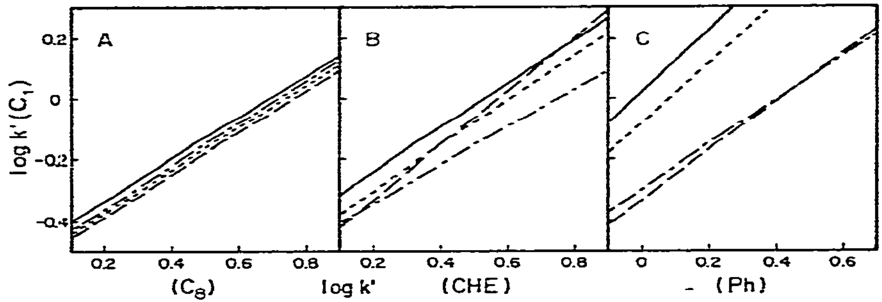


Fig. 2. Plots of log k' values on C_1 phase against log k' values on C_8, CHE and Ph phases in 80% methanol. See Fig. 1 for the solute identification.

Fig. 3 shows a comparison between the C_{18} phase, the most commonly used stationary phase in RPLC, and five stationary phases that be used as complementary stationary phases owing to their differing retention characteristics from the C_{18} phase. As shown in Fig. 3A, the difference between the C_{18} and the C_8 phase is not particularly great, especially for non-planar aromatic compounds and saturated compounds, although the C_8 phase is commonly used as an alternative to obtain separations which are not possible with the C_{18} phase. The largest difference in retention between the PAHs and non-planar aromatic compounds is seen in Fig. 3B, which compares the C_{18} and CHE phases. The planarity of the stationary phase is the factor to give such a difference. Figs. 3C–E show plots between the C_{18} and the three aromatic stationary phases. Large differences in retention behaviour between aromatic compounds and saturated compounds were observed, which was not the case with the C_8 phase. Aromatic compounds were preferentially retained by the aromatic stationary phase, and saturated compounds by the C_{18} phase. Any of these three combinations can also provide different selectivity between alicyclic compounds and *n*-alkanes. The largest increase in retention was seen for PAHs with an increase in the size of the aromatic ring system in the stationary phase, as with increase in alkyl chain length in the stationary phase from the C_1 to the C_{18} phase[1].

The PE phase could be a possible choice as a complementary stationary phase to the C_{18} phase in Fig. 3. When considering the versatility of a stationary phase, however, the absolute retention of the stationary phase has to be taken into account. The retention times of hydrocarbons on the PE phase were three to six times smaller than on the C_{18} phase. The commercially available Ph phase showed even smaller retention. Thus the solvent strength has to be lowered when these stationary phases are used instead of the C_{18} phase. Large retention times are also preferable with preparative separations, because they allow the use of stronger eluents. Large sample volumes and higher elution strengths of the sample solvents often cause a decrease in column performance in preparative separations[12,13] and the undesirable effect can be minimized by the use of stronger mobile phases. The PYE phase is more retentive, and is considered to be better in this respect.

The stationary phase still needs improvement to provide a greater selectivity difference to the C_{18} phase as the PAHs and non-planar aromatic compounds behaved very similarly on both the C_{18} phase and the PYE phase in this mobile phase. The PAHs and the non-planar aromatic compounds behaved differently on the two stationary phases in other mobile phases such as 80% acetonitrile and absolute methanol. This is probably due to the change in the alkyl chain structure of the C_{18}

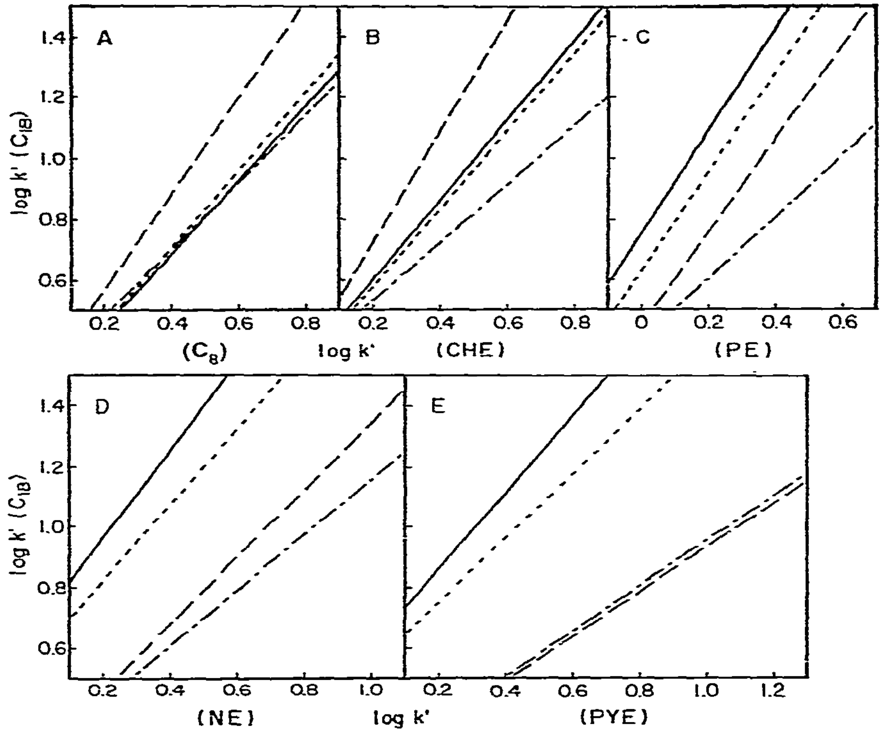


Fig. 3. Plots of log k' values on C_{18} phase against log k' values on C_8, CHE, PE, NE and PYE phases in 80% methanol. See Fig. 1 for the solute identification.

phase and the extent of solvation of the PYE phase. The more extended C_{18} chains seem to favour the planar PAHs in stronger mobile phases than the PYE phase, which is expected to show little structural change with change in the mobile phase.

There is no doubt that any of the five stationary phases in Fig. 3 can enhance the capability of RPLC, as the C_8 phase, which showed a relatively small difference from the C_{18} phase, has proved useful. If one considers an alternative stationary phase to the C_{18} phase which can provide a greater separation capability and versatility in RPLC, it will be a stationary phase with large aromatic groups such as the PYE phase used in this study. Some advantages of such a phase over other phases are mentioned below.

First, the PYE phase gave the greatest retention among the five stationary phases in Fig. 3, almost similar to the C_{18} phase for aromatic compounds and slightly less for aliphatic polar compounds, as shown in Fig. 4. Large k' values, which enable the use of strong solvents, are needed with preparative separations, as mentioned before, to allow large sample volumes and strong sample solvents to be used, because large aromatic compounds sometimes have limited solubility in aqueous solvents. Second, a large difference in the retention characteristics was seen between the PYE phase and the C_{18} phase with respect to the degree of unsaturation of the solutes. Aromatic and polar compounds, especially aromatic nitro compounds, were selectively retained by the PYE phase. The chromatograms in Fig. 5 show a difference in selectivity between the two phases. Peak reversal can be seen in the same k' range in the same mobile phase. Selectivity difference of this magnitude for hydrocarbons in the

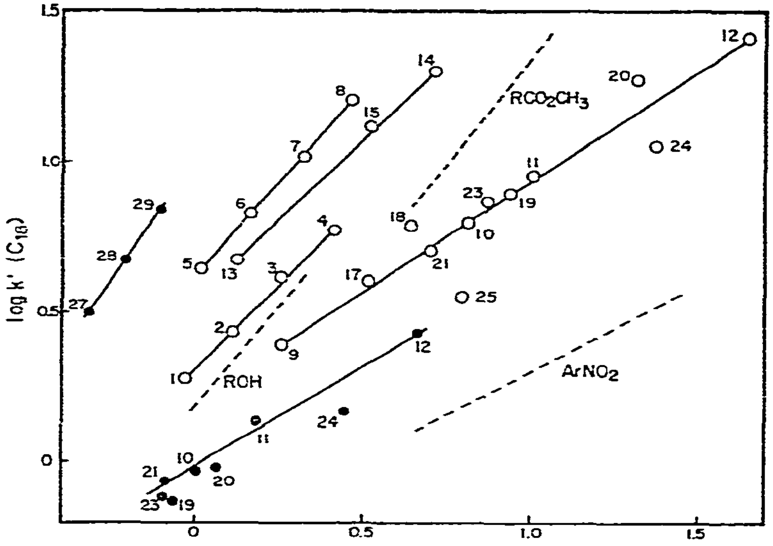


Fig. 4. Plots of log k' values on C_{18} phase against log k' values on PYE phase. The numbers indicate the compounds listed in the Experimental section. ○, 80% methanol; ●, absolute methanol.

same mobile phase is unprecedented. As seen in Fig. 4, an increase in retention on the PYE phase relative to the C_{18} phase is seen with increase in the rigidity and the extent of interaction with the aromatic stationary phase. The preference by the PYE phase decreases in the following order: aromatic nitro compounds, aromatic hydrocarbons, aliphatic esters, aliphatic alcohols, alkylbenzenes and alicyclic compounds. The scattered plot in Fig. 4 illustrates the large difference in selectivity in the similar k' range in the same mobile phase. The comparison between the two stationary phases in absolute methanol is also shown in Fig. 4. The difference in selectivity is even larger in this mobile phase, and planar PAHs showed a slightly different behaviour to non-planar aromatic compounds. The third point of the versatility of aromatic stationary phases is that a change in the mobile phase causes more variation of retention and selectivity on the PYE phase than on the C_{18} phase. As shown in Table II, a change in the mobile phase from 80% methanol to 80% acetonitrile did not affect the relative retention between pyrene and triphenylmethane on the C_{18} phase, whereas the same change in the mobile phase gave a significant difference in selectivity on the PYE phase. Triptycene, a rigid non-planar aromatic compound, was selectively retained by the PYE phase relative to triphenylmethane, which also has three phenyl rings in its structure. These facts imply that, in addition to the effect of solvents in the stationary phase[1], some other factors that depend on the orientation of aromatic rings of solutes in the stationary phase, such as π–π interaction, are responsible. The magnitude of the solvent effect on k' was strongly dependent on solute structure, providing the sensitivity towards a change of solvents. This can be an advantage, as the greater solvent effect may allow the use of less complicated and less expensive solvents.

There are cases where a very slight change in a particular portion of a chromatogram is desirable. There are other cases, however, where transfer of a group of peaks to another region of the chromatogram to prevent overlap with interfering

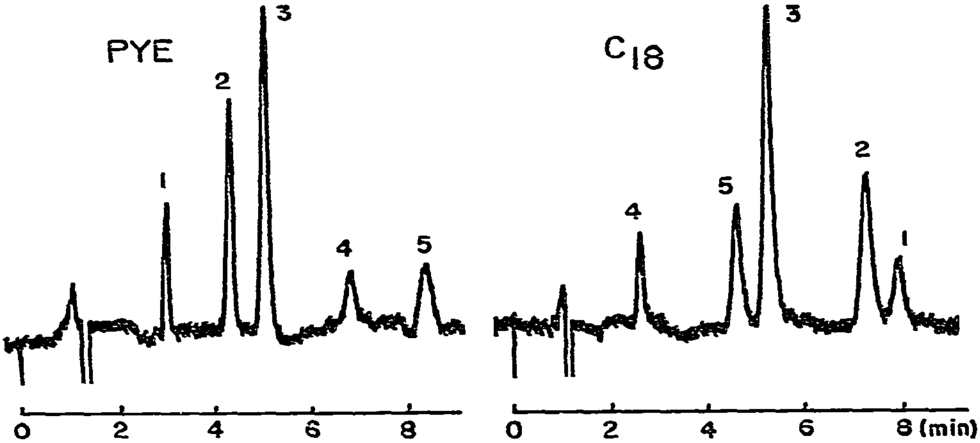


Fig. 5. Chromatograms of *n*-hexane (1), *n*-butylbenzene (2), diphenylmethane (3), 1-nitronaphthalene (4) and triptycene (5) on PYE and C_{18} phases in 80% methanol. Flow-rate: 1 ml/min. Detector sensitivity: ×2 on the R401 RI detector. Column: 10 cm × 4.6 mm I.D.

peaks is needed. Complementary use of the C_{18} and the PYE phases may make this kind of operation possible.

Another interesting point is that a group of compounds with the same carbon skeleton with different functional groups, *e.g.*, C_8H_{18}, $C_8H_{17}OH$ and $C_7H_{15}CO_2CH_3$, were eluted closer to each other on the PYE phase than on the C_{18} phase, making the analysis time shorter. The *k'* of *n*-octane was nine times greater than that of 1-octanol on the C_{18} phase, but the difference was less than three-fold on the PYE phase in 80% methanol.

In order to understand the difference among the stationary phases in more detail, separation factors generated by changes in solute structure were plotted against the retention increase caused by one additional methylene group derived from *n*-alkanes on each stationary phase in Fig. 6. As a very small specific interaction is expected with a methylene group, the line representing the methylene group increment with the slope of unity follows the contribution of simple solvophobic interaction on these stationary phases. Aromatic stationary phases showed much smaller hydro-

TABLE II

EFFECT OF MOBILE PHASE ON *k'* VALUES ON C_{18} AND PYE PHASES

Solute	*k'*					
	C_{18} phase			*PYE phase*		
	*I**	*II**	*k'_I/k'_{II}*	*I**	*II**	*k'_I/k'_{II}*
n-C_8H_{18}	16.0	12.9	1.24	2.93	1.97	1.49
Pyrene	8.95	4.80	1.86	10.2	3.22	3.17
Triphenylmethane	7.82	4.15	1.88	8.74	3.20	2.73
1-Nitronaphthalene	1.34	1.30	1.03	4.90	1.51	3.25
Triphenylcarbinol	2.46	1.78	1.38	3.24	1.61	2.01
n-$C_{12}H_{25}OH$	9.35	6.26	1.49	3.72	1.57	2.37
n-$C_9H_{19}CO_2CH_3$	7.24	5.66	1.28	4.54	1.82	2.49

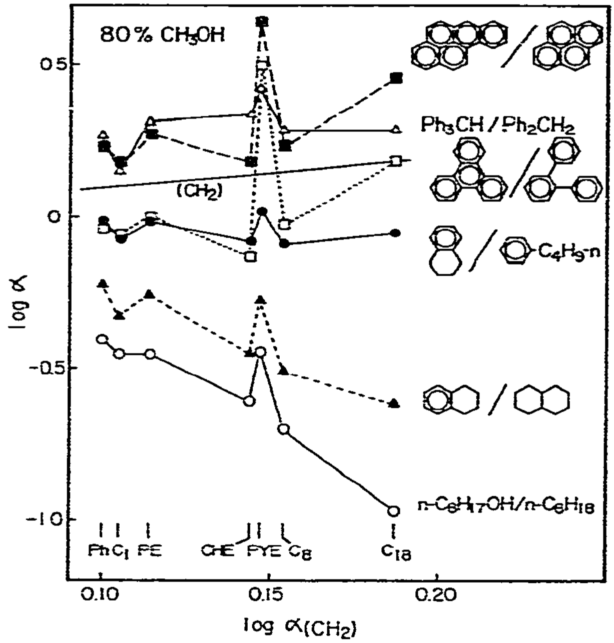


Fig. 6. Plots of separation factors (log α) for the pair of compounds shown on the right against the separation factor caused by one methylene group (log α_{CH_2}) on each stationary phase in 80% methanol.

phobicity than saturated stationary phases having the same carbon numbers. As can be seen in Fig. 6, no other change follows the tendency of the methylene group increment. The measurements were made in the same mobile phase throughout, and therefore the difference was attributed to the stationary phase effects. An increase of one fused aromatic ring from pyrene to 3,4-benzpyrene caused a large increase in retention on the PYE and aromatic stationary phases as well as on the C_{18} phase. A similar tendency was observed for the change from *o*-terphenyl to triphenylene, with an increase in planarity and rigidity. An increase of one phenyl ring in non-planar aromatic compounds, however, produced a relatively small retention increase with the PYE and C_{18} phases, and a large increase on stationary phases with one six-membered ring. Cyclization within a molecule, or a change from *n*-butylbenzene to tetrahydronaphthalene caused a decrease in retention on small saturated stationary phases. However, aromatic stationary phases and the C_{18} phase showed a slight upward shift in the plot. This was also the case with aromatization, *i.e.*, a change from decahydronaphthalene to tetrahydronaphthalene, which makes the solute more rigid and planar. Addition of a hydroxyl group produced a smaller retention decrease on aromatic stationary phases than on saturated stationary phases. The conclusion from Fig. 6 is that the C_{18} phase has the characteristics of a planar aromatic stationary phase with respect to selectivity based on steric recognition toward the solutes, only slightly less in magnitude than the PYE phase. This suggests the contribution of the aligned alkyl chains in the C_{18} phase for its preference toward planar solutes.

A test of RPLC stationary phases using a non-polar eluent such as *n*-heptane has been recommended for characterizing chemically bonded phase[14]. Table III shows the *k'* values obtained in *n*-hexane containing *ca.* 28 ppm of water at 30°C. We

TABLE III

k' VALUES IN n-HEXANE (30°C)

The solvent contained *ca.* 28 ppm of water. The elution time of n-dodecane was used as t_0.

Solute	*Stationary phase*				
	C_1	C_{18}	*CHE*	*PE*	*PYE*
1-Nitronaphthalene	0.52	0.31	0.11	0.46	2.2
Ethyl benzoate	1.3	0.35	0.14	0.26	0.87
Acetophenone	4.9	2.0	0.80	0.91	6.3
n-$C_{12}H_{25}OH$	~16	2.0	1.5	1.6	6.0
Benzene	0.04	0.04	0.03	0.10	0.06
Pyrene	0.07	0.22	0.06	0.25	0.76
Triphenylene	0.14	0.29	0.04	0.30	1.5
3,4-Benzpyrene	0.15	0.50	0.06	0.34	2.1
Triphenylmethane	0.09	<0.01	0.02	0.20	0.62
Triptycene	0.22	<0.01	0.03	0.25	0.88
Tetraphenylethylene	0.15	<0.01	0.06	0.20	0.88

used a single batch of n-hexane and measured the retention after constant k' values were attained with a minimum amount of sample. The magnitude of the k' values for polar compounds gave an estimate of how much silanol groups was left and accessible to the solutes. Although similar reaction conditions were used throughout the bonding reaction and the end capping, considerable differences were found among the five stationary phases. The C_1 phase allows the polar solutes to come close to the silanols easily, as expected. The PYE phase showed considerable retention of polar solutes. Specific retention of aromatic nitro compounds on the PYE phase was seen, as noted in aqueous methanol. Hemetsberger *et al.*[15] proposed the use of this type of interaction in normal-phase chromatography.

The results with aromatic hydrocarbons as solutes gave useful information on the mechanism of retention in RPLC. The C_1 and PE phases showed significant retentions for these aromatic compounds regardless of planarity, probably owing to accessible silanols and π–π interaction in the latter. The CHE phase showed very small retentions for all of the hydrocarbons tested. On the C_{18} phase, considerable retention was seen only for large PAHs. The PYE phase showed significant retention for both types of aromatic compounds, but a preference was seen for the PAHs. This observation is very similar to that in RPLC. The results with the PYE phase are understandable on the basis of the residual silanols and π–π interaction. As the only driving force of retention in the non-polar eluent with the C_{18} phase is the affinity to silanols, supposedly extended C_{18} chains in hexane seem positively to assist the adsorption of PAHs on to the silanols. The location of the PAHs in the C_{18} stationary phase must be close to the bottom of the brush-type structure between the long chains. The common observation of the steric recognition provided by the C_{18} chains in both polar and non-polar mobile phases is indicative of the similar retention scheme under RPLC conditions, as suggested before. The driving force of retention is different, but the steric interaction between the solutes and stationary phases seems to be common. With the PYE phase, the planar PAHs are accomodated in the slots made by pyrenyl groups just like slides in slide trays. The preference of planar compounds over non-polar ones is easily understood. The results with the CHE phase suggest that the non-planar stationary phase does not have a positive effect on the preferred retention of non-planar aromatic compounds, which was seen in Fig. 3B. The different behavior between PAHs and non-planar aromatic compounds in Fig. 3B was probably due to the fact that non-planar compounds are rejected by the C_{18} phase. Although a significant retention was seen for many compounds in *n*-hexane, the retention is much less in 10% benzene in *n*-hexane, and almost no retention was seen in tetrahydrofuran. Hence the effect of silanols on the retention of these hydrocarbons in RPLC is considered to be very small.

CONCLUSION

The stationary phase effects such as steric recognition and π–π interactions between solutes and stationary phases and the solvent effect were found to be important in determining the retention and selectivity in RPLC, in addition to the solvophobic interaction. Unsaturated compounds were selectively retained by aromatic stationary phases. Rigid solutes were favoured by rigid stationary phases. Planar solutes were preferentially retained under both reversed-phase and normal-phase condition by stationary phases such as C_{18} and PYE, which are assumed to have a planar structure and to accommodate easily planar molecules between the bonded hydrocarbon moieties. It was shown to be possible to control the magnitude of the stationary phase effects by selecting the structure of the stationary phase to make it more sensitive to specific interactions. Stationary phases with a large aromatic functionality were shown to have some merits such as the good retentivity, widely different selectivity from the C_{18} phase and the large solvent effect on retention and selectivity. They can be a good alternative for the C_{18} phase, and the complementary use of such stationary phases with the C_{18} phase will increase the capability of RPLC.

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