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EFFECT OF ALKYL-CHAIN LENGTH OF THE STATIONARY PHASE ON RETENTION AND SELECTIVITY IN REVERSED-PHASE LIQUID CHROMATOGRAPHY

PARTICIPATION OF SOLVENT MOLECULES IN THE STATIONARY PHASE*

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SUMMARY

The retention behaviour of various hydrocarbons, including n-alkanes, poly-nuclear aromatic hydrocarbons (PAHs) and alicyclic compounds, were examined on alkylsilylated stationary phases with trimethylsilyl (C_1), octyldimethylsilyl (C_8) and octadecyldimethylsilyl (C_{18}) functionality. In general, a longer alkyl chain in the stationary phase provided a longer retention time. Although the retention of rigid planar aromatic hydrocarbons relative to non-planar aromatic compounds and alkanes was much larger on the C_{18} phase than on the C_8 and C_1 phases in a methanolic mobile phase, the effect of chain length was much smaller in a mobile phase containing acetonitrile. A change in the organic solvent in the mobile phase from methanol to acetonitrile resulted in grossly different selectivities between aromatic hydrocarbons and the saturated hydrocarbons regardless of the rigidity or planarity in structure on the C_{18} phase, in contrast with minor changes on the C_1 and C_8 phases. The increase of organic solvent content in the mobile phase resulted in much larger retention of rigid planar aromatic compounds relative to other aromatic compounds on the C_{18} phase, whereas on the C_1 and C_8 phases this effect was smaller. The retention characteristics of the C_8 phase for the hydrocarbons were found to be relatively close to those of the C_1 phase. The results suggest that the effect of chain length is influenced by both the molecular structure of the solutes and the type of organic solvents used in the mobile phase.

INTRODUCTION

Whereas C_{18} stationary phases are most popular in reversed-phase liquid chromatography (RPLC), C_8 and other stationary phases with even shorter chain lengths are gaining increasing popularity owing to their high performance and particular retention characteristics.

For stationary phases with maximum coverage, retention in RPLC was explained by mobile phase effects such as solvophobic interaction[1], or solubility[2], or by adsorption[3-6] or partition[7] involving the alkyl chain layer[8] or organic solvents preferentially extracted on to alkyl chains[9]. Although these mechanisms would predict little selectivity differences among stationary phases with different chain lengths, practising chromatographers utilized the different selectivities provided by the stationary phases with various chain lengths that are now commercially available. Little and co-workers tried chain lengths up to C_{22} and found a better performance[10,11]. In some instances difficult separations on a C_{18} stationary phase were easily achieved on a C_8 stationary phase[12].

There have been numerous reports on the important effect of chain length[4,7,8,11,13-21], but most of them have just described the increase of both retention and separation factor with increase in chain length. Some workers tried to explain the different selectivities found for stationary phases with different chain lengths by steric factors[4,8] or by different surface structures of the stationary phases[7,19]. The results, however, were not clear enough to allow further mechanistic understanding.

We started the examination of the effect of alkyl chain length in RPLC with two major objectives in mind: firstly to understand the mechanistic implications of the effect of chain length on retention and selectivity, and secondly to examine the possibility of the prediction of retentions on other stationary phases, which is required in order to be able to choose the best stationary phase for particular separations. It has been pointed out that organic solvents in the mobile phase affect the retention of various compounds in RPLC[22,23]. However, there has been little evidence that the different selectivities on stationary phases with different chain lengths is actually provided by the difference in the alkyl chain length itself.

There seemed to be several points to be considered when dealing with this problem. Firstly, the sample solutes should be carefully selected. Several series of compounds should be chromatographed rather than a few particular pairs of compounds in order to obtain a clear view on the effect for certain type of compounds. Secondly, sample solutes should be selected so that the problem can be simplified; for example, polar effects should first be eliminated when considering steric effects. Thirdly, the effects of solvents should be examined to show if there is any specific effect provided by solvents on the appearance of the chain length effect. Stable columns packed with particles of maximum rather than partial coverage are essential for this task, as disturbances due to silanols in the stationary phase[20,23,24] are not desirable for our present purposes.

We report here an investigation of the effect of chain length in RPLC, leading to the suggestion of the participation of solvent molecules in the stationary phase in addition to the steric effect caused by the structural differences between solutes in the determination of retention in RPLC.

EXPERIMENTAL

Equipment

An HPLC system was constructed with a B-100-S single plunger pump (Eldex Lab., Menlo Park, CA, U.S.A.), with a pressure gauge and a pulse damper, 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.). Columns (10 cm × 4.6 mm I.D.) were maintained at 30 ± 0.1°C by submerging them in a thermostated bath.

Materials

Spherical silica gel (Develosil; Nomura Chem., Seto, Japan) of average particle diameter 5 μm (surface area *ca.* 330 m²/g) was used. Alkyldimethylchlorosilanes were obtained from Petrarch System (Levittown, PA, U.S.A.). The chemical bonding reaction and column packing were carried out as previously described[23].

Sample substances were either purchased or prepared by standard methods. The following hydrocarbons were used as solutes: 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), tetraphenyl-ethylene (20), fluorene (21) and bidiphenyleneethylene (22). Samples were made up in the mobile phase in most cases.

Mobile phases were made up by volume from LC-grade solvents (Nakarai Chem., Kyoto, Japan) and distilled water. Absolute acetonitrile was obtained by successive distillation, five times over phosphorus pentoxide and once over anhydrous potassium carbonate prior to final fractionation. Absolute methanol was obtained by distillation following reflux over magnesium for several hours.

Chromatographic measurement

Chromatographic runs were duplicated and the results were averaged. The reproducibility between the runs was better than ±0.5%. The void volume (t_0) was obtained by averaging the retention times of glycerine, water, methanol, and aceto-nitrile, all of which agreed within 1% of each other. The time lag between the UV detector and the refractive index detector was corrected. The amount of sample did not affect the retention times at the concentrations used.

RESULTS AND DISCUSSION

Elemental analysis of alkylsilylated silica gel showed coverages of 4.2, 3.4 and 3.3 μmol/m² for C_1, C_8 and C_{18} stationary phases, respectively. These values agree well with those in the literature when considered as a bonded phase of maximum coverage for each chain length[19,20,23]. We treated the bonded phase with smaller silylating reagents, trimethylchlorosilane and hexamethyldisilazane, as the retention and selectivity were known to be influenced by residual silanols[23].

Figs. 1 and 2 show the plots of log k' values obtained on the C_{18} phase against log k' values obtained on the C_1 and C_8 phases in 80% methanol and in 80% aceton-itrile. Solid lines were drawn based on *n*-alkanes (5–8), and the broken lines indicate the location of the PAHs. PAHs consistently gave higher log k' values in Fig. 1 than other hydrocarbons on the C_{18} phase compared to the C_1 or C_8 phase. The large differences shown in Fig. 1 must be explained by factors originating from the stationary phase, as the mobile phase was common in all instances. Here a com-parison of the chromatographic behaviour of two pairs of compounds, 17 and 21,
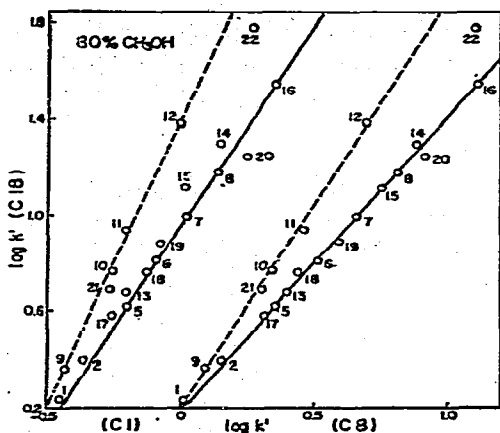
Fig. 1. Plots of log k' values on C_{18} phase against log k' values on C_1 and C_8 phases in 80% methanol. The numbers in Figs. 1–11 indicate the compounds listed in the Experimental section
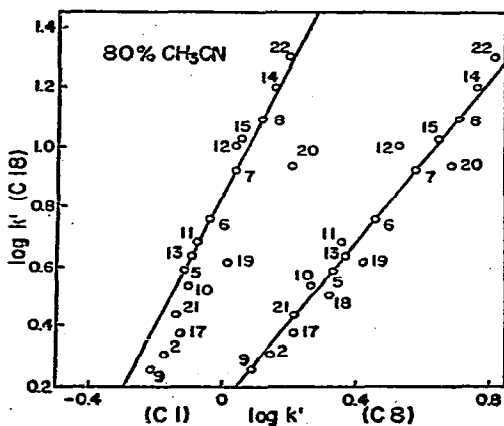

Fig. 2. Plots of log k' values on C_{18} phase against log k' values on C_1 and C_8 phases in 80% acetonitrile.

and 20 and 22, is instructive. Compounds 21 and 22 have the same numbers of carbon atoms and phenyl rings as 17 and 20, respectively. The only difference is that the phenyl rings on one carbon atom were bonded directly in 21 and 22, which increases the rigidity and planarity of the molecules, as shown in Scheme 1. In compounds 17 and 20 the phenyl rings are not in one plane due to steric repulsion between the phenyl rings. The planar rigid compounds (21 and 22) showed much larger reten-
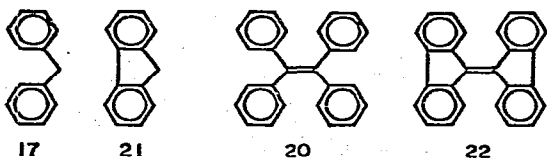
Scheme 1.

tions on the C_{18} phase than the non-rigid aromatic compounds (17 and 20); however, the difference was much smaller on the C_8 and almost none on the C_1 phase. Planarity or rigidity, which have been discussed in terms of compactness[26,27], thus are able to visualize the difference in length of carbon chain in stationary phase with the use of common mobile phase.

Although a similar tendency was observed in 80% acetonitrile as shown in Fig. 2 in the relation between molecular structure of solutes, chain length of stationary phase, and retention, aromatic compounds as a whole showed smaller retention relative to saturated compounds in this mobile phase compared to 80% methanol. When the plots of log k' on C_{18} against C_1 phase were compared, higher log k' values were seen for PAHs in 80% methanol, whereas non-rigid aromatic compounds showed lower log k' values in 80% acetonitrile.

These results indicate that the effect of chain length on retention and selectivity among these hydrocarbons includes the participation of solvent molecules in the stationary phase in addition to the steric effect caused by the differing chain lengths of the stationary phases. This will be discussed below.

One of the common features of Figs. 1 and 2 is the similarity of the behaviour of the C_1 and C_8 phases. When log k' values on the C_8 phase were plotted against those on the C_1 phase, PAHs and alicyclic compounds showed minor deviations from the straight line drawn for n-alkanes, as shown in Fig. 3. Hence the nature or the state of the C_8 phase is considered to be relatively close to that of the C_1 phase.

Past reports on the effect of chain length showed continuous changes in retention and separation factor from the C_1 to the C_8 phase, and the apparent dissimilarity between the two phases was attributed to the polat nature of the C_1 phase[7,19]. In contrast, the present results show a similarity between these two phases. Individual
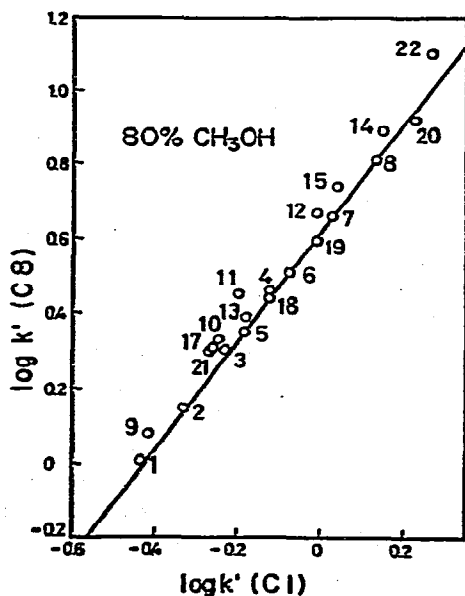

Fig. 3. Plot of log k' values on C_8 phase against log k' values on C_1 phase in 80% methanol.

k' values and separation factors might show the difference between the C_1 and C_{18} phases, whereas the plots in Figs. 1–3 indicate a similarity between the two phases. This result is remarkable if one considers the structural difference between the two phases.

Figs 4 and 5 show the plots between log k' values in methanol and in acetonitrile mobile phases on the three stationary phases. On the C_1 phase all compounds were very close to the solid line drawn for n-alkanes. The plot for the C_8 phase showed slightly higher log k' values for aromatic compounds than n-alkanes. As shown in Fig. 5, all aromatic compounds showed much larger log k' values on the C_{18} phase, regardless of rigidity or planarity in structure which caused the large differences in Figs. 1 and 2. It is notable that the three stationary phases gave completely different selectivities between aromatic compounds and saturated hydrocarbons in the two mobile phases. This precludes the possibility that the deviation for aromatic compounds was caused by the difference between aromatic compounds and saturated compouns with regard to the extent of interaction with solvent molecules in the mobile phase. The deviation could not be due to steric effects either, since rigidity or planarity did not influence the behaviour. Thus the large deviation of aromatic compounds on the C_{18} phase in Fig. 5 is most likely due to the interaction with methanol in the stationary phase which is much stronger for the C_{18} phase than for the C_1 or C_8 phases.

Complexation of proton donors with phenyl rings has been reported from nuclear magnetic resonance and UV spectroscopy[27,28], and this seems to be responsible for the results shown here. Although such complexation was also suggested for acetonitrile[29], the results indicate that the effect is more dominant with methanol than with acetonitrile, as expected from the difference in acidity between these two solvents. Figs. 4 and 5, in addition to Figs. 1 and 2, seem to show clear evidence for the participation of solvent molecules in the stationary phase in determining the retention of solutes in RPLC.

Very similar free energies of solution have been reported for gaseous alkanes in methanol and acetonitrile[30]. This suggests that the extents of interaction, considered to be very small except non-specific Van der Waals interaction, between saturated hydrocarbons and the two types of organic solvents, are similar. Hence, it seems to be more reasonable to discuss the difference in solvent effect seen in Figs. 4 and 5 based on the complex formation of aromatic compounds mentioned above, than on the solvation of the hydrocarbonaceous part of the stationary phase with these solvents.

When the mobile phase was changed from 60% to 80% methanol with the C_1 phase, the retention of alicyclic compounds decreased just as much as that of n-alkanes, and all aromatic compounds showed a much larger decrease as shown in Fig. 6. With the C_8 phase, alicyclic compounds showed a slightly larger slope and a positive deviation from the line for n-alkanes with change in the mobile phase from 70% to 90% methanol, as shown in Fig. 7. The negative deviation of aromatic compounds in these plots can be attributed to the increased mobile phase solvation of these compounds which is expected to be more dominant in mobile phases with a higher methanol content. In Fig. 4, essentially no difference was observed in the behaviour of n-alkanes and alicyclic compounds with change in the type of organic solvent. Hence the deviation for the alicyclic compounds in Fig. 7 was probably caused by the change in the alkyl chain structure in the stationary phase with the increase of methanol content in the mobile phase, which did not occur with the C_1
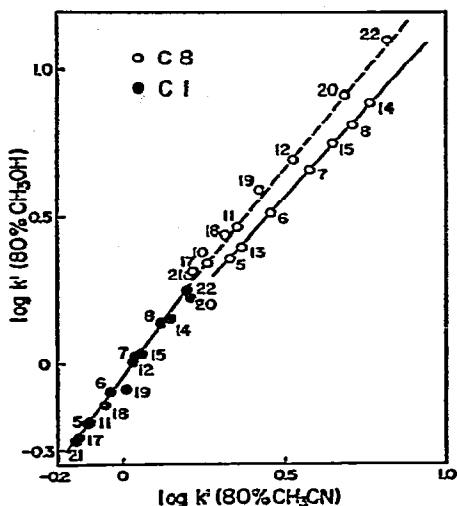
Fig. 4. Plots of log k' values in 80% methanol against log k' values in 80% acetonitrile on C_1 and C_8 phases.
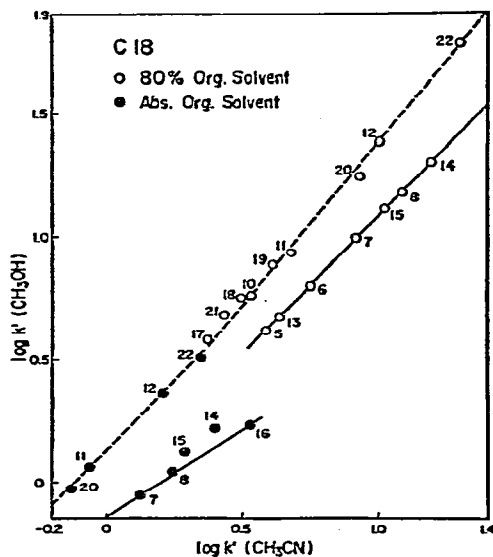
Fig. 5. Plots of log k' values in methanol mobile phase against log k' values in acetonitrile mobile phase on C_{18} phase
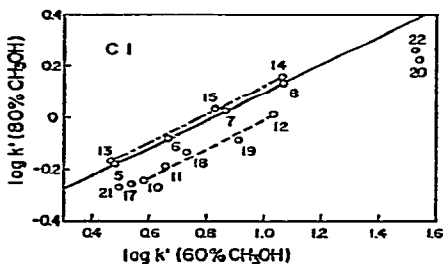

Fig. 6. Plot of log k' values on C_1 phase in 80% methanol against log k' values in 60% methanol.
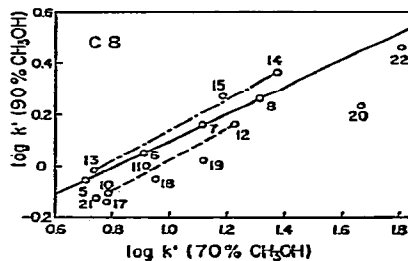
Fig. 7. Plot of log k' values on C_8 phase in 90% methanol against log k' values in 70% methanol.

phase. The behaviour of PAHs on the C_8 phase is explained by the combination of the two effects, one in the mobile phase and the other in the stationary phase. Supporting this explanation, non-rigid aromatic hydrocarbons behaved as in the C_1 phase. Very similar results were obtained with a change in the acetonitrile concentration on C_1 and C_8 phases, as shown in Figs. 8 and 9.

The effect of organic solvent content is considerably different on the C_{18} phase, as shown in Figs. 10 and 11. PAHs showed a large increase in relative retention compared with n-alkanes with increase in methanol content, while non-rigid aromatic compounds showed a much lower deviation. As shown in Fig. 11, the plot in acetonitrile mobile phase showed a similar tendency: larger retention of rigid planar aromatic compounds than of non-rigid ones with the increase of acetonitrile content. Note that this effect was much smaller on the C_8 phase and none on the C_1 phase.
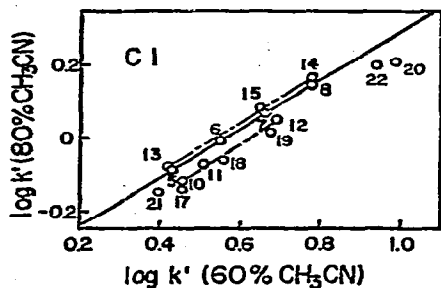
Fig. 8. Plot of log k' values on C_1 phase in 80% acetonitrile against log k' values in 60% acetonitrile.
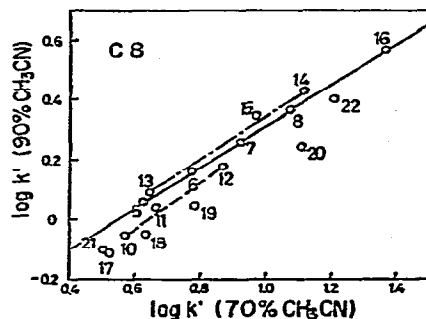
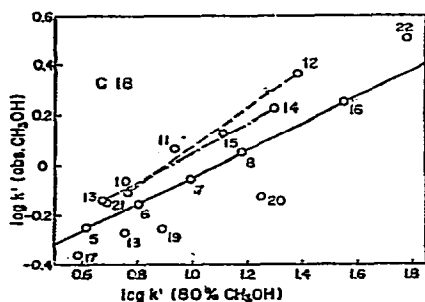Fig. 9. Plot of log k' values on C_8 phase in 90% acetonitrile against log k' values in 70% acetonitrile.

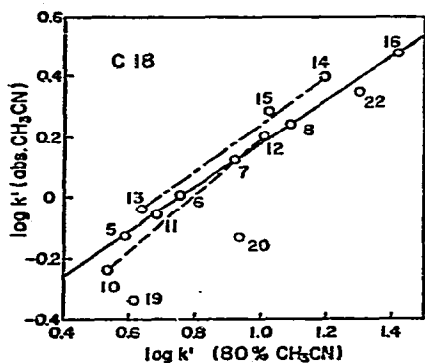Fig. 10. Plot of log k' values on C_{18} phase in absolute methanol against log k' values in 80% methanol.

Fig. 11. Plot of log k' values on C_{18} phase in absolute acetonitrile against log k' values in 80% acetonitrile.

One will notice the similarity between the plots in Figs. 1 and 10, and also in Figs. 2 and 11 with respect to the behaviour of the two types of aromatic compounds, taking into account the increase in organic solvent content which should produce a lower deviation for all aromatic compounds in Figs. 10 and 11, as shown for C_1 phase in Figs. 6 and 8. In other words, increase of the organic solvent content from 80 to ca. 100% on the C_{18} phase gave the results as if the alkyl chains in the stationary

phase had been lengthened, differentiating rigid planar aromatics from non-rigid ones, and at the same time increasing the effect of solvent change from methanol to acetonitrile on retention, as seen in Fig. 5. Solute–solvent interactions have been observed regardless of rigidity or planarity of aromatic compounds. Thus the different behaviour of PAHs and non-rigid aromatics shown in Figs. 10 and 11 was considered to arise from structural factors of alkyl chains in the C_{18} phase.

An aggregated structure of octadecyl chains was suggested for the C_{18} phase rather than a brush-type structure[8]. And it is reasonable to assume that the alkyl chains in the stationary phase are extended more in mobile phases of higher organic solvent content, which could produce results similar to the increase of chain length. It should be emphasized here that the effect of solute–solvent interactions on the C_{18} phase was found to be very large in 80% organic solvent and even larger in absolute organic solvents, regardless of structure of aromatic compounds. These results imply that the hydrocarbon molecules in stationary phase can realize chain restructuring due to the change of mobile phase composition, and simultaneously participate in complexation with solvent molecules in the C_{18} phase, leading to the mechanism based on partition of solutes between mobile phase and the effective stationary phase, relatively ordered long alkyl chains[26] containing solvent molecules. The stationary phase seems to be more ordered in methanol which is known to associate with each other by hydrogen bonding. However, the effective stationary phase should not be taken as simple mixed solvent since C_8 and C_{18} showed considerable differences in their response to the change in mobile phase. Insensitivity of the C_8 phase toward the change of mobile phase implies that the chains are almost free from overlap with each other in this range of mobile phase as would be the case in the C_1 phase. Then the description of "brush-type structure" may be appropriate for these two phases.

The effect of solute structure in RPLC has been discussed based on entropy effects[25,26]. Chmielowiec and Sawatzky[25] showed that compact PAHs gave a smaller retention than non-rigid aromatic compounds at higher temperatures. Temperature change is expected to affect both the structure of alkyl chains in the stationary phase and the steric requirement of solutes. Although in the present study it seems that ordered long alkyl chains in the stationary phase favour rigid planar PAHs and disfavour molecules having larger steric requirement, further investigation is required for better understanding.

As mentioned earlier, retention in RPLC has been explained in the past by solubility of solutes[2], solvophobic interactions[1], adsorption on to alkyl chains[3–6], or on to organic solvents on alkyl chains[9], and partition between the mobile phase and adsorbed mobile phase[7]. The present results suggest that the retention of aromatic compounds is influenced by solvent molecules in the stationary phase and that the molecular structure of the solutes also influences the appearance of the effect of chain length. There is no doubt that the mobile phase effect, including a hydrophobic interaction, is predominant in determining the retention in RPLC[1,31], but a kind of partition between the mobile phase and the effective stationary phase must be taken into account to explain the chromatographic behaviour of various hydrocarbons observed in this study. This mechanism does not necessarily require the displacement of solvent molecules from the stationary phase.

numbers of compounds, the exact nature of the solute–solvent interaction in either phase and a mechanistic interpretation of the effect of chain length in relation to the solute structure must await further studies. We are currently examing the chromatographic behaviour of other types of compounds on a variety of stationary phases.

CONCLUSION

The two major factors responsible for the difference in selectivity on stationary phases of various chain length are the molecular structure of the solutes and solute–solvent interactions in the stationary phase. Rigid planar aromatic compounds were preferentially retained by C_{18} phase in methanol mobile phase. The change of organic solvent in mobile phase from methanol to acetonitrile caused a major change in selectivity between aromatic compounds and saturated hydrocarbons on C_{18} phase regardless of rigidity or planarity of solutes, whereas only a minor change occurred on the C_1 and C_8 phases. The C_1 and C_8 phases showed relatively similar retention characteristics for the hydrocarbons in the mobile phases employed in this study.

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