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

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Effect of alkyl chain length of stationary phase on retention and selectivity of various hydrocarbons was found to be strongly dependent on the type of organic solvents in mobile phase in reversed phase liquid chromatography. The results suggest the participation of methanol in the retention of polynuclear aromatic hydrocarbons in stationary phase having long alkyl chains.

In reversed phase liquid chromatography the difference in selectivity on alkylsilylated silica gel of various chain length has been explained either by steric effect1) or by the difference in surface structure of stationary phase.2) The authors wish to report here an evidence that the effect of chain length on retention and selectivity is greatly influenced by the type of organic solvents in mobile phase. In aqueous methanol, polynuclear aromatic hydrocarbons(PAH) showed much greater retention relative to n-alkanes on octadecyldimethylsilylated stationary phase(C18) than on stationary phases having octyldimethylsilyl(C8) or trimethylsilyl (C1)moiety, while the selectivity among the hydrocarbons varied very little in aqueous acetonitrile on the same stationary phases. Actually the change of mobile phase from 80% methanol to 80% acetonitrile caused large change of selectivity among the hydrocarbons on the C18 phase while essentially no change was observed on the C1 phase. The finding has an important implication in retention mechanism in this mode of chromatography using alkylsilylated silica gel that in some cases solvent molecules participate in controlling retention of solutes at the inside of the stationary phase rather than in bulk mobile phase or at the interface between mobile phase and stationary phase as usually suggested.3,4)

Stationary phases were prepared by silylation of silica gel(average particle diameter: 5μm, surface area: ca. 330m^2/g) with alkyldimethylchlorosilanes.5)

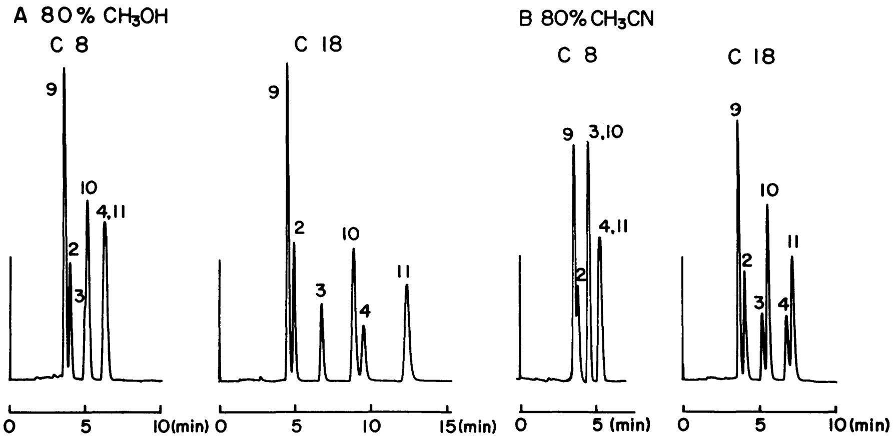

Fig. 1. Chromatograms of alkylbenzenes(2-4) and PAH(9-11) in 80% methanol(A) and 80% acetonitrile(B). Identity of peaks is stated in the text.

Fifteen 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), t-decahydronaphthalene(14), and adamantane(15). Aqueous methanol and aqueous acetonitrile were used as eluents. Chromatographic measurements were carried out at 30°C.

Fig. 1A shows the chromatograms of some alkylbenzenes and PAH obtained on the C8 and C18 phases. Note the large change in selectivity between the two stationary phases in 80% methanol. Fig. 1B shows the chromatograms of the same solutes on the two stationary phases in 80% acetonitrile. In this case selectivity varied very little between the C8 and the C18 phase.

When log k' values obtained on the C18 phase are plotted against those on the C1 and the C8 phase, the dependence of the effect of chain length on the type of

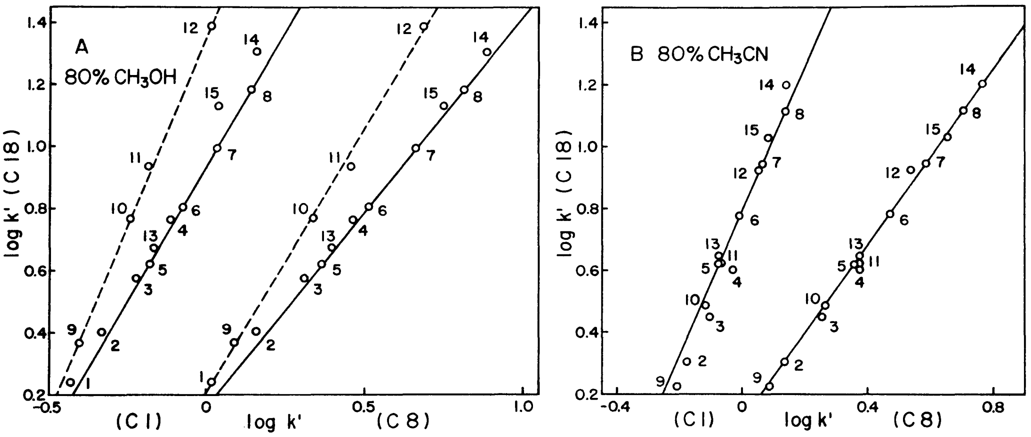

Fig. 2. Plot of log k' on the C18 phase against log k' on the C1 and the C8 phase in 80% methanol(A) and 80% acetonitrile(B).

solvents can be seen very clearly. In 80% methanol PAH showed large upper deviation compared with n-alkanes(shown by the solid line) and alicyclic compounds. Since all k' values in Fig. 2A were obtained in the same mobile phase, 80% methanol, the cause of different selectivity must be sought in the stationary phases. PAH behaved very similarly to n-alkanes in 80% acetonitrile, as shown in Fig. 2B. These facts indicate that the different selectivity on the stationary phases of different chain length in 80% methanol is likely due to the participation of solvent molecules in the retention of certain type of compounds. One of the features of Fig. 2 is the close similarity in tendency between the C1 and the C8 phase. As a matter of fact the plot of log k' on the C8 phase against those on the C1 phase either in 80% methanol or in 80% acetonitrile was close to straight line, indicating similar nature of the two stationary phases in contrast to the suggestion by others.[2]

The log k' values in 80% methanol were plotted against those in 80% acetonitrile in Fig. 3. On the C1 phase all hydrocarbons are found to be close to the straight line drawn for n-alkanes, showing similar selectivity in the two mobile phases on this stationary phase. PAH showed small upper deviation on the C8 phase. On the C18 phase, PAH were retained much more favorably in 80% methanol than in 80% acetonitrile compared with saturated hydrocarbons. Steric factors cannot explain the deviation of PAH, since relatively bulky alicyclic compounds behaved just like n-alkanes. Alkylbenzenes were located between the solid line for n-alkanes and the dotted line for PAH in Fig. 3. Similar observation was made at other mobile phase composition.

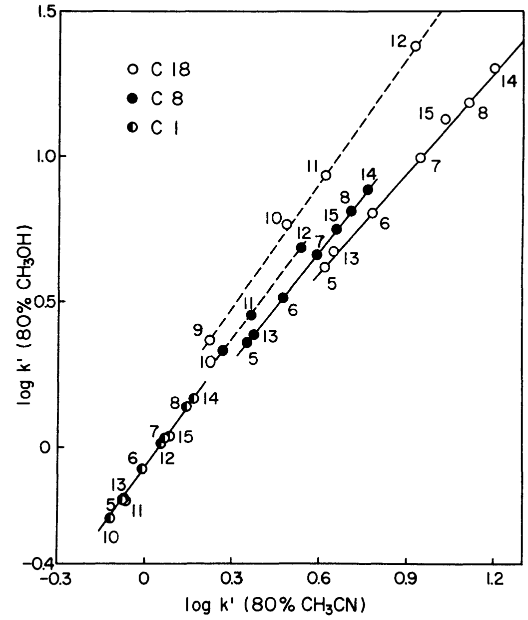

Fig.3. Plot of log k' in 80% methanol against log k' in 80% acetonitrile.

The results seem to indicate that solvent molecules in bulk mobile phase or at the interface between mobile phase and stationary phase cannot cause the difference in solvent effect among the three stationary phases, since contribution of solvents in such a way would have common features for all stationary phases. According to Unger and co-workers,[2] total surface areas of single alkyldimethylsilyl chain on C1, C8, and C18 phases are about 1.3nm^2, 2.9nm^2, and 5.1nm^2, respectively. The absence of solvent effect on the C1 phase and large solvent effect on the C18 phase imply the existence of solvent molecules specific to the C18 phase which affect the retention of PAH. It requires further study to explain the exact nature of solvent molecules associated with C18 phase and the mode of solute-solvent interaction in C18 phase. There is no doubt that in most cases mobile phase effect such as solvophobic interaction3) is predominant in determining the retention in reversed phase liquid chromatography. It is shown here, however, that specific solute-solvent interaction in stationary phase can significantly influence retention of some compounds, resulting different selectivity on certain stationary phase. Since the existence of such interaction is rather surprising for PAH which have been thought to have small specific interaction with stationary phases under normal chromatographic conditions,1,5) study of chromatographic behavior of other type of compounds including polar ones at various mobile phase composition will help understanding the different features of stationary phases of various chain length. From the practical point of view, this approach is expected to explain the fact that change of stationary phase or change of mobile phase does not improve separation in some cases and does it nicely in other cases.6)

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