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RETENTION BEHAVIOUR OF BENZENE DERIVATIVES ON BONDED REVERSED-PHASE COLUMNS

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SUMMARY

The retention behaviour of fourteen benzene derivatives has been studied in reversed-phase chromatography, eluting them with various aqueous organic solvent systems from the hydrocarbon chemically bonded reversed phase using a microchromatograph. The effects of eluent composition and of packings are discussed using the capacity factor, k. A linear relationship was observed between log k and the logarithm of the molarity of the organic moiety, log $[C]$, in the mobile phase. A p value for each solute was obtained from the slope of the linear relationship of log k vs. log $[C]$. This value was recognized as the number of the released solvent molecules after the formation of the bristle–solvent–solute complex when the solvated solute molecules were adsorbed on the solvated bristles of the bonded materials. Consideration of p offered a key to the explanation of the role of the eluent on chemically bonded reversed-phase chromatography.

INTRODUCTION

Reversed-phase chromatography using chemically bonded phases is one of the most frequently selected separation modes in high-performance liquid chromatography (HPLC). The usefulness of the technique arises from the development of suitable packings and the wide variety of eluting solvents.

Many interesting investigations have been directed towards the understanding of the retention mechanism on a bonded phase column[1–7]. However, much effort is still required to evaluate the effect on the selectivity of the separation conditions, such as the mobile phase composition and the characteristic of packings.

The purpose of this work is to elucidate the relationship between the separation condition and retention behaviour and to explain the retention mechanism of benzene derivatives on n-alkyl bonded phases.

EXPERIMENTAL

A Familic-100 microchromatographic system (Jasco, Tokyo, Japan) was used throughout this work. The system consists of a PTFE column (8 cm × 0.5 mm), a

syringe type micropump (volume capacity 250 μl) or a Model FLC 350 syringe type pump (volume capacity 200 ml) and a micro-UV detector.

The column packings used were LiChrosorb RP-18, RP-8 (E. Merk, Darmstadt, G.F.R.), Nucleosil C_{18} (Macherey, Nagel & Co., Düren, G.F.R.) and Permaphase ODS (DuPont, Wilmington, Del., U.S.A.). Benzene and its derivatives and mobile phase solvents, alcohols, dioxane and acetonitrile were obtained from Nakarai, Kyoto, Japan. Chromatographic separations of fourteen benzene derivatives were carried out using four different columns, eluting with 0.6–1.4 M (30–90%) water-miscible organic eluents. A 0.1-μl volume of the methanol solution containing ca. 50 pg of each solute was chromatographed at a constant flow-rate of 4 μl/min. The column volume used was so small that the analysis was carried out with 30 atm. Therefore, this chromatographic technique avoided the systematic errors otherwise due to the compressibility of liquids at inlet pressures above 200 atm. The retention volume of each solute was determined by averaging three experimental points.

RESULTS AND DISCUSSION

The effect of the molecular size of the solute on retention is shown in Fig. 1. Each solute was eluted with ethanol–water (60:40) from various columns, such as Nucleosil C_{18}, LiChrosorb RP-18, RP-8 and Permaphase ODS. The retention behaviour of solutes examined here is described by using the capacity factor, k. A linear relationship was obtained between log k and the carbon number C_n of the solute molecule in homologous groups, such as the substituted benzene derivatives and the polyaromatic hydrocarbon group. Comparing the experimental results on the three packings consisting of C_{18} bristles, the log k values obtained on the Permaphase ODS

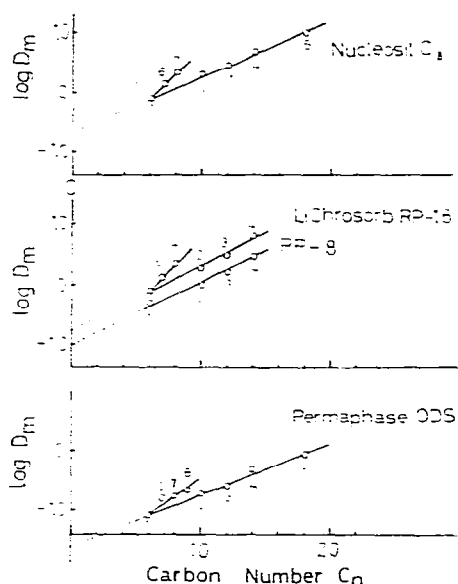

Fig. 1. Relationship between log k and carbon number in solute. Eluent: ethanol–water (60:40) Solutes: 1 = benzene; 2 = naphthalene; 3 = diphenyl; 4 = anthracene; 5 = p-terphenyl; 6 = toluene; 7 = xylene; 8 = cumene.

column were much smaller than those on the two other columns, Nucleosil C_{18} and LiChrosorb RP-18. However, the slopes of log k vs. C_n obtained on these packings were nearly equal, 0.16–0.21 for the substituted benzene derivatives, 0.09–0.11 for the polyaromatic hydrocarbon compounds. This result shows that the hydrophobic selectivity of the solute to the bristles is roughly equal for these packings. It may be assumed that the log k values depend on the capacity of packings, provided that the adsorption of the solute to unreacted silanol groups of the silica surface is negligibly small. From the intercept in Fig. 1, the relative capacity of packing was calculated, viz., Nucleosil C_{18}:LiChrosorb RP-18:LiChrosorb RP-8:Permaphase ODS = 1.45: 1.0:0.62:0.19. This ratio is comparable to the value of the carbon content as recorded in the literature[8].

The slope of log k vs. C_n was effected by the type and the concentration of the organic modifier in the mobile phase. The slope obtained with ethanol–water (60:40; 10.3 M ethanol) eluent was found to be similar to that with methanol–water (80:20; 19.8 M methanol) or isopropanol–water (50:50; 6.5 M isopropanol). This suggests that the solvent strength of these alcohols decreased in the order of isopropanol, ethanol and methanol.

In order to investigate the effect of the organic modifiers on the selectivity, five different water-miscible organic eluents, namely methanol–water, ethanol–water, isopropanol–water, dioxane–water and acetonitrile–water, were used. Fig. 2 shows the plots of log k values on Nucleosil C_{18} column against the logarithm of the molarity of three alcohol components in the mobile phase. The retention volume of each solute decreased with increasing amount of organic component. A linear relationship was observed between log k and the logarithm of the molarity of the organic solvent, log [C]. Figs. 3 and 4 show the results on LiChrosorb RP-18 and Permaphase ODS, columns, respectively. A linear relation was also observed for each solute eluted with acetonitrile–water or dioxane–water.
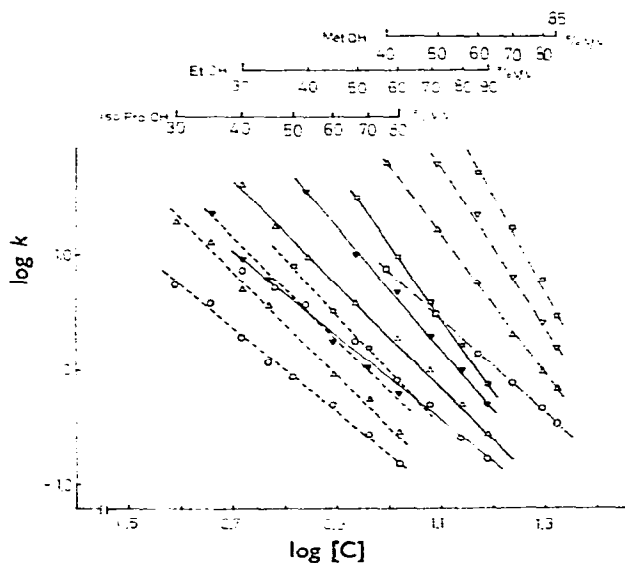

Fig. 2. Plots of log k vs. log [C] on Nucleosil C_{18} column. ○, Benzene; △, ethylbenzene; ▼, anthracene; □, p-terphenyl. Eluent: — · — · —, methanol–water; ————, ethanol–water; — — —, isopropanol–water.
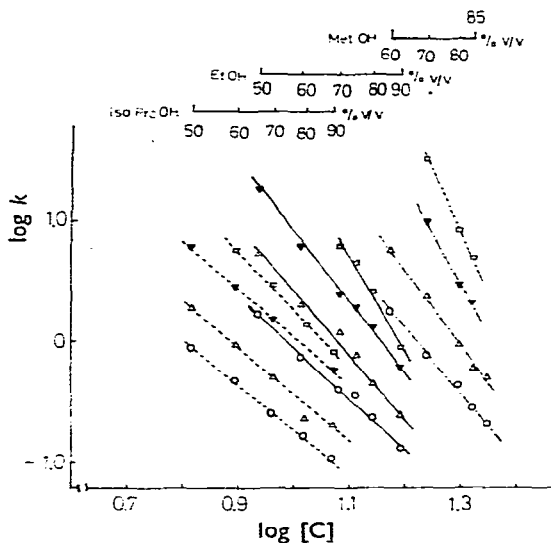
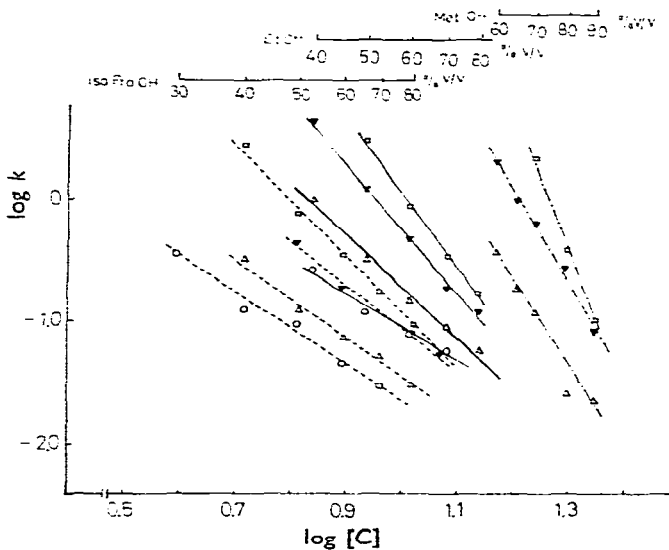Fig. 3. Plots of log k vs. log [C] on LiChrosorb RP-18 column. Solutes and eluents as in Fig. 2.

Fig. 4. Plots of log k vs. log [C] on Permaphase ODS column. Solutes and eluents as in Fig. 2.

It has been already reported that the bristles of chemically bonded packings are solvated with a monolayer of the organic solvent molecules in the aqueous organic solvent, and solvated solute molecules are distributed into the stationary liquid layer on the bristles and the mobile phase. Solvophobic interaction takes place between a solute molecule and a hydrocarbon chain of the bristle[9].

In this work, the following assumption was made in order to explain a linear relationship between log k and log [C]. Some organic solvating molecules may be released after the formation of a bristle–solvent–solute complex when the solvated

solute molecules are adsorbed on the solvated bristle. Thus the following equilibrium equations:

$$C_mB + C_nS \overset{K}{\rightleftharpoons} C_{m+n-p}BS + pC$$

and

$$K = \frac{[C_{m+n-p} \, BS] \, [C]^p}{[C_m \, B] \, [C_n \, S]} \tag{1}$$

The solvated bristle in the packings is represented as C_mB, and the solute in the mobile phase is also solvated to form C_nS. The symbol m indicates the number of organic solvent molecules associated with the bristle B, n those associated with solute S. The numbers m and n are assumed to be constant for a given solvent and a given solute in the relatively high concentration of organic solvent used here. p represents the number of the released solvent molecules. If capacity factor, k, is defined as

$$k = \frac{[C_{m+n-p} \, BS] \, V_s}{[C_n \, S] \, V_m}$$

then

$$k = \frac{K \, [C_m \, B] \, V_s}{[C]^p \, V_m}$$

$$\log k = -p \log [C] + \log K \, [C_mB] + \log (V_s/V_m) \tag{2}$$

where V_s/V_m is the ratio of the volume of the stationary phase, V_s, to that of the mobile phase, V_m, in a column. In a given system, the second and third terms in eqn. 2 were constant, so that eqn. 2 shows the linear relationship between $\log k$ and $\log [C]$, and satisfies the results shown in Figs. 2–4. The p value for each solute under various eluting conditions was obtained from the slope of $\log k$ vs. $\log [C]$.

Since the value of p reflects directly the retention mechanism, the relationships between p values and elution conditions were examined. Fig. 5 shows the plot of the p value vs. carbon number in the solute. Each solute was eluted with three different aqueous organic eluents from the LiChrosorb RP-18 column. The p value increased linearly with the molecular sizes of the solute in each homologous series. The p values for substituted benzene derivatives such as toluene, xylene, ethylbenzene and cumene were larger than those for polyaromatic hydrocarbons having a corresponding carbon number. The alkylbenzene derivatives seem to be solvated more than the polyaromatic hydrocarbons. It is suggested that the number of released solvent molecules p depends not only on the molecular weight but also on the molecular shape and the substituted group. The increment of the p values for increasing number, Δp, was obtained from p vs. C_n plots. The Δp value is written as

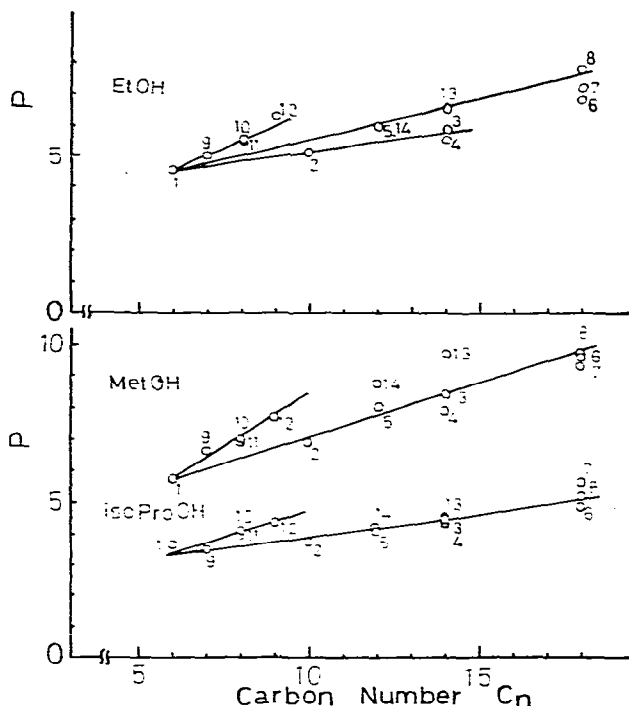$$\Delta p = \Delta \log (k_{C_{n-1}}/k_{C_n})/\Delta \log [C]$$

Fig. 5. Plots of p value *vs.* carbon number in solute. Solutes: 1 = benzene; 2 = naphthalene; 3 = anthracene; 4 = phenanthrene; 5 = diphenyl; 6 = *o*-terphenyl; 7 = *m*-terphenyl; 8 = *p*-terphenyl; 9 = toluene; 10 = xylene; 11 = ethylbenzene; 12 = cumene; 13 = dibenzyl; 14 = 2,3-dimethyl-naphthalene.

where $k_{C_{n-1}}$ and k_{C_n} represent the capacity factor for the solutes whose carbon numbers are $n-1$ and n, respectively. If the separation factor is defined as $f = k_{C_{n-1}}/k_{C_n}$, then $\varDelta p$ can be related to the separation factor by

$$\varDelta p = \varDelta \log f / \varDelta \log [C] \tag{3}$$

On the basis of their molecular structure the solutes were classified into three groups, The $\varDelta p$ values for group A (benzene, toluene, xylene), group B (benzene, diphenyl, p-terphenyl) and group C (benzene, naphthalene, anthracene) for the three packings are shown in Table I. The $\varDelta p$ value increased for the groups in the order of A > B ⩾ C. This order shows that the separation factor in the analysis of the substituted benzene derivatives is more affected by the organic modifier composition than that of the polyaromatic hydrocarbon.

The $\varDelta p$ value for a given solute group was largest for the elution with aqueous methanol, followed by aqueous ethanol and finally by aqueous isopropanol. Comparing the $\varDelta p$ value obtained from the elution with methanol to that obtained from the elution with the other alcohol eluents, the ratio on Nucleosil C_{18} or LiChrosorb RP-18 was $\varDelta p$ (methanol):$\varDelta p$ (ethanol):$\varDelta p$ (isopropanol) = 1.0:0.65:0.45. A slightly .arger ratio was obtained from the result on the Permaphase ODS column. This order suggests that the smaller molecular volume of the organic solvent gave the larger

TABLE I

VARIATION OF Δp VALUES WITH CARBON ATOM INCREMENT OF SOLUTE

A = Benzene, toluene, xylene; B = benzene, diphenyl, p-terphenyl; C = benzene, naphthalene, anthracene.

Column	Solutes	Methanol	Ethanol	Isopropanol	Dioxane	Acetonitrile
Nucleosil C18	A	1.00	0.70	0.37	0.39	0.25
	B	0.34	0.23	0.10	0.21	0.18
	C	0.34	0.18	0.10	0.08	0.09
LiChrosorb RP-18	A	0.73	0.50	0.33	0.60	0.35
	B	0.34	0.27	0.15	0.35	0.16
	C	0.34	0.17	0.15	0.28	0.09
Permaphase ODS	A	2.1	1.28	1.05	1.56	0.80
	B	0.42	0.37	0.20	0.18	0.18
	C	0.42	0.32	0.20	0.18	0.18

variation of the separation factor. However, the Δp value for acetonitrile–water eluent was smaller and that for dioxane–water was larger than expected from the molecular volume of these solvents. It is believed that these results are probably due to the character of the water structure in aqueous solutions of these organic solvents.

CONCLUSION

The effect of the mobile phase composition on the capacity factor in chemically bonded reversed-phase chromatography revealed a linear relationship between log k and log [C] when using hydrocarbon bonded phases and water-miscible organic eluents. In the retention mechanism proposed, the solvated solute interacts with the solvated bristle to form a solute–solvent–bristle complex, some solvent molecules being excluded from the newly formed solvated layer on the solute and the bristle. The number of released solvent molecules p can be obtained from the slope of log k vs. log [C]. The p value characterizes the interaction of solute, packing and eluent.

ACKNOWLEDGEMENT

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