Retention Behavior of *p*-Alkylphenols in Reversed-Phase Liquid Chromatography Using Ethanol/Water Mixtures

Kanji MIYABE[†], Noboru NOMURA, Fumie MORISHITA, Sugako KURATA,
Hiroko ASAMURA and Yukiko IMADA

Chemistry Section, Faculty of Education, Toyama University, Gofuku, Toyama 930, Japan

The retention behavior of *p*-alkylphenols was studied in reversed-phase liquid chromatography using octadecylsilyl-silica gel and ethanol/water mobile phases. The results were compared with those in methanol/water systems. Linear correlations were observed between the logarithmic retention factor ($\ln k$) and the carbon number of side chains in the solutes, irrespective of the type and volumetric fraction (ϕ) of the organic modifiers in the mobile phases. The straight lines measured at different ϕ converged at almost one point. By analyzing the slope of the linear correlations, a functional contribution of a methylene group to k and a reduction of the hydrophobic surface area of the solutes and octadecylsilyl ligands due to the retention were estimated. It was also attempted to analyze the characteristics of the retention in reversed-phase liquid chromatography on the basis of solvophobic theory.

Keywords Reversed-phase liquid chromatography, retention factor, solvophobic theory, retention contribution, *p*-alkylphenol, alcohol/water eluent

The chromatographic behavior in the reversed-phase mode is affected by various physicochemical properties of the solutes as well as the mobile and stationary phases, which constitute separation systems. Extremely extensive studies have been carried out so far concerning the correlation between the retention behavior and the chromatographic conditions. A great deal of information about the separation mechanism in reversed-phase liquid chromatography (RP-LC) has been reported. The results of the studies have also been applied to establish optimization strategies for the chromatographic conditions.

The quantitative structure-retention relationship (QSRR) has been studied in order to specify the influence of the various conditions on the chromatographic behavior.¹ Separation profiles in RP-LC have been analyzed from the viewpoint of the hydrophobicity of the solutes.² The partition coefficient (P) of a solute between 1-octanol and water is commonly used as a descriptor for the hydrophobic character.³ The retention behavior in RP-LC has been widely studied in connection with P . It was reported that linear relationships could be obtained between the logarithm of the retention factor (k) and $\log P$ for homologous compounds, and that the slope of the linear correlation depends on the composition of an organic modifier (ϕ) in a mobile phase.^{3,4} Though a convergence of the linear correlations was phenomenally observed over a limited range of ϕ , there are some subjects concerning the theoretical interpretation for the retention behavior in RP-LC.

In neat water, solute molecules are retained on a stationary phase by only the hydrophobic effect. Because organic solvents provide no influence on the retention behavior under such conditions, k in 100% water (k_w) was also regarded to be a descriptor for the hydrophobic character of solutes.⁴ The value of k_w can be determined by means of a chromatographic method using pure water as a mobile phase. However, k_w has usually been estimated by extrapolating the correlation between $\log k$ and ϕ , because it is hard to measure k_w in practice under isocratic conditions using water. The correlation between $\log k$ and ϕ has been frequently formulated as a linear or quadratic function, because the profile of the correlation varies according to the type of organic modifiers and ϕ . The value of the estimated k_w is affected by the experimental conditions related to the mobile phase, and by the functionality of the correlation. Because there is no convenient procedure for estimating k_w accurately, k_w cannot be used as a general descriptor for the hydrophobicity.

A functional-group contribution (τ) has also been introduced to correlate the retention behavior with the structure and physicochemical properties of the solutes.⁵ It is assumed that the retention of a solute results from the contributions of functional groups in the solute molecule. The value of τ is experimentally determined from the k values of two solutes with and without the functional group. However, it is well known that τ of a certain functional group is not constant under various conditions of the solute, mobile and stationary phases. According to the change in the chromatographic conditions, different values of τ have been

[†] To whom correspondence should be addressed.

determined. Both the accumulation and quantitative analysis of much experimental data determined under various conditions of chromatography should be required.

This paper concerns the retention behavior of *p*-alkylphenols in a RP-LC system using an ODS column. Mixed solvents of ethanol and water were used as mobile phases, because there have been a few studies concerning the retention behavior in RP-LC using ethanol/water eluents. Comparable experiments were made by using methanol/water systems as a reference. Ethanol is not as popular as an organic modifier in the mobile phases compared with methanol and acetonitrile in RP-LC. However, it should be required to analyze the separation properties of RP-LC using different organic modifiers other than methanol and acetonitrile, such as ethanol, in order to prove the universal validity of the theory for the retention mechanism developed in RP-LC systems using methanol or acetonitrile mobile phases. For example, some studies concerning the retention behavior in RP-LC have been made by using various organic modifiers other than methanol and acetonitrile.⁵⁻⁷ In this paper, quantitative analyses of some characteristics of the retention behavior in RP-LC were also attempted by applying the solvophobic theory.⁸⁻¹⁰

Experimental

A chromatograph system was assembled with a Model TRIROTOR high-pressure pump, a Model UVIDEK-100 UV spectrophotometric detector (JASCO Co., Ltd.), and a Chromatopac C-R1A recorder (Shimadzu Sci. Ind. Co. Ltd.). The column temperature controller used was a Chemco LC column conditioner Model 360 (Chemco Sci. Co., Ltd.).

The reversed-phase column packing used was a Develosil ODS-3 (Nomura Chem. Co., Ltd.). The physical properties of the ODS are as follows: particle diameter, 3 μm ; surface area, 350 $\text{m}^2 \text{g}^{-1}$; mean pore diameter, 10 nm; pore volume, 1.00 $\text{cm}^3 \text{g}^{-1}$; carbon content, 21 wt%. End capping was carried out. The ODS was packed in a stainless-steel tube (15 $\text{cm} \times 4.6 \text{ mm}$ i.d.) by a slurry packing method using a Chemco Slurry Packing Apparatus (Model 124A; Chemco Sci. Co., Ltd.) under $6.06 \times 10^7 \text{ Pa}$ pressured by nitrogen gas.

Phenol, *p*-cresol, and *p*-ethylphenol were purchased from Wako Pure Chem. Ind., Ltd., and *p*-propylphenol was purchased from Tokyo Kasei Co., Ltd., respectively. These phenol derivatives were purified by reduced-pressure distillation under a nitrogen stream. Deionized water was distilled and further sub-boiling distilled with a quartz sub-boiling still. HPLC-grade methanol and ethanol (Wako Chem. Ind., Ltd.) were used. The concentration of the standard samples were 0.2 mM in 50 (v/v)% of aqueous methanol, and the injection volume was 5 μl each.

Results and Discussion

Figures 1 and 2 illustrate the linear correlations between the logarithm of k of phenol and *p*-alkylphenols in RP-HPLC using alcohol/water mixtures and the carbon number (N_c) of the side chains in the solutes. The slopes of the linear relationship increase along with an increase in the water content of the eluents; these lines converge approximately at one point. Similar results have been reported between $\log k$ and $\log P$.¹¹

Fig. 1 Convergence of correlative lines between $\ln k$ of *p*-alkylphenols and carbon number of alkyl chains. Conditions: column, Develosil ODS-3, 150 $\times$ 4.6 mm i.d.; temperature, 313 K; mobile phase, ethanol/water (50/50–60/40, v/v); flow rate, 0.5 $\text{cm}^3 \text{min}^{-1}$.

Fig. 2 Convergence of correlative lines between $\ln k$ of *p*-alkylphenols and carbon number of alkyl chains. Conditions: column, Develosil ODS-3, 150 $\times$ 4.6 mm i.d.; temperature, 313 K; mobile phase, methanol/water (60/40–70/30, v/v); flow rate, 0.5 $\text{cm}^3 \text{min}^{-1}$.

An interpretation has been provided concerning the dependence of k on the size of the solutes and the mobile-phase composition on the basis of the lattice statistical thermodynamic theory.^{11,12} In this work, another quantitative interpretation of the results in Figs.1 and 2 was attempted by applying solvophobic theory.

Functional contribution of the methylene group to k

The contribution of a functional group to the retention (τ) is calculated as follows:⁵

$$\tau = \log k_2 - \log k_1 = \log(k_2/k_1). \quad (1)$$

The values of k_2 and k_1 are the retention factors of two solutes having a functional group or not, respectively. A number of data for the retention contributions have been reported for various functional groups and for the effect of the position of the groups.⁵ However, there is little data concerning τ in ethanol/water mobile-phase systems.

The slope of the linear lines in Figs.1 and 2 represents the group contribution of the methylene unit to k . Table 1 shows the τ of methylene in p -alkylphenols determined in the RP-LC system using Develosil ODS-3. In methanol/water mobile-phase systems ($\phi=60-70$ vol%), τ was about 0.22–0.28. These values are in agreement with previous data.⁵ When ϕ was 50–60 vol% in ethanol/water mixtures, τ ranged from 0.20 to 0.25, which were relatively smaller than those in methanol/water eluents. The values of τ in 60 vol% ethanol are intermediate between those in 60 vol% methanol and in 60 vol% acetonitrile.⁵

Reduction of hydrophobic surface area

In solvophobic theory^{2,8-10}, it is assumed that the contact area between polar solvents and the hydrophobic surfaces of both solute molecules and the ODS ligands decreases when the solute molecules are retained on the ODS ligands, and that the reduction of the hydrophobic surface area (ΔA) is proportional to a surface area of the solute (A_s). The free-energy change due to the retention of the solutes in a liquid-phase system (ΔG_{liq}) is represented as follows:⁸⁻¹⁰

$$\begin{aligned} \Delta G_{\text{liq}} = & \Delta G_{\text{gas}} - \Delta G_{\text{vdw},s} + N_A(\lambda - 1)\mu_s^2\Psi P / (2\lambda v_s) \\ & - N_A\Delta A - N_A A_s(\kappa^e - 1)V^{2/3}\gamma / V_s^{2/3} \\ & - R_g T \ln(R_g T / P_0 V), \end{aligned} \quad (2)$$

where ΔG_{gas} and $\Delta G_{\text{vdw},s}$ are the free-energy changes due to the retention of the solute in a gaseous system and the van der Waals interaction between the solute and surrounding polar solvents, respectively; γ and V are the surface tension and the molar volume of the solvent; κ^e is an energy correction parameter for a curved surface; R_g and N_A are the gas constant and the Avogadro number; T is the temperature; P_0 is the atmospheric pressure; μ_s , v_s , and V_s are dipole moment, molecular volume and molar volume of the solute, respectively; and λ is the ratio of the molecular volume of a complex consisting of the solute and the ODS ligand to v_s . The values of Ψ and P are calculated as follows:

$$\Psi = 2(\epsilon' - 1) / (2\epsilon' + 1), \quad (3)$$

$$P = [4\pi\epsilon_0(1 - \Psi\beta_s/v_s)]^{-1}, \quad (4)$$

where ϵ' is the dielectric constant of the solvent, ϵ_0 is the free-space permittivity constant, and β_s is the polarizability of the solute. The contribution of the electrostatic term (the third term on the right-hand side (RHS) of Eq.(2)) to ΔG_{liq} was assumed to be negligibly small in the case of a hydrophobic interaction. The value of k is related to ΔG_{liq} by considering the phase ratio of both the stationary and mobile phases (F_R),

$$\Delta G_{\text{liq}} = -R_g T (\ln k - \ln F_R). \quad (5)$$

From Eqs.(2) and (5), k can be expressed by assuming that the shape of the solute is spherical.

$$\begin{aligned} \ln k = & \ln F_R - \Delta G_{\text{gas}} / R_g T + \Delta G_{\text{vdw},s} / R_g T \\ & + N_A \Delta A \gamma / R_g T + 4.836 N_A^{1/3} (\kappa^e - 1) V^{2/3} \gamma / R_g T \\ & + \ln(R_g T / P_0 V) \end{aligned} \quad (6)$$

According to Eq.(6), Horvath *et al.* estimated the ratio of $\Delta A/A_s$ (α) for three kinds of homologies by assuming the changes in the second and third terms on the RHS of Eq.(6) to be the same.

Similarly, α for p -alkylphenols was estimated from the slope of the linear relationships in Figs.1 and 2. The values of α at $\phi=60$ were calculated to be about 0.4 and 0.34 in methanol/water and ethanol/water mixtures, respectively. Though the mobile-phase conditions were not entirely identical, similar values of α were reported, *i.e.*, 20–30% and 35%.^{8,9} It was indicated that the reduction of the hydrophobic surface area due to retention was smaller in ethanol/water than in methanol/water when ϕ was identical in both mobile-phase systems.

Table 1 Functional contribution of methylene group to retention^a

N_C	Ethanol, vol%			Methanol, vol%		
	50	55	60	60	65	70
$C_1 - C_0$	0.23	—	0.20	0.25	0.24	0.22
$C_2 - C_1$	0.24	—	0.20	0.26	0.25	0.22
$C_3 - C_2$	0.25	—	0.21	0.28	0.25	0.23
$(C_3 - C_0)/3$	0.24	0.22	0.20	0.26	0.25	0.22

a. Conditions: RP packing materials, Develosil ODS-3; temperature, 313 K.

N_C =carbon number in alkyl chains of p -alkylphenols.

Convergence of the correlation between k and carbon number

Equation (6) is simplified to

$$\ln k = a'A_s + b', \quad (7)$$

where coefficients a' and b' are given by

$$a' = N_A \alpha \gamma / R_g T, \quad (8)$$

$$b' = \ln F_R - \Delta G_{\text{gas}} / R_g T + \Delta G_{\text{vdw,s}} / R_g T + 4.836 N_A^{1/3} (\kappa^e - 1) V^{2/3} \gamma / R_g T + \ln(R_g T / P_0 V). \quad (9)$$

As illustrated in Figs. 1 and 2, the linear relationships between $\ln k$ and N_c in p -alkylphenols converge approximately at one point. The first term on the RHS of Eq.(9) is almost constant over a narrow range of ϕ . The second term is independent of ϕ . Table 2 shows the calculated values of the other terms on the RHS of Eq.(9). Even when ϕ was changed, the contributions of the three terms changed only slightly. Though the absolute values of the terms may not be very accurate, it was approximately confirmed that the contributions changed little. In such a case, b' for a certain solute is almost constant, irrespective of ϕ . On the other hand, if it is assumed that the contributions of the second and third terms on the RHS of Eq.(9) are canceled by each other, as indicated by Horvath *et al.*⁸, b' is approximately constant for various solutes at a certain ϕ . The other terms on the RHS of Eq.(9) are independent of the properties of the solutes. An almost constant value of b' may be expected regardless of ϕ and N_c under such conditions. This is one explanation for the convergence observed in Figs. 1 and 2, and is probably a sufficient condition.

Comparison of k in 60 vol% methanol and 60 vol% ethanol

The values of k of p -alkylphenols in the RP-LC systems using the two alcohol/water mixtures (60/40, v/v) are compared in Fig. 3. The values of k in 60 vol% ethanol were smaller than those in 60 vol% methanol, indicating that the interaction between the ODS ligands and the solutes was weaker in 60 vol% ethanol than in 60 vol% methanol. The difference in k increased with increasing k . The results given in Fig. 3 indicate a well-known conclusion that the elution strength of ethanol is larger than that of methanol. An attempt was

Fig. 3 Comparison of retention factor in 60 vol% methanol and 60 vol% ethanol. Conditions: column, Develosil ODS-3, 150×4.6 mm i.d.; temperature, 313 K; flow rate, 0.5 cm³ min⁻¹.

made to quantitatively interpret the results in Fig. 3 by solvophobic theory.

According to Eq.(6), the difference in k in both mobile phases can be represented as follows:

$$\begin{aligned} \ln(k_M/k_E) = & (\Delta G_{\text{vdw,s,M}} - \Delta G_{\text{vdw,s,E}}) / R_g T \\ & + N_A A_s (\alpha_M \gamma_M - \alpha_E \gamma_E) / R_g T \\ & + N_A A_s [(\kappa_M^e - 1) V_M^{2/3} \gamma_M - (\kappa_E^e - 1) V_E^{2/3} \gamma_E] / V_s^{2/3} R_g T \\ & + [\ln(R_g T / P_0 V_M) - \ln(R_g T / P_0 V_E)]. \end{aligned} \quad (10)$$

Subscripts M and E represent 60 vol% methanol and 60 vol% ethanol, respectively. The values of F_R and ΔG_{gas} are assumed to be constant, irrespective of the mobile-phase conditions. Horvath *et al.* indicated that $\Delta G_{\text{vdw,s}}$ was almost equivalent in RP systems, though they compared $\Delta G_{\text{vdw,s}}$ of toluic acid in methanol/water and in acetonitrile/water mobile phases. For example, they reported $\Delta G_{\text{vdw,s}} / R_g T$ as being -14.9 and -14.3 in 60 vol% methanol and acetonitrile, respectively.⁸ Horvath *et al.* also indicated that the contribution of the fourth term in the RHS of Eq.(10) was negligible. They reported $\ln(R_g T / P_0 V)$ as 7.8 and 7.7 for 60 vol% methanol and acetonitrile, respectively.⁸ If the contributions of the first and fourth terms in Eq.(10) can be neglected, it can be rearranged as follows:

$$\begin{aligned} \ln(k_M/k_E) = & N_A A_s (\alpha_M \gamma_M - \alpha_E \gamma_E) / R_g T \\ & + 4.836 N_A^{1/3} [(\kappa_M^e - 1) V_M^{2/3} \gamma_M \\ & - (\kappa_E^e - 1) V_E^{2/3} \gamma_E] / R_g T. \end{aligned} \quad (11)$$

Figure 4 illustrates the correlation of $\ln(k_M/k_E)$ with A_s . The experimental data for p -alkylphenols could be correlated by each straight line. The linear correlation between $\ln(k_M/k_E)$ and A_s indicates that the difference in

Table 2 Comparison of solvophobic contributions

Mobile phase	VDW	CAV	CRAT
60 vol% MeOH	28.9	9.2	6.9
65 vol% MeOH	28.6	8.4	6.8
70 vol% MeOH	28.4	7.8	6.8

VDW = $\Delta G_{\text{vdw,s}} / R_g T$, CAV = $4.836 N_A^{1/3} (\kappa^e - 1) V^{2/3} \gamma / R_g T$, CRAT = $\ln(R_g T / P_0 V)$.

Fig. 4 Correlation between $\ln(k_M/k_E)$ and A_s .

k in both mobile-phase systems depends on the size of the solutes. Equation (11) provides an explanation for the influence of the type of organic modifiers on k , which is correlated with the size of the solutes. However, it should be required to accurately determine several parameters in solvophobic theory in order to

quantitatively analyze the retention behavior in RP-LC in more detail.

The authors are indebted to Dr. Toshihiko Hanai for his useful discussion. The authors are grateful to the late Koichi Mochizuki, JASCO Ind. Co., Ltd., for his effective support concerning technique, and wish to thank Mrs. Kimiko Fujii and Miss Sonoe Zenkoji for carrying out some of the experiments.

References

1. R. Kaliszan, *J. Chromatogr. A*, **656**, 417 (1993).
2. A. Tchaplal, S. Heron, E. Lesellier and H. Colin, *J. Chromatogr. A*, **656**, 81 (1993).
3. W. J. Lambert, *J. Chromatogr. A*, **656**, 469 (1993).
4. K. Valko, L. R. Snyder and J. L. Glajch, *J. Chromatogr. A*, **656**, 501 (1993).
5. R. M. Smith, *J. Chromatogr. A*, **656**, 381 (1993).
6. T. Hanai, *Chromatographia*, **12**, 77 (1979).
7. T. Hanai, *J. Chromatogr.*, **550**, 313 (1991).
8. C. Horvath, W. Melander and I. Molnar, *J. Chromatogr.*, **125**, 129 (1976).
9. G. Belfort, G. L. Altshuler, K. K. Thallam, C. P. Feerick, Jr. and K. L. Woodfield, *AIChE J.*, **30**, 197 (1984).
10. K. Miyabe and M. Suzuki, *AIChE J.*, **41**, 536 (1995).
11. J. G. Dorsey and K. A. Dill, *Chem. Rev.*, **89**, 331 (1989).
12. K. A. Dill, *J. Phys. Chem.*, **91**, 1980 (1987).

(Received August 7, 1997)

(Accepted October 7, 1997)