

# Relationship Between Retention Behavior of Substituted Benzene Derivatives and Properties of the Mobile Phase in RPLC

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## Abstract

The linear dependence of the logarithm of the capacity factor ( $\log k'$ ) on the composition of the aqueous binary mobile phase is examined systematically in reversed-phase liquid chromatography. In discussing the physical significance of the intercept ( $\log k_w$ ) and the slope ( $S$ ), it is found that  $\log k_w$  is linearly related to  $S$  by regression analysis. Simultaneously, the nonlinear quantitative relationships between either  $\log k_w$  or  $S$  and the structure parameters of the solutes are derived from the chromatographic thermodynamic theory, which involves two-parameter models consisting of the total solubility parameter ( $\delta_T$ ) and the *n*-octanol–water partition coefficient ( $P$ ). Results indicate that the indices of  $\log k_w$  and  $S$  are solute-related constants that reflect the influences of hydrophobicity and hydrophilicity, respectively, and play opposite roles in solute retention. Additionally, the methods estimating  $\log P$  with molecular structure parameters are proposed and justified by the practical calculation of  $P$ , which provides the possibility of predicting the  $P$  values of compounds. On the basis of the linear summation of free energy change, we establish a fundamental retention equation that includes the influences of the properties of solute and mobile phase on the retention process, and we use the general retention equation to successfully predict the retention values of the tested solutes. We have also done multi-regression analysis of a great deal of data and have proved the quantitative equations discussed above. Finally, the selectivity of the chromatographic system is discussed in detail, and a basic relationship between the relative retention and the influencing factors is given.

## Introduction

It is well-known that the study of the quantitative structure–retention relationship (QSRR) is an important topic in chromatographic thermodynamics because it can be applied not only to predict chromatographic retention behavior and to measure physicochemical parameters, but also to lead to an understanding of the retention mechanism. Many experiments

have proven that, for a given column, the retention behavior depends only on the molecular structure of the solute and the property of the mobile phase; the solute–mobile phase interactions cannot be ignored and play a dominant role in the retention mechanism of reversed-phase liquid chromatography (RPLC)(1–4). Therefore, it is possible to enable the chromatographer to choose a suitable mobile phase composition for optimal separation.

In most investigations, the composition and polarity of the mobile phase have been used to estimate the retention data of the solute. A linear relationship for the logarithm of the capacity factor,  $\log k'$ , as a function of the log of the total solubility parameter of the normal-phase binary mobile phase ( $\log \delta_{m,T}$ ) was experimentally observed by Cooper et al.(5), as shown:

$$\log k' = S_0 - S_1 \log \delta_{m,T} \quad \text{Eq 1}$$

where  $S_0$  is the intercept and  $S_1$  is the slope. Although the linear correlation between slope and intercept was found for structurally similar compounds, a nonlinear relationship was often observed for other compounds. Kaibara et al.(3,6) examined how the hydrophobic interaction between the solute and the stationary ligand can be evaluated from the dependence of the capacity factor on the concentration of methanol ( $C$ ) in an aqueous binary mobile phase; it was found that the plot of  $\log k'$  versus  $\log (1/C)$  presented a straight line:

$$\log k' = q + Q \times \log (1/C) \quad \text{Eq 2}$$

where  $Q$  is the slope of the correlation line and  $q$  is a constant. The  $Q$  value is well-correlated to the hydrophobic properties of the solute, such as the 1-octanol–water partition coefficient ( $P$ )(3).

According to the solubility parameter concept (7), the quadratic equation between  $\log k'$  and the volume fraction of the organic modifier ( $\phi$ ) can be expressed as:

$$\log k' = \log k_w + S\phi + A\phi^2 \quad \text{Eq 3}$$

where  $A$  and  $S$  are constants for a given solute–eluent combination and  $\log k_w$  is the capacity factor obtained by extrapola-

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**Table I. Molecular Structure Parameters and log  $k'$  of Substituted Benzene Derivatives**

No.	Compound	Volume fraction of methanol ( $\phi$ )					$\delta_T^*$	log $P^\dagger$
		50.0%	57.1%	60.8%	67.7%	79.2%		
1	Aniline	0.18	0.01	-0.03	-0.20	-0.46	12.06	0.90
2	Benzylalcohol	0.25	0.09	0.02	-0.15	-0.40	11.51	1.10
3	4-Nitrophenol	0.43	0.13	0.09	-0.12	-0.41	13.35	1.91
4	Benzaldehyde	0.49	0.31	0.23	0.03	-0.24	11.18	1.48
5	Nitrobenzene	0.72	0.53	0.42	0.22	-0.11	11.70	1.85
6	Methoxybenzene	0.88	0.68	0.57	0.36	0.03	10.34	2.11
7	4-Nitrotoluene	1.02	0.79	0.66	0.43	0.06	10.79	2.37
8	2-Nitrotoluene	0.95	0.78	0.63	0.39	0.03	10.90	2.30
9	Benzene	0.87	0.69	0.58	0.37	0.04	10.02	2.13
10	Chlorobenzene	1.19	1.01	0.85	0.60	0.20	10.32	2.84
11	Toluene	1.22	0.99	0.85	0.62	0.24	9.61	2.69
12	Bromobenzene	1.31	1.08	0.92	0.67	0.25	10.58	2.99
13	Ethylbenzene	1.52	1.27	1.11	0.83	0.39	9.24	3.15
14	<i>n</i> -Propylbenzene	1.89	1.59	1.39	1.07	0.56	8.87	3.68
15	4-Bromotoluene	1.68	1.40	1.21	0.92	0.45	10.09	3.53
16	2-Bromotoluene	1.67		1.23	0.93	0.49	10.15	3.50
17	1,4-Dimethylbenzene	1.56		1.15	0.87	0.45	9.24	3.20
18	1,2-Dimethylbenzene	1.53		1.10	0.83	0.41	9.49	3.12

\* Calculated from Equation 6.  
† Taken from reference 3.

tion of retention data from binary eluents to 100% water. The curvature of the log  $k'$  versus  $\phi$  plot, as described by Equation 3, was mainly caused by capacity factors measured at the upper or lower end of the volume fraction range. Many experiments have shown that, over a volume fraction range of at most 0.1–0.9, Equation 3 can be simplified to become an approximate linear function:

$$\log k' = \log k_w + S\phi \quad \text{Eq 4}$$

Equation 4 is an adequate expression for the dependence of log  $k'$  on  $\phi$  restricted to the intermediate volume fraction range and has been verified by various studies (8–10). More recent studies have added strong evidence that log  $k_w$  is closely related to  $P$  and is a hydrophobic parameter and a linear function of log  $P$  (9,11,12). Because the hydrophobic constant ( $P$ ) is one of the fundamental natures of a substance, affecting not only its physicochemical properties but also its biological activities, it has been widely accepted as an important parameter in QSRR and quantitative structure–activity relationship (QSAR) studies (3,13,14). Therefore, it is necessary to determine  $P$  values with experimental and RPLC methods. In the review reported by Braumann (12), the linear correlation between log  $P$  and log  $k_w$  that can be used to calculate log  $P$  values is discussed in detail. In addition, a computer program has been developed for estimating both log  $P$  and the aqueous solubility from the structural formulas of 497 compounds (15).

Studies have justified that log  $k_w$  and  $S$  of Equation 4 are functions of solute molecular structure parameters (e.g., van der Waals volume [16]) for homologous series. Additionally, more complex equations containing four structure parameters can be used to predict log  $k_w$  and  $S$  (17–20); however, it is difficult to use them because of the inconvenience of obtaining these parameters by experiment and reference. We emphasize that it is necessary and rather practical to establish a simple model indicating log  $k_w$  and  $S$  that can be used for other compounds besides homologous series.

The purpose of this paper was to explore the retention mechanism of substituted benzene derivatives based on the correlation between log  $k'$  and  $\phi$  and to find the dependence of log  $k'$  on  $S$  in RPLC. In addition, we wished to systematically investigate the relationships between either log  $k_w$  or  $S$  and

**Table II. Regression Results of 18 Compounds with Equation 4 and the Predicted Data and Deviation of log  $k_w$  from Equation 7**

No.	Regression results from Equation 4				log $k_w$ from Equation 7	
	log $k_w$	$S$	$r$	SD	Predicted*	AD†
1	1.272	-2.179	0.9981	0.017	1.313	0.041
2	1.369	-2.234	0.9997	0.007	1.381	0.012
3	1.780	-2.789	0.9925	0.044	2.073	0.293
4	1.750	-2.518	0.9994	0.011	1.735	-0.015
5	2.154	-2.857	0.9999	0.005	2.158	0.004
6	2.346	-2.925	0.9999	0.005	2.242	-0.104
7	2.668	-3.297	0.9999	0.005	2.706	0.038
8	2.587	-3.226	0.9981	0.026	2.618	0.031
9	2.319	-2.873	0.9995	0.011	2.178	-0.141
10	2.951	-3.464	0.9981	0.028	2.914	-0.037
11	2.905	-3.368	0.9999	0.007	2.795	-0.110
12	3.151	-3.662	0.9996	0.013	3.161	0.010
13	3.484	-3.908	0.9997	0.013	3.468	-0.016
14	4.191	-4.592	0.9997	0.016	4.320	0.129
15	3.803	-4.242	0.9996	0.016	3.884	0.081
16	3.693	-4.056	0.9997	0.014	3.652	-0.041
17	3.465	-3.814	0.9998	0.010	3.350	-0.115
18	3.440	-3.838	0.9997	0.013	3.380	-0.060

\* Predicted log  $k_w$  from Equation 7.  
† Absolute deviations of experimental and predicted values of log  $k_w$ .

the molecular structure characteristics of solutes. We also wished to establish corresponding quantitative models between them and to estimate  $\log P$  values of compounds by using molecular structure parameters from these models. We also applied the linear summation of free energy change to set up a general retention equation and used it to investigate chromatographic selectivity in detail.

## Experimental

### Apparatus

The liquid chromatograph used in this work consisted of a model LC-4A pump (Shimadzu, Kyoto, Japan) and a Shimadzu model SPD-2A ultraviolet (UV) detector. The reversed-phase stationary phase was 5- $\mu\text{m}$  Zorbax ODS, and the column (Waters, Milfold, MA) dimensions were 15 cm  $\times$  4.6-mm i.d. in all cases.

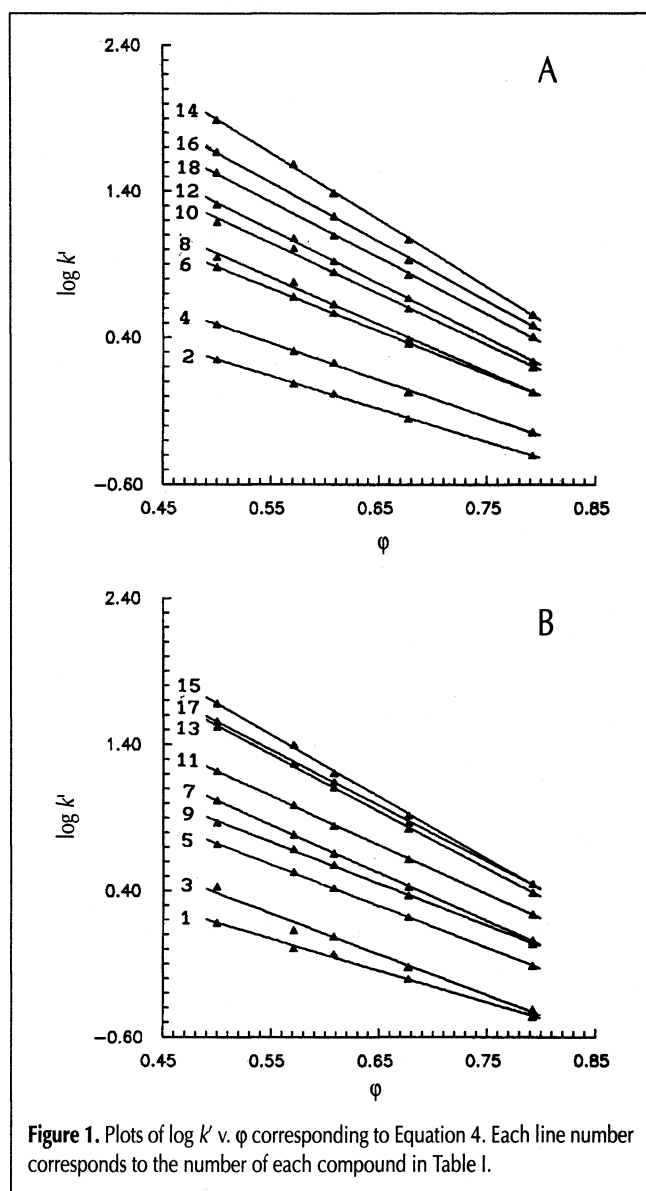


Figure 1. Plots of  $\log k'$  v.  $\phi$  corresponding to Equation 4. Each line number corresponds to the number of each compound in Table I.

### Chemicals

Analytical-grade solutes and methanol were purchased from Tianjin Chemical Regent Factory (Tianjin, P.R. China). The methanol and water were distilled before being used. Methanol-water mixtures consisting of different volume fractions of methanol were used as eluents at 25°C.

### Determination of mobile phase composition

McCown et al. (21) reported the influence of dissolved gases on gradient high-performance liquid chromatography (HPLC). Much of the detrimental effect of dissolved air in the mobile phase is attributed to dissolved oxygen. It is essential that the mixed mobile phase be degassed by an ultrasound method to eliminate the influence of dissolved gases. The organic modifier composition in the mixture is damaged by the degassing process. A simple method to determine the precise composition of organic modifier with the refractive index ( $\eta$ ) of a mixed mobile phase was reported in our previous paper (22). The  $\phi$  values of methanol in five binary mobile phases were calculated according to the  $\eta$  values of the degassed mobile phase and are listed in Table I.

### Calculation of capacity factor

The capacity factors of 18 substituted benzene derivatives in five different compositions of binary mobile phases were calculated by the following equation:

$$k' = \frac{V_R - V_0}{V_0} \quad \text{Eq 5}$$

where  $V_R$  is the retention volume of the solute, which is equal to the product of the retention time and the flow rate.  $V_0$  is the column holdup volume, which is the difference between the methanol retention volume and the extracolumn volume. All solutes were detected by a UV detector at 254 nm. The logarithms of capacity factors are given in Table I.

### Calculation of $\delta_T$ and $\log P$

The total solubility parameters,  $\delta_T$  ( $\text{cal}^{1/2} \text{cm}^{-3/2}$ ), of tested compounds were computed by the quantitative equation published in our previous paper (23):

$$\delta_T^2 = -0.02085 \frac{T_b^2}{V} + 58.93 \frac{T_b}{V} - 6892.14 \frac{1}{V} - 26.76 \quad \text{Eq 6}$$

where  $T_b$  and  $V$  are the absolute boiling point temperature (at 760 mm Hg) and the molar volume of a compound, respectively. The  $\log P$  data for all solutes were taken from the literature (3);  $\delta_T$  values are listed in Table I.

Other experimental results that were utilized in this work were taken from papers by Smith et al. (24) and Schoenmakers et al. (25), which give exact descriptions of the analytical conditions employed.

## Results and Discussion

### Relationship between capacity factor and mobile phase composition

To determine whether Equation 3 or Equation 4 is the best model depicting the retention behavior of these solutes

**Table III. Regression Results from Equation 4 and Molecular Structure Parameters of Some Substituted Benzene Compounds**

No. Compound	$\log k_w$	$S$	$r$	SD	$\delta_T^*$	$\log P^\dagger$
<b>A) Methanol-buffer, pH = 7) (40–90%) (24)</b>						
1 Aniline	0.881	-1.686	0.9962	0.036	12.06	0.90
2 Benzamide	0.585	-1.473	0.9778	0.076	12.19	0.64
3 Benzene	2.113	-2.579	0.9987	0.032	10.02	2.13
4 Benzonitrile	1.608	-2.354	0.9952	0.056	11.38	1.56
5 Benzyl alcohol	1.075	-1.880	0.9950	0.046	11.51	1.10
6 Benzyl bromide	2.884	-3.477	0.9969	0.066	10.53	2.92
7 Benzyl chloride	2.713	-3.326	0.9992	0.032	10.39	2.30
8 Benzyl cyanide	1.605	-2.455	0.9952	0.058	11.26	1.58
9 Bromobenzene	2.970	-3.405	0.9987	0.041	10.58	2.99
10 Chlorobenzene	2.813	-3.281	0.9989	0.038	11.32	2.84
11 Methyl benzoate	2.257	-2.881	0.9962	0.061	10.18	2.12
12 Nitrobenzene	1.915	-2.534	0.9988	0.030	11.70	1.85
13 Phenol	1.075	-1.917	0.9940	0.051	12.19	1.46
14 Toluene	2.678	-3.062	0.9998	0.015	9.61	2.69
15 Anisole	2.126	-2.649	0.9992	0.020	10.34	2.11
16 Benzaldehyde	1.544	-2.284	0.9962	0.037	11.18	1.48
17 Biphenyl	4.058	-4.468	0.9987	0.038	8.77	4.09
<b>B) (Acetonitrile-buffer, pH=7) (30–90%) (24)</b>						
1 Aniline	0.828	-1.590	0.9928	0.051	12.06	0.90
2 Benzamide	0.144	-0.919	0.9551	0.067	12.19	0.64
3 Benzene	1.748	-2.328	0.9968	0.044	10.02	2.13
4 Benzonitrile	1.359	-2.108	0.9961	0.044	11.38	1.56
5 Benzyl alcohol	0.549	-1.225	0.9816	0.056	11.51	1.10
6 Benzyl bromide	2.252	-2.820	0.9897	0.097	10.53	2.92
7 Benzyl chloride	2.218	-2.898	0.9946	0.072	10.39	2.30
8 Benzyl cyanide	1.388	-2.105	0.9920	0.063	11.26	1.58
9 Bromobenzene	2.296	-2.820	0.9938	0.074	10.58	2.99
10 Chlorobenzene	2.190	-2.739	0.9945	0.068	11.32	2.84
11 Methyl benzoate	1.657	-2.318	0.9952	0.054	10.18	2.12
12 Nitrobenzene	1.622	-2.371	0.9965	0.047	11.70	1.85
13 Phenol	0.889	-1.754	0.9956	0.039	12.19	1.46
14 Toluene	2.210	-2.740	0.9938	0.072	9.61	2.69
15 Anisole	1.820	-2.468	0.9945	0.053	10.34	2.11
16 Benzaldehyde	1.340	-2.114	0.9987	0.022	11.18	1.48
17 Biphenyl	3.223	-3.863	0.9895	0.118	8.77	4.09
<b>C) (Methanol-water) (50–100%) (25)</b>						
1 Acetophenone	1.686	-2.337	0.9869	0.072	10.67	1.58
2 Aniline	1.089	-1.883	0.9935	0.041	12.06	0.90
3 Anisole	2.187	-2.693	0.9673	0.133	10.34	2.11
4 Benzaldehyde	1.529	-2.207	0.9834	0.077	11.18	1.48
5 Benzene	2.243	-2.687	0.9982	0.031	10.02	2.13
6 Benzonitrile	1.516	-2.254	0.9919	0.055	11.38	1.56
7 Benzyl alcohol	1.203	-2.013	0.9778	0.081	11.51	1.10
8 Chlorobenzene	2.932	-3.332	0.9980	0.039	11.32	2.84
9 <i>o</i> -Cresol	1.772	-2.513	0.9936	0.054	9.92	2.02
10 Diethyl phthalate	2.741	-3.466	0.9928	0.079	8.58	3.15
11 2,4-Dimethyl phenol	2.294	-2.951	0.9963	0.048	10.26	2.58
12 Dimethyl phthalate	1.763	-2.568	0.9857	0.083	9.62	2.11

\* Calculated from Equation 6.

† Taken from reference 3.

consisting of mono- and multi-substituted benzene derivatives (see Table I), we completed the regression analysis based on both equations, and the overall average results are given below:

$$\text{Eq 3: } r = 0.9993, \text{ SD} = 0.0148, \text{ AD} = 0.0071$$

$$\text{Eq 4: } r = 0.9991, \text{ SD} = 0.0145, \text{ AD} = 0.0086$$

where  $r$  is the mean correlation coefficient, SD is the mean standard deviation, and AD is the mean absolute deviation of  $\log k'$  for 18 tested compounds. Through the comparison of these statistical results, it was verified that there was no remarkable difference between Equations 3 and 4. To simplify the calculating process and avoid trouble in the prediction of  $k'$ , the linear equation, Equation 4, can satisfy the requirement for the prediction of the retention values. The conclusion can be proven by using the plots of  $\log k'$  versus the volume fraction of methanol ( $\phi$ ) shown in Figure 1. A linear relationship exists between  $\log k'$  and  $\phi$ . The detailed regression results of Equation 4 are listed in Table II.

The results in Table II show that each regression line corresponds to a correlation coefficient greater than 0.998 (except 4-nitrophenol, for which  $r = 0.9925$ ), and a standard deviation less than or equal to 0.044. In addition, we studied other substituted benzene compounds (24,25) on various columns and in five mobile phases, and the linear correlation between the retention behavior and composition of the mobile phase was satisfied. The regression results are listed in Table III:  $r$  was greater than or equal to 0.9161, and SD was less than or equal to 0.184. Therefore, the linear correlation between the retention behavior and the composition of the mobile phase in the region of the intermediate volume fraction was justified by the results of the comparison discussed above.

#### Correlation between slope and intercept

Based on the net hydrogen bonding properties of compounds, Minick and coworkers (11) classified solutes into two types, donors and acceptors, and found approximately parallel lines corresponding to  $\log k_w$  versus  $S$ . In a report by Tan and Carr (26), the  $S$ - $\log k_w$  linear correlation is discussed, and it is pointed out that the linear correlation is universal and is restricted to a homologous series. To illustrate the relationship between slope and intercept, a graph of the  $\log k_w$

**Table III (Continued). Regression Results from Equation 4 and Molecular Structure Parameters of Some Substituted Benzene Compounds**

No. Compound	log $k_w$	S	r	SD	$\delta_r^*$	log $P^\dagger$
13 <i>m</i> -Dinitrobenzene	1.909	-2.627	0.9949	0.050	12.57	1.57
14 <i>n</i> -Methylaniline	2.028	-2.656	0.9958	0.046	11.07	1.66
15 <i>o</i> -Nitroaniline	1.528	-2.273	0.9937	0.049	13.82	1.62
16 Nitrobenzene	1.943	-2.559	0.9952	0.048	11.70	1.85
17 <i>m</i> -Nitrophenol	1.585	-2.382	0.9931	0.053	11.01	1.18
18 Phenol	1.149	-1.976	0.9884	0.057	12.19	1.46
19 2-Phenylethanol	1.558	-2.320	0.9901	0.062	10.75	1.36
20 Toluene	2.837	-3.180	0.9984	0.034	9.61	2.69
<b>D) (Methanol-water)</b>						
<b>(60–100%) (25)</b>						
1 Acetophenone	2.058	-2.955	0.9795	0.111	10.67	1.58
2 Anisole	2.105	-2.625	0.9895	0.070	10.34	2.11
3 Benzaldehyde	1.469	-2.191	0.9910	0.054	11.17	1.48
4 Benzene	1.756	-2.087	0.9380	0.141	10.01	2.13
5 Benzonitrile	1.484	-2.248	0.9903	0.058	11.38	1.56
6 Benzyl alcohol	1.331	-2.351	0.9913	0.057	11.51	1.10
7 Biphenyl	3.842	-4.158	0.9957	0.070	8.77	4.09
8 Chlorobenzene	2.765	-3.224	0.9933	0.069	11.32	2.84
9 <i>p</i> -Chlorophenol	2.053	-2.872	0.9963	0.045	11.31	2.17
10 <i>p</i> -Chlorotoluene	3.344	-3.728	0.9958	0.058	11.89	3.40
11 <i>o</i> -Cresol	1.569	-2.336	0.9917	0.055	9.92	2.02
12 <i>o</i> -Dichlorobenzene	3.244	-3.604	0.9934	0.076	10.55	3.55
13 Diethyl phthalate	1.569	-2.336	0.9891	0.090	8.58	3.15
14 Dimethyl phthalate	1.735	-2.587	0.9904	0.066	9.62	2.11
15 <i>m</i> -Dinitrobenzene	2.012	-2.762	0.9966	0.042	12.57	1.57
16 <i>o</i> -Dinitrobenzene	2.001	-2.886	0.9890	0.079	11.11	1.57
17 <i>p</i> -Dinitrobenzene	1.888	-2.740	0.9949	0.051	12.89	1.57
18 2,4-Dinitrobenzene	2.468	-3.159	0.9947	0.059	10.89	2.13
19 Ethylbenzene	3.182	-3.560	0.9947	0.067	9.24	3.15
20 <i>m</i> -Fluoronitrobenzene	2.218	-2.851	0.9917	0.067	11.32	1.99
21 <i>o</i> -Fluoronitrobenzene	2.006	-2.747	0.9954	0.049	11.51	1.99
22 <i>p</i> -Fluoronitrobenzene	2.026	-2.755	0.9977	0.034	11.38	1.99
23 <i>p</i> -Methoxybenzaldehyde	1.570	-2.256	0.9894	0.060	11.09	1.46
24 <i>p</i> -Methylbenzaldehyde	1.910	-2.656	0.9357	0.183	10.64	2.04
25 Methylbenzoate	2.046	-2.583	0.9861	0.079	10.18	2.12
26 Naphthalene	3.206	-3.565	0.9949	0.066	10.46	3.36
27 Nitrobenzene	1.944	-2.583	0.9945	0.050	11.70	1.85
28 <i>m</i> -Nitrophenol	1.620	-2.485	0.9949	0.046	11.01	1.18
29 <i>o</i> -Nitrophenol	1.824	-2.467	0.9874	0.072	11.44	1.18
30 <i>p</i> -Nitrophenol	1.512	-2.387	0.9890	0.065	13.36	1.18
31 Phenol	1.272	-2.247	0.9918	0.053	12.18	1.46
32 2-Phenylethanol	1.524	-2.390	0.9948	0.045	10.75	1.36
33 <i>p</i> -Phenylphenol	2.774	-3.450	0.9937	0.071	8.94	3.42
34 <i>n</i> -Propylbenzene	3.799	-4.123	0.9964	0.064	8.87	3.68
35 Toluene	2.709	-3.143	0.9957	0.053	9.60	2.69
36 2,4-Dimethylphenol	2.171	-2.902	0.9937	0.060	10.26	2.58
<b>E) (Acetonitrile-water)</b>						
<b>(40–100%) (25)</b>						
1 Acetophenone	1.145	-1.823	0.9921	0.055	10.67	1.58
2 Anisole	1.596	-2.182	0.9957	0.048	10.34	2.11
3 Benzaldehyde	1.135	-1.843	0.9961	0.039	11.17	1.48
4 Benzene	1.637	-2.206	0.9974	0.038	10.01	2.13

\* Calculated from Equation 6.

† Taken from reference 3.

versus  $S$  data from Table II is shown in Figure 2. Because the results demonstrate that  $\log k_w$  is directly related to  $S$ , all of the solutes can be classified as one type of compound consisting of mono- and multi-substituted benzene derivatives in the examination of the correlation between slope and intercept.

From Figure 2, a better linear relationship can be observed; we can express it with the following equation:

$$\log k_w = a_0 + a_1 S \quad \text{Eq 7}$$

where  $a_0$  and  $a_1$  are constants. The values of  $a_0$  and  $a_1$  were obtained from linear regression analysis ( $r = 0.9930$ ,  $SD = 0.105$ ); all of the results are given in Table IV. The predicted values and deviations of  $\log k_w$  on the basis of Equation 7 are listed in Table II and agree with the experimental data. The analytical results further indicate the linear dependence of  $\log k_w$  on  $S$  in all tested solutes including mono- and multi-substituted benzene derivatives, but the intercept and slope have converse values and play different roles in the retention mechanism. The positive value of  $\log k_w$  indicates that the retention time of the solute is in direct proportion to  $\log k_w$ , whereas the negative value of  $S$  indicates that the capacity factor will decrease with the increasing  $S$  values.

Additionally, the  $a_i$  coefficients of Equation 7 that correspond to the five data groups in Table III were obtained by multi-regression analysis. The results are listed in Table IV;  $r \geq 0.9538$ , and  $SD \leq 0.209$ . It is clear that Equation 7 corresponds to a linear dependence of  $\log k_w$  on  $S$  shown in Figure 3. From this figure, we can see that  $\log k_w$  is decreasing while  $S$  is increasing. It was confirmed that  $\log k_w$  and  $S$  play opposite roles in the retention mechanism of RPLC.

#### Relationship between $\log k_w$ and molecular structure parameters

$\log k_w$  is an extrapolated value for the capacity factor in pure water obtained from Equation 4. It is a constant depending only on the nature of the solutes, and it plays an important role in our understanding of the retention mechanism and the interactions of two phases and a solute in RPLC. In fact,  $\log k_w$  is the logarithm of the capacity factor of a solute in water. According to the chromatographic thermodynamic theory, the relationship between  $\log k_w$  and free energy change ( $\Delta G$ ) can be expressed as:

$$\log k_w = \log \phi - \frac{\Delta G}{2.3 RT} \quad \text{Eq 8}$$

where  $R$  and  $T$  are gas constants and absolute temperature, respectively, and  $\phi$  is the phase ratio and a constant in a given condition. Chemically bonded stationary phases are the most widely used column packing materials in RPLC, especially silica (ODS) gel. Because of steric restrictions, it is impossible to couple alkyl chains with all hydroxyl groups on the silica gel surface, depending on the bonding procedure, so a variable number of residual silanol groups remain accessible to "silanophilic" interactions with solute molecules. Because the silanol groups are strongly hydrated and the alkyl chains display violent hydrophobicity, a mixed retention mechanism exists in RPLC (27). Therefore, the solute retention is dominated by both hydrophobic and silanophilic (or hydrophilic) interactions. On the basis of chromatographic thermodynamic theory, we determined that the free energy change,  $\Delta G$ , is made up of a linear combination of contributions of the polar (or hydrophilic) part, the nonpolar (or hydrophobic) part, and their cooperative interaction in a molecule, which can be described by the following:

$$\Delta G = \Delta G_p + \Delta G_{np} + \Delta G_p \times \Delta G_{np} \quad \text{Eq 9}$$

where  $\Delta G_p$  and  $\Delta G_{np}$  are the contributions of hydrophilicity and hydrophobicity of a solute, respectively, to the free energy change.  $\Delta G_p \times \Delta G_{np}$  is the product of  $\Delta G_p$  and  $\Delta G_{np}$ , which indicates the ability of their cooperative interaction to participate in the retention mechanism.

According to the definition of total solubility parameters,  $\delta_T$  is one of the basic parameters that represent the magnitude of polarity and the ability of a substance to participate in dispersive, dipole orientation as well as inductive and hydrogen-bonding interactions. Because the total solubility parameter is regarded as the quantitative standard of compound polarity, which had been widely used in various fields detailed in our previous work (23) and others (28), we assumed that  $\Delta G_p$  could be expressed as a nonlinear function of  $\delta_T$ :

$$\Delta G_p = f(\delta_T) \quad \text{Eq 10}$$

On the other hand, the hydrophobicity of a compound is defined as its relative tendency to be readily soluble in most nonpolar solvents but only sparingly soluble in water. Solute hydrophobicity is usually expressed as the partition coefficient ( $P$ ) derived from distribution studies of the compound between water and immiscible nonpolar solvents. It is now generally accepted that  $P$  values obtained from  $n$ -octanol-water partition systems are particularly suitable for characterizing biological activities and physicochemical phenomena of compounds. The logarithm of the partition coefficient in  $n$ -octanol-water,  $\log P$ , had been regarded as the quantitative standard of hydrophobicity of a solute. From the thermodynamic equilibrium principle, the partition coefficient of solute  $i$  can be described as:

$$\Delta G_i = G_i^0 - 2.3RT \times \log P_i \quad \text{Eq 11}$$

where  $G_i^0$  is the standard free energy of solute  $i$ , a constant to a substance in a given condition. Because  $\log P$  is suitable for quantitatively describing the ability of a solute to participate in hydrophobic interactions, we can assume that  $\Delta G_{np}$  can be expressed a nonlinear function of  $\log P$ :

$$\Delta G_{np} = f(\log P) \quad \text{Eq 12}$$

Substituting Equations 11 and 12 into Equation 9 yields the following:

$$\Delta G = f(\delta_T) + f(\log P) + f(\delta_T) \times f(\log P) \quad \text{Eq 13}$$

The correlation between free energy change in the solute retention process and

**Table III (Continued). Regression Results from Equation 4 and Molecular Structure Parameters of Some Substituted Benzene Compounds**

No. Compound	$\log k_w$	$S$	$r$	SD	$\delta_T^*$	$\log P^\dagger$
5 Benzonitrile	1.307	-2.049	0.9958	0.045	11.38	1.56
6 Biphenyl	2.719	-3.125	0.9957	0.069	8.77	4.09
7 Chlorobenzene	1.986	-2.448	0.9952	0.057	11.32	2.84
8 <i>p</i> -Chlorophenol	1.204	-1.995	0.9678	0.123	11.31	2.17
9 <i>p</i> -Chlorotoluene	2.339	-2.716	0.9950	0.065	11.89	3.40
10 <i>o</i> -Cresol	1.113	-1.916	0.9782	0.096	9.92	2.02
11 <i>o</i> -Dichlorobenzene	2.284	-2.651	0.9952	0.062	10.55	3.55
12 Diethyl phthalate	1.886	-2.543	0.9934	0.070	8.58	3.15
13 <i>m</i> -Dinitrobenzene	1.615	-2.468	0.9958	0.053	12.57	1.57
14 <i>o</i> -Dinitrobenzene	1.684	-2.599	0.9971	0.047	11.61	1.57
15 <i>p</i> -Dinitrobenzene	1.664	-2.543	0.9961	0.053	12.89	1.57
16 2,4-Dinitrotoluene	1.948	-2.787	0.9981	0.041	10.89	2.13
17 <i>m</i> -Fluoronitrobenzene	1.669	-2.375	0.9949	0.057	11.23	1.99
18 <i>o</i> -Fluoronitrobenzene	1.549	-2.316	0.9941	0.060	11.51	1.99
19 <i>p</i> -Fluoronitrobenzene	1.639	-2.391	0.9976	0.039	11.38	1.99
20 <i>p</i> -Methoxybenzaldehyde	1.145	-1.875	0.9927	0.054	11.09	1.46
21 <i>p</i> -Methylbenzaldehyde	1.437	-2.066	0.9941	0.053	10.64	2.04
22 Methyl benzoate	1.484	-2.057	0.9926	0.059	10.18	2.12
23 Naphthalene	2.300	-2.718	0.9947	0.066	10.46	3.36
24 Nitrobenzene	1.571	-2.281	0.9967	0.044	11.70	1.85
25 <i>m</i> -Nitrobenzene	0.957	-1.825	0.9198	0.184	11.01	1.18
26 <i>o</i> -Nitrobenzene	1.211	-1.830	0.9615	0.124	11.44	1.18
27 <i>p</i> -Nitrobenzene	1.140	-2.158	0.9490	0.170	13.35	1.18
28 Phenol	0.767	-1.690	0.9719	0.097	12.18	1.46
29 2-Phenylethanol	0.558	-1.264	0.9161	0.131	10.75	1.36
30 <i>p</i> -Phenylphenol	1.764	-2.461	0.9739	0.136	8.94	3.42
31 <i>n</i> -Propylbenzene	2.678	-3.020	0.9960	0.064	8.68	3.68
32 Toluene	2.116	-2.698	0.9956	0.060	9.60	3.69
33 2,4-Dimethylphenol	1.344	-2.027	0.9767	0.105	10.26	2.58

\* Calculated from Equation 6.

† Taken from reference 3.

hydrophilic and hydrophobic properties is described in Equation 13. By combining Equation 13 with Equation 8, the relationship between  $\log k_w$  and the molecular structure parameters is described in the following:

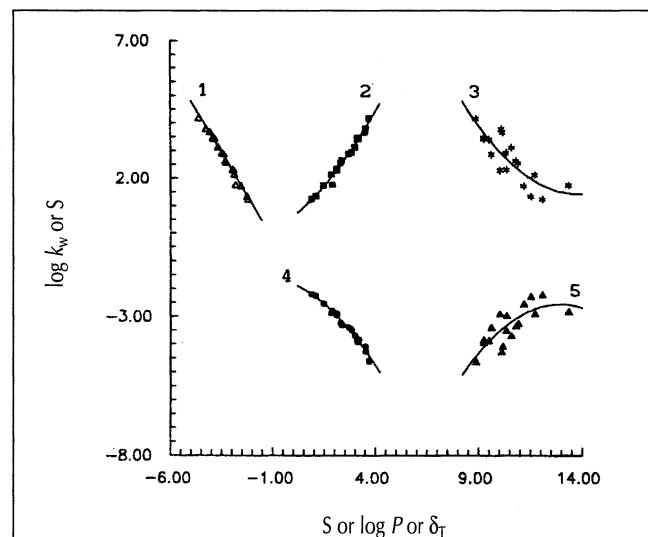
$$\log k_w = b_0 + b_1\delta_T + b_2\log P + b_3\delta_T\log P \quad \text{Eq 14}$$

where  $b_i$  ( $i = 0, 1, 2, 3$ ) is a constant depending on the employed conditions. It is clear that Equation 14 corresponds to a nonlinear correlation between  $\log k_w$  and the molecular nature of the solute, instead of a linear dependence of  $\log k_w$  on  $\log P$ , as described in various investigations. This conclusion has also been proven by the plots of  $\log k_w$  versus  $\log P$  and  $\delta_T$ , as shown in Figure 2 (data from Tables I and II, respectively).

To determine Equation 14, multi-regression analysis was performed in this paper; the  $b_i$  values and regression results are listed in Table IV. The correlation coefficient is 0.9953, and the standard deviation is 0.092. Additionally, we used the equation to estimate the  $\log k_w$  values with the total solubility parameters and hydrophobic constants of 18 substituted benzene derivatives to verify Equation 14. The predicted  $\log k_w$  values given in Table V correspond with the experimental data listed in Table II.

In order to further harmonize the nonlinear correlation between  $\log k_w$  and molecular structure parameters, we performed the multi-regression analysis for other substituted benzene compounds (see Table III). The  $b_i$  coefficients and the regression results are also listed in Table IV;  $r \geq 0.877$ , and  $SD \leq 0.258$ . This verified that Equation 14 could be employed to estimate the  $\log k_w$  values of mono- and multi-substituted benzenes in a large volume fraction range and that the correlation between  $\log k_w$  and the molecular structure parameters of substituted benzene derivatives exists as depicted in Equation 14.

The nonlinear correlation can also be validated by the plots of  $\log k_w$  versus  $\delta_T$  and  $\log P$  shown in Figure 3. From these



**Figure 2.** Relationship between  $\log k_w$  and  $S$  corresponding to Equation 7 and relationship between either  $\log k_w$  or  $S$  and either  $\log P$  or  $\delta_T$  corresponding to Equations 14 and 15 in methanol-water. 1 =  $\log k_w$  versus  $S$ ; 2 =  $\log k_w$  versus  $\log P$ ; 3 =  $\log k_w$  versus  $\delta_T$ ; 4 =  $S$  versus  $\log P$ ; 5 =  $S$  versus  $\delta_T$ .

figures, we can see that  $\log k_w$  has a general tendency to increase with decreasing hydrophilicity and increasing hydrophobicity. This agrees with the experiments.

#### Relationship between $S$ and molecular structure parameters

The slope,  $S$ , is a characteristic constant of the solvent strength. Its magnitude reflects the influence of the aqueous mobile phase on  $\log k'$  and appears to reflect both hydrophobic and hydrogen bonding properties simultaneously (10). However, we found that  $S$  is variable and tends to decrease with increasing solute retention; also,  $S$  values generally tend to decrease as the molecular size and hydrophobicity of the solute increase. Because the value of  $S$  is negative,  $S$  exerts an opposite influence on the retention mechanism. Specifically, the retention time of the solute decreases as the concentration of organic modifier in the binary mobile phase increases. All of the conclusions were justified in the data in Tables I and II.

As discussed above,  $\log k_w$  is a solute-related constant. The  $S$  value must depend on the solute properties from Equation 7. Therefore,  $S$  is also a function of the solute structure parameters based on Equation 14, which is given in the following equation:

$$S = c_0 + c_1\delta_T + c_2\log P + c_3\delta_T\log P \quad \text{Eq 15}$$

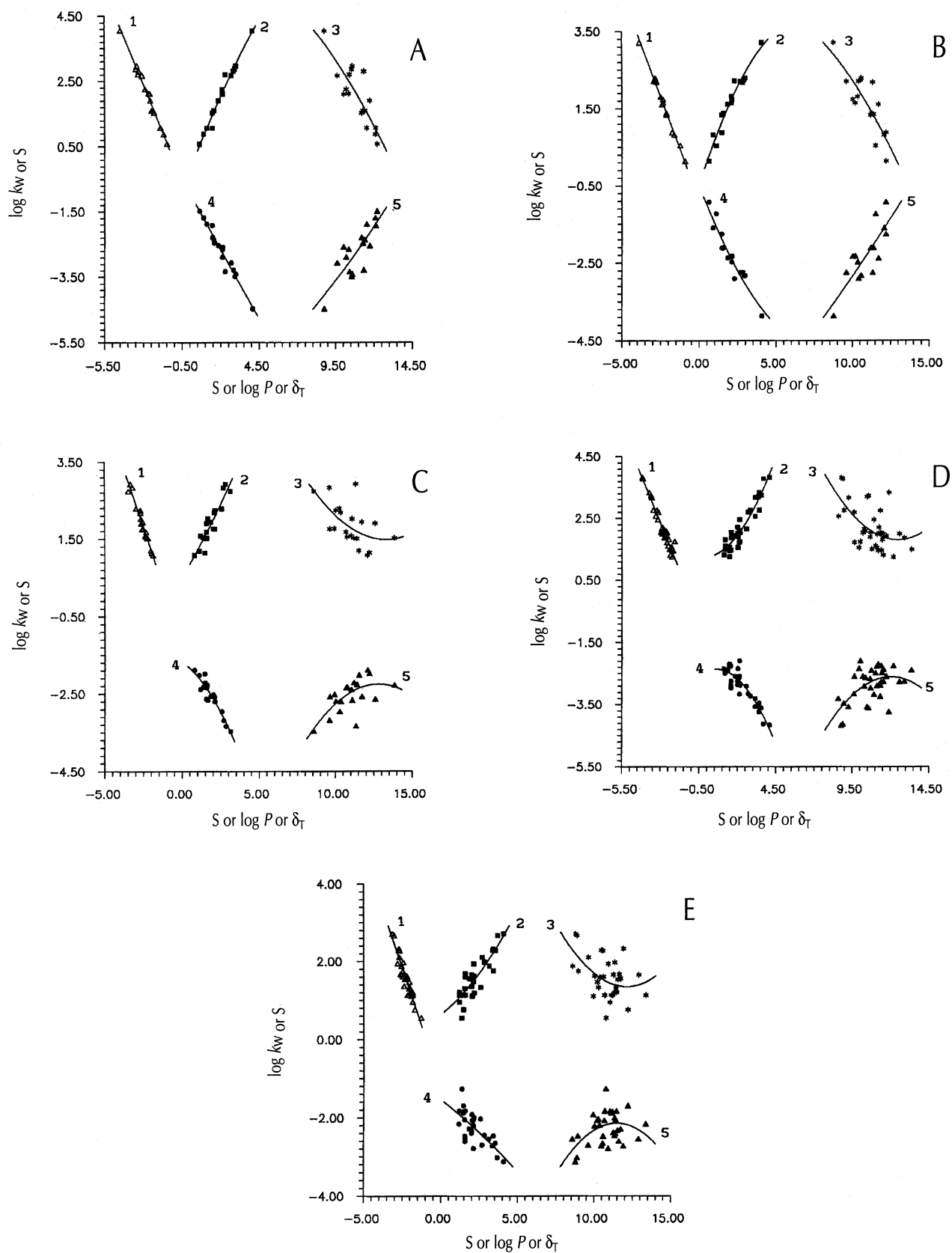
where  $c_i$  ( $i = 0, 1, 2, 3$ ) is a constant depending on the employed conditions. Equation 15 indicates a nonlinear relationship between  $S$  and either  $\log P$  or  $\delta_T$ , which can be observed in Figure 2. The nonlinear regression results are included in Table IV;  $r = 0.9922$ , and  $SD = 0.049$ . In addition, the predicted  $S$  values from Equation 15 and the  $\log P$  and  $\delta_T$  values of 18 tested compounds listed in Table V agree closely with the experimental values in Table II. All of the relative deviations are less than 4.5%. The results showed that  $S$  is related not only to hydrophobic but to hydrophilic characteristics of solutes.

In addition, the  $c_i$  coefficients of Equation 15 that correspond to the five data groups in Table III were obtained through multi-regression analysis. The regression results are also listed in Table IV;  $r \geq 0.8106$ , and  $SD \leq 0.252$ . Therefore, we can apply Equation 15 to evaluate  $S$ . The nonlinear correlation between  $S$  and molecular structures is also verified in the plots of  $S$  versus  $\delta_T$  and  $\log P$ , shown in Figure 3. From this figure, it can be concluded that  $S$  is increasing as hydrophilicity increases and hydrophobicity decreases; it is the opposite of  $\log k_w$ .

#### Establishment of retention model and prediction of capacity factor

We found it to be meaningful and necessary to establish a general retention model that included the influences of properties of the solutes and the mobile phase and that could be used to predict the retention values and selectivity of the chromatographic system. On the basis of Equations 4, 14, and 15, we proposed that  $\log k'$  could be expressed as:

$$\log k' = (-0.2036 + 0.0586\delta_T + 1.6742\log P - 0.0722\delta_T\log P) + (1.3988 - 0.2485\delta_T - 1.9548\log P + 0.1081\delta_T\log P)\phi \quad \text{Eq 16}$$



**Figure 3.** Relationship between  $\log k$  and  $S$  corresponding to Equation 7 and relationship between either  $\log k_w$  or  $S$  and either  $\log P$  or  $\delta_T$  corresponding to Equations 14 and 15, respectively. 1 =  $\log k_w$  versus  $S$ ; 2 =  $\log k_w$  versus  $\log P$ ; 3 =  $\log k_w$  versus  $\delta_T$ ; 4 =  $S$  versus  $\log P$ ; 5 =  $S$  versus  $\delta_T$ . Figure letters correspond to group letters in Table III.



Equation 16 is a fundamental retention equation indicating that the retention behavior of the solute is a characteristic function of the solute and the mobile phase (eluent: methanol–water, Zorbax ODS column). To verify its predictive ability, we used Equation 16 to estimate the capacity factors with  $\delta_T$  and  $\log P$  and the volume fraction of methanol in five different composition binary phases and to calculate the deviations of these solutes, which are given in Table VI. As Table VI indicates, the absolute deviations of all solutes rarely exceed 0.04 (only 4-nitrophenol is 0.06 in a 57.1% methanol system). The predicted values are consistent with the experimental values of  $\log k'$  for all solutes.

Equation 16 is a three-parameter model, which may satisfy the practical requirement of predicting the capacity factors of solutes in given conditions. If the  $\delta_T$  and  $\log P$  values of solutes are given, the  $\log k'$  values can be estimated by using Equation 16 with the  $\phi$  data (e.g., 2-bromotoluene, 1,4-dimethylbenzene, and 1,2-dimethylbenzene in 57.1% methanol mobile phase).

#### Evaluation of $\log P$ with $\log k_w$ , $S$ , and $\delta_T$

$\log P$  values are usually determined by the shake-flask method. However, because this method is elaborate and troublesome, for highly lipophilic compounds in particular, RPLC

has been proposed as a convenient and precise alternative (9). From systematic analysis and comparison, it is evident that there is no single relationship between the  $\log k'$  and  $\log P$  values of solutes. Therefore, it is difficult to predict  $\log P$  values using the  $\log k'$  values of solutes, which can be affected by the retention mechanism. The best method for predicting  $\log P$  is to use either  $\log k_w$  or  $S$  (11,12). Because the slope parameter exhibits hydrophobic and hydrophilic properties,  $S$  may be useful as an alternative to quantitatively estimate the hydrophobicity of a solute.

To explore the possibility of estimating  $\log P$ , Equations 14 and 15 were even applied to calculate the  $\log P$  values of the tested compounds with their  $\log k_w$ ,  $S$ , and  $\delta_T$  values separately. The predicted  $\log P$  values are given in Table V. From the comparison of the two methods, it was found that either of the two equations could be used to successfully estimate the  $\log P$  values of compounds, but Equation 14 was more accurate than Equation 15 because of their mean absolute deviations (AD = 0.076 in Equation 14, and AD = 0.084 in Equation 15). The reason may be that the  $S$  value is affected by more factors than  $\log k_w$  (e.g., the natures of eluents);  $S$  is the coefficient of  $\phi$  in Equation 4. In any case, using RPLC to estimate the partition coefficient in *n*-octanol–water has many advantages. The RPLC experiment is faster and more readily automated than the shake-flask method and is not dependent on the purity of the sample.

#### Selectivity of the RPLC system

The quantitative standard of whether two solutes can be separated by a chromatographic system is regarded as a separation factor. The ratio of two capacity factors,  $\alpha$ , is defined as the following:

$$\alpha = k'_2 / k'_1 \quad \text{Eq 17}$$

According to Equation 4, Equation 17 can be written as:

$$\log \alpha = \Delta \log k_w + \Delta S \phi \quad \text{Eq 18}$$

where  $\Delta \log k_w = \log k_{w,2} - \log k_{w,1}$  Eq 19

and  $\Delta S = S_2 - S_1$  Eq 20

where  $\log k_{w,1}$  and  $S_1$  are the intercept and the slope values of the first solute, and  $\log k_{w,2}$  and  $S_2$  are the intercept and slope values of the second solute, respectively. Equation 18 indicates that the selectivity,  $\alpha$ , is related to the properties of the solutes and mobile phase. For a given column and composition of eluents,  $\phi$  is a constant, and Equation 18 can be expressed as:

$$\log \alpha = \Delta \log k_w + \Delta S \times \text{constant} \quad \text{Eq 21}$$

Based on Equations 14 and 15,  $\Delta \log k_w$

Table IV. Results of Regression Analysis from Equations 7, 14, and 15\*

Solute groups	Regression equation coefficients				Statistical results	
	Equation 7				<i>r</i>	SD
<i>a</i> <sub>0</sub>	<i>a</i> <sub>1</sub>					
O	-1.4032	-1.2462			0.9930	0.105
A	-1.1150	-1.1782			0.9939	0.104
B	-0.8678	-1.0844			0.9944	0.083
C	-1.2144	-1.2143			0.9792	0.111
D	-1.4352	-1.2790			0.9580	0.209
E	-1.1080	-1.1889			0.9538	0.156
	Equation 14				<i>r</i>	SD
<i>b</i> <sub>0</sub>	<i>b</i> <sub>1</sub>	<i>b</i> <sub>2</sub>	<i>b</i> <sub>3</sub>			
O	-0.2036	0.0586	1.6742	-0.0722	0.9953	0.092
A	0.7367	-0.0638	0.8784	0.0072	0.9812	0.191
B	2.9960	-0.2633	-0.0203	0.0714	0.9753	0.185
C	1.9686	-0.1472	-0.0473	0.0813	0.9236	0.224
D	-1.7961	0.1951	1.3318	-4.4677	0.9389	0.247
E	-2.0649	0.2082	1.0526	-0.0393	0.8770	0.258
	Equation 15				<i>r</i>	SD
<i>c</i> <sub>0</sub>	<i>c</i> <sub>1</sub>	<i>c</i> <sub>2</sub>	<i>c</i> <sub>3</sub>			
O	1.3988	-0.2485	-1.9548	0.1081	0.9922	0.049
A	-0.7210	-0.0168	-0.6166	-0.0270	0.9316	0.318
B	-2.5977	0.1558	-0.2942	-0.0371	0.9572	0.224
C	-1.4567	1.1334	-0.6133	-0.0041	0.9377	0.163
D	1.2973	-0.2515	-1.4357	0.0736	0.9102	0.234
E	2.4100	-0.3341	-1.4060	0.0875	0.8106	0.252

\* O: data in Table II; A,B,C,D,E: data in Table III.

and  $\Delta S$  depend on the natures of the two solutes. Therefore, the differences in their hydrophobicities and hydrophilicities will determine the separation efficiency. The bigger the differences, the easier the two compounds are separated.

For a given column and two solutes,  $\Delta \log k_w$  and  $\Delta S$  are constants. In this case, Equation 18 can be written as:

$$\log \alpha = \text{constant } 1 + \text{constant } 2 \times \phi \quad \text{Eq 22}$$

Equation 22 indicates that selectivity is dominated only by the characteristics of the mobile phase; the composition of eluents can be adjusted to improve the separation efficiency. Generally, Equation 18 provides a fundamental correlation between  $\alpha$  and properties of solutes and mobile phases that can be used to predict the separation effect and optimize the mobile phase system in RPLC.

## Conclusion

The relationships between  $\log k'$  and either molecular structure parameters of solutes or natures of eluents have been discussed in this paper. Based on systematic analysis of the retention of solute varying with the total solubility parameter and the composition of the mobile phase, we justified the linear correlation between  $\log k'$  and the volume fraction of methanol in binary mobile phase from the experiments.

To discuss the characteristics and physical meaning of slope and intercept ( $S$  and  $\log k_w$ , which were obtained from the plot of  $\log k'$  versus  $\phi$ ), a linear relationship between  $\log k_w$  and  $S$

was derived from regression analysis. On the basis of chromatographic thermodynamic theory and the assumption of the linear sum of free energy, we found that  $\log k_w$  and  $S$  depend on the properties of solutes, which relate not only to hydrophobicities but to hydrophilicities of solutes, and the nonlinear relationships between them can be expressed by the total solubility parameter  $\delta_T$  and the *n*-octanol–water partition coefficient  $\log P$  as described in Equations 14 and 15. From these two equations, the mixed retention mechanism was proven. The retention process of solutes was controlled by the hydrophobicity and hydrophilicity as well as their cooperative interactions. Experimental results showed that  $\log k_w$  and  $S$  are related to the structure parameters of solutes, though they play different roles in the solute retention process. Through the multi-regression analysis of a great deal of data, we achieved further confirmation of the above conclusions.

We also established a general retention equation, Equation 16, which depicts the quantitative relationship between  $\log k'$  and either the molecular structure parameters or the nature of the mobile phase, and we used the equation to successfully predict the  $\log k'$  values of substituted benzene derivatives on a Zorbax ODS column.

In addition, the comparison of the experimental and estimated  $\log P$  values of tested compounds proved that methods of calculating  $\log P_w$  with  $\log k_w$ ,  $S$ , and  $\delta_T$  by using Equations 14 and 15 are not only possible but more convenient than the shake-flask method. This exploration gives us a new way to calculate  $\log P$  values by using molecular parameters of compounds. Finally, the general relationship between the relative retention ( $\alpha$ ) and the affecting factors was derived from the concept of chromatographic selectivity, which was detailed in some situations.

**Table V. Predicted Values and Deviation of  $\log k_w$ ,  $\log P$ , and  $S$  of 18 Tested Solutes from Equations 14 and 15**

No.	Equation 14				Equation 15			
	$\log k_w$		$\log P$		$S$		$\log P$	
	Predicted	AD <sup>†</sup>	Predicted	AD <sup>†</sup>	Predicted	AD <sup>†</sup>	Predicted	AD <sup>†</sup>
1	1.226	-0.046	0.96	0.06	-2.184	-0.005	0.89	-0.01
2	1.399	0.030	1.06	-0.04	-2.243	-0.009	1.09	-0.01
3	1.936	0.156	1.69	-0.22	-2.895	-0.106	1.70	-0.21
4	1.735	-0.015	1.50	0.02	-2.483	0.035	1.53	0.05
5	2.017	-0.137	2.02	0.17	-2.784	0.073	1.96	0.11
6	2.360	0.014	2.09	-0.02	-2.936	-0.011	2.10	-0.01
7	2.551	-0.117	2.50	0.13	-3.150	0.147	2.56	0.19
8	2.476	-0.111	2.42	0.12	-3.095	0.131	2.47	0.17
9	2.409	0.090	2.04	-0.09	-2.947	-0.074	2.05	-0.08
10	3.040	0.089	2.74	-0.10	-3.548	-0.084	2.74	-0.10
11	2.997	0.092	2.60	-0.09	-3.452	-0.084	2.60	-0.09
12	3.139	-0.012	3.00	0.01	-3.654	0.008	3.00	0.01
13	3.511	0.027	3.12	-0.03	-3.908	0.000	3.15	0.00
14	4.121	-0.070	3.75	0.07	-4.469	0.123	3.80	0.12
15	3.726	-0.077	3.61	0.08	-4.157	0.085	3.63	0.10
16	3.686	-0.007	3.51	0.01	-4.124	-0.068	3.42	-0.08
17	3.561	0.096	3.10	-0.10	-3.955	-0.141	3.05	-0.15
18	3.439	-0.001	3.12	0.00	-3.857	-0.019	3.10	-0.02

<sup>†</sup> Absolute deviations.

Table VI. Predicted Values and log  $k'$  Deviation of 18 Tested Solutes from Equation 16

No.	Volume fraction of methanol ( $\phi$ )									
	50.0%		57.1%		60.8%		67.7%		79.2%	
	Predicted*	AD <sup>†</sup>	Predicted*	AD <sup>†</sup>	Predicted*	AD <sup>†</sup>	Predicted*	AD <sup>†</sup>	Predicted*	AD <sup>†</sup>
1	0.18	0.00	0.03	0.02	-0.05	-0.02	-0.20	0.00	-0.45	0.01
2	0.25	0.00	0.09	0.00	0.01	-0.01	-0.14	0.01	-0.40	0.00
3	0.39	-0.04	0.19	0.06	0.08	-0.01	-0.11	0.01	-0.43	0.02
4	0.49	0.00	0.31	0.00	0.22	-0.01	0.05	0.02	-0.24	0.00
5	0.73	0.01	0.52	-0.01	0.42	0.00	0.22	0.00	-0.11	0.00
6	0.88	0.00	0.68	0.00	0.57	0.00	0.37	0.01	0.03	0.00
7	1.02	0.00	0.79	0.00	0.66	0.00	0.44	0.01	0.06	0.00
8	0.97	0.02	0.74	-0.04	0.63	0.00	0.40	0.01	0.03	0.00
9	0.88	0.01	0.68	-0.01	0.57	-0.01	0.37	0.00	0.04	0.00
10	1.22	0.03	0.97	-0.04	0.84	-0.01	0.61	0.01	0.21	0.01
11	1.22	0.00	0.98	-0.01	0.86	0.01	0.62	0.00	0.24	0.00
12	1.32	0.01	1.06	-0.02	0.92	0.00	0.67	0.00	0.25	0.00
13	1.53	0.01	1.25	-0.02	1.11	0.00	0.84	0.01	0.39	0.00
14	1.90	0.01	1.57	-0.02	1.40	0.01	1.08	0.01	0.55	-0.01
15	1.68	0.00	1.38	-0.02	1.22	0.01	0.93	0.01	0.44	-0.01
16	1.67	0.00	1.38 <sup>‡</sup>		1.23	0.00	0.95	0.02	0.48	-0.01
17	1.56	0.00	1.29 <sup>‡</sup>		1.15	0.00	0.88	0.01	0.44	-0.01
18	1.52	-0.01	1.25 <sup>‡</sup>		1.11	0.01	0.84	0.01	0.40	-0.01

\* Predicted values of log  $k'$  from Equation 16.  
<sup>†</sup> Absolute deviations of experimental and predicted values of log  $k'$ .  
<sup>‡</sup> No experimental data.

## Acknowledgment

The authors thank the National Science Foundation of China for its financial support.

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Manuscript accepted April 10, 1996.