Hydrophobicity Parameters Determined by Reversed-Phase Liquid Chromatography. XIV.[1] Application of a New Hydrogen-accepting Scale of Monosubstituted Pyrazines to Analysis of the Relationship between Octanol–Water Partition Coefficients and Retention Factors Measured in Different Mobile Phases

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We recently proposed a new H-accepting scale, S_{HA}, for monosubstituted pyrazines, and demonstrated that this parameter works effectively in expressing the relationship between $\log P$ (P: 1-octanol/water partition coefficient) and $\log k'$ (k': retention factor derived from reversed phase liquid chromatography) with aqueous methanol solutions as the mobile phase, according to the equation: $\log k' = a \log P + \rho\sigma_I + sS_{HA} + \text{const.}$, where σ_I represents the electronic substituent constant. In this work, we have extended the same treatment to analysis of $\log k'$ measured in mobile phases containing different organic modifiers such as 1-propanol, acetonitrile, and dioxane, and found that the above equation is still useful. By comparing the correlations obtained, it was confirmed that the parameter S_{HA} could be universally utilized for representing the difference in H-bonding effects involved in different partitioning systems.

Key words H-bonding; H-accepting scale; hydrophobicity; partition coefficient; retention factor; reversed phase liquid chromatography

The hydrophobicity of molecules plays an important role in structure–activity relationship studies for various bioactive compounds, along with other physicochemical properties such as electronic and steric (including three-dimensional structure) factors.[2—4] In connection with recently developed high throughput screening techniques, the necessity has been increasing that such prospective parameters should be measured or predicted, not only accurately but also rapidly. To express hydrophobicity, the logarithm of the octanol/water partition coefficient, $\log P$, has been used[2,3] and large compilations[5] are available. As an alternative approach, the retention factor, $\log k'$, obtained by reversed phase liquid chromatography, RPLC, has been used as a convenient proxy for $\log P$. Extensive studies for predicting $\log P$ from $\log k'$ have been reported.[6—9] One of the difficulties in utilizing this RPLC method lies in interference from H-bonding between the solutes and the environmental medium that affects linearity between $\log P$ and $\log k'$.[6—9]

We have been systematically studying the relationship between $\log P$ and $\log k'$ for various series of heteroaromatic compounds under different RPLC conditions.[1,10—13] In our earlier work,[11] we analyzed the relationship between $\log P$ and $\log k'$ determined for monosubstituted diazines (pyrazines and pyrimidines) by using various combinations of stationary and mobile phases. These studies demonstrated that $\log k'$ can be described by Eq. 1.

$$\log k' = a \log P + \rho\sigma_I + h_{CO}HB_{CO} + h_AHB_A + h_{AM}HB_{AM} + \text{const.} \quad (1)$$

In Eq. 1, σ_I represents the inductive-electronic substituent constant[14] expressing the electronic effect of substituents on the hydrogen bonding capacity of the ring *N*-atom(s). The *HB* terms serve to correct for variation in H-bonding associated with substituents.[15] The parameters HB_{CO} and HB_A are indicator variables which take either the value 1 for strong H-accepting substituents with two sites (*e.g.* CO_2R and $CONMe_2$) and one site (*e.g.* CN and Ac) respectively, or 0 for non-H-bonders (*e.g.* H, alkyls, halogens, OR, SMe, and NMe_2); the parameter HB_{AM} is that for amphiprotic substituents.

Although Eq. 1 works well for each series, classification of substituents into an appropriate category has to be done arbitrarily; it is clearly desirable to seek an alternative, universal H-bonding parameter. The new H-acceptor scale, S_{HA}, which we have recently proposed,[1] appears to satisfy this need. The value of S_{HA} for each member of the (di)azine series is derived from heats of formation calculated by the COSMO method.[1] When this parameter is applied to analyses of the data used with Eq. 1, omitting the data for amphiprotic substituents, the results show that when the organic modifier in the eluent is methanol, the two *HB* terms, HB_{CO} and HB_A, can be replaced by the single term sS_{HA} (Eq. 2).

$$\log k' = a \log P + \rho\sigma_I + sS_{HA} + \text{const.} \quad (2)$$

Accordingly, we were interested in examining the possibility that Eq. 2 might be applied to other mobile phases in order to evaluate the validity of using the S_{HA} parameter generally as an H-acceptor scale. In this study, we have re-analyzed, by means of Eq. 2, the values of $\log k'$ that had been obtained for eluents containing a wide range of organic modifiers and previously analyzed by Eq. 1.

Materials and Methods

Compounds Compounds used in this work are the same as those used previously but excluding those containing amphiprotic substituents (see Table 2).[11]

$\log P$ and $\log k'$ Values The 1-octanol/water $\log P$ (Table 1) and $\log k'$ obtained with eluents other than aqueous methanol solutions are taken from our previous work unless otherwise noted.[11] The RPLC conditions used for obtaining $\log k'$ values were as follows:

Columns: C18 (CAPCELL PAK C18-AG or SG type, Shiseido), Ph (CAPCELL PAK Ph, Shiseido); the size of each column is 4.6×150 mm; packing materials of C18 and Ph columns are silicone-polymer coated silica gels chemically modified with octadecyl and phenyl groups, respectively.

Eluents: Organic modifiers used in this work are acetonitrile (A), dioxane

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Table 1. Physicochemical Parameters Used for Analyses by Eq. 2.

Substituent	$\log P$ [a]	S_{HA} [b]	σ_I [c]
H	−0.26	1.00	0.00
F	0.29	0.99	0.54
Cl	0.70	0.97	0.47
Me	0.21	0.96	−0.01
Et	0.69	0.91	−0.01
OMe	0.73	1.02	0.30
OEt	1.28	1.00	0.28
OPr	1.84	0.99	0.28
SMe	1.17	0.96	0.30
NMe_2	0.93	1.09	0.17
CN	−0.01	1.21	0.57
Ac	0.20	1.31	0.30
COOMe	−0.23	1.62	0.32
COOEt	0.28	1.60	0.30
$CONMe_2$	−0.80	1.78[c]	0.28

a) Taken from ref. 10. *b*) Taken from ref. 1. *c*) Taken from ref. 14.

Table 2. $\log k'$ Values for Monosubstituted Pyrazines.[a]

Substituent	M5[b]	M15	M30	M50	M70
H	0.443	0.102	−0.263	−0.590	−0.786
F	0.583	0.296	0.000	−0.317	−0.570
Cl	0.934	0.637	0.336	−0.068	−0.369
Me	0.949	0.474	0.031	−0.383	−0.671
Et	1.417	0.897	0.401	−0.084	−0.463
OMe	1.202	0.834	0.430	0.000	−0.341
OEt	1.730	1.318	0.850	0.329	−0.112
OPr	—[c]	1.846	1.317	0.685	0.140
SMe	1.547	1.153	0.694	0.205	−0.193
NMe_2	1.653	1.846	0.551	−0.021	−0.409
CN	0.493	0.177	−0.154	−0.481	−0.766
Ac	0.940	0.526	0.097	−0.296	−0.600
CO_2Me	0.921	0.441	−0.065	−0.514	−0.822
CO_2Et	1.456	0.917	0.352	−0.179	−0.574
$CONMe_2$	0.684	0.140	−0.386	−0.834	−1.096

a) Remeasured in this work. *b*) In 0.01 M phosphate buffer (pH 7.4). The figures after M represent the % volume of MeOH. *c*) The retention time was too long to be measured.

(D), 1-propanol (P) and trimethylene glycol (TMG). An appropriate composition of eluent was prepared by volume using a phosphate buffer solution (0.01 M, pH 7.4) as the aqueous component. As the retention times of the solutes eluted by the mobile phases mentioned above were shorter than those eluted by aqueous methanol solutions with the same percentage of water, the data could be obtained only for highly water-rich eluents. To compare the analytical results with those from aqueous methanol eluents under equivalent conditions, we remeasured $\log k'$ values with methanol–buffer (pH 7.4) mixtures containing 5, 15, 30, 50 and 70% MeOH (M5, M15, M30, M50 and M70, respectively), designated as the M-series. The newly obtained $\log k'$ values are given in Table 2.

Hydrogen Accepting Scale, S_{HA} Values for the H-accepting parameter, S_{HA}, are taken from our previous work.[1] The heat of formation, ΔH_f, of the minimum energy conformation of a given compound (Ar-X) was calculated for the gaseous phase and also for several dielectric (ε) environments with the AM1 Hamiltonian[16] and by using the eps command to perform the COSMO procedure[17] incorporated in the MOPAC 93 program package.[18] By plotting ΔH_f values for Ar-X, calculated at these ε values, against the corresponding ΔH_f values for the unsubstituted compound (Ar-H), a straight line was obtained; the slope of this line was defined as S_{HA} for the X substituent, $S_{HA} = \delta\Delta H_f(\text{Ar-X})/\delta\Delta H_f(\text{Ar-H})$.

Analyses for the Relationship between $\log P$ and $\log k'$ Regression analyses were performed as previously described by means of Eq. 2 using the parameters given in Table 1. The level of significance for the correlation and for each term was estimated by the F and t tests, respectively.

Results and Discussion

Correlations between $\log P$ and $\log k'$ under various RPLC conditions are summarized in Tables 3—5. For each set, two results are reported: (a) first, those from fitting the data to the equation $\log k' = a \log P + \text{const.}$; and (b) from fitting the data to Eq. 2. When inclusion of neither σ_I nor S_{HA} parameters improved the correlation, only the result in the case of (a) is given. Confidence intervals (95%) for the parameters are shown in parentheses for the statistically most significant correlation, designated "best correlation" in subsequent discussion. The results (Table 3) obtained for $\log k'$ from the M-series shown in Table 2 were essentially the same as those previously reported.[1] It can be clearly seen that the contributions of σ_I and S_{HA} terms increase with decreased methanol concentration: ρ becomes more negative and s more positive, confirming that H-bonding effects are more significant in more water-rich solvents.

Correlations for other organic modifiers measured on C18 and Ph columns are in Tables 4 and 5, respectively. Inspection of these tables indicates that the $\log k'$ values are also well expressed by Eq. 2 for each set regardless of the properties of the organic solvents. Here again, the contributions of the correction terms become more significant as the modifier concentration approaches zero, reaching their highest values at organic modifier concentrations of 5%. The excellent predictive ability of Eq. 2 is shown in Fig. 1, for 5% modifier-containing eluents with the Ph column, in comparison with that of the uncorrected equation.

By examining the coefficients of each term in the correlations thus obtained, we can evaluate the contributions provided by the components of Eq. 2. The most significant is the coefficient, a, of the $\log P$ term. In Fig. 2, the value of a at each mobile phase composition is plotted as a function of the volume fraction, f, of methanol (M-series) and acetonitrile (A-series). In each case, whereas values of a from the uncorrected equation provide no linear correlation against f (dotted line), those from the best correlations show good linear correlations (solid line), yielding Eqs. 3—6. It is particularly noteworthy that good linear correlation covers the full range of methanol concentrations in the M-series.

M-series

$$\text{C18:}\quad a = -0.783f + 1.006 \quad n=5,\ r=1.000,\ s=0.007 \tag{3}$$

$$\text{Ph:}\quad a = -0.823f + 0.898 \quad n=5,\ r=0.999,\ s=0.009 \tag{4}$$

A-series

$$\text{C18:}\quad a = -1.334f + 0.998 \quad n=3,\ r=1.000,\ s=0.003 \tag{5}$$

$$\text{Ph:}\quad a = -1.249f + 0.767 \quad n=3,\ r=0.997,\ s=0.017 \tag{6}$$

We have previously shown that $\log k'$ for pyrazines increases non-linearly with decreases in f. It follows that the good linearities indicated for Eqs. 3—6 must arise from relative changes in pure hydrophobicity ascribable to substituent effects. This demonstrates the validity of analyzing the chromatographic hydrophobicity ($\log k'$) in terms of an intrinsic factor reflecting $\log P$ and separable specific substituent-modified H-bonding effects by Eq. 2. The intercepts for Ph column are smaller than those for C18, consistent with the fact that the stationary phase bonded with phenyl groups is more polar than that with octadecyl hydrocarbon groups.

The dependence of a on f is greater for acetonitrile-containing eluents (Eqs. 5 and 6) than for methanolic eluents

Table 3. Analyses of $\log k'$ for Pyrazines Determined with Methanol as Organic Modifier by Using Eq. 2

Set	Mobile phase[a)]	Coefficient			Const.	n[b)]	r[c)]	s[d)]	F[e)]
		$\log P$	σ_I	S_{HA}					
				C18 column					
1	M5	0.588			0.851	14	0.790	0.279	19.9
		0.958 (0.088)[f)]	−0.888 (0.202)	1.135 (0.181)	−0.372 (0.232)	14	0.992	0.062	215.1
2	M15	0.673			0.410	15	0.925	0.195	77.5
		0.893 (0.038)	−0.543 (0.108)	0.832 (0.092)	−0.509 (0.117)	15	0.998	0.033	1033.0
3	M30	0.651			−0.025	15	0.971	0.112	218.0
		0.778 (0.042)	−0.226 (0.120)[g)]	0.481 (0.102)	−0.580 (0.130)	15	0.997	0.037	710.8
4	M50	0.564			−0.434	15	0.989	0.061	562.2
		0.614 (0.050)		0.187 (0.120)[h)]	−0.675 (0.157)	15	0.994	0.045	516.2
5	M70	0.454 (0.040)			−0.721 (0.032)	15	0.990	0.047	608.5
				Ph column					
6	M5	0.578			0.534	15	0.850	0.253	33.8
		0.849 (0.079)	−0.790 (0.225)	1.024 (0.192)	−0.563 (0.245)	15	0.991	0.069	204.9
7	M15	0.562			0.227	15	0.898	0.194	54.3
		0.774 (0.061)	−0.558 (0.174)	0.803 (0.149)	−0.650 (0.189)	15	0.994	0.054	291.3
8	M30	0.518			−0.085	15	0.943	0.129	105.1
		0.664 (0.045)	−0.282 (0.129)	0.550 (0.110)	−0.714 (0.140)	15	0.996	0.040	411.1
9	M50	0.413			−0.463	15	0.976	0.065	259.1
		0.484 (0.030)		0.266 (0.071)	−0.805 (0.093)	15	0.996	0.027	818.8
10	M70	0.295			−0.750	15	0.980	0.038	287.9
		0.318 (0.033)		0.123 (0.084)[i)]	−0.900 (0.105)	15	0.990	0.028	261.1

a) In 0.01 M phosphate buffer (pH 7.4). The figures after M represent the % volume of MeOH. *b*) Number of compounds used for correlations. *c*) Correlation coefficients. *d*) Standard deviations. *e*) Values of *F*-ratio between regression and residual variances. *f*) Figures in parentheses are 95% confidence intervals. The terms except the constant term are justified >99. 9% unless otherwise noted. *g*) Justified at the 99.8% by *t*-test. *h*) Justified at the 99.4% by *t*-test. *i*) Justified at the 99.1% by *t*-test.

Table 4. Analyses of $\log k'$ for Pyrazines with Various Mobile Phases on the C18 Column by Using Eq. 2

Set	Mobile phase[a)]	Coefficient			Const.	n[b)]	r[c)]	s[d)]	F[e)]
		$\log P$	σ_I	S_{HA}					
				C18 column					
11	A5	0.658			0.495	15	0.895	0.232	52.1
		0.933 (0.058)[f)]	−0.313 (0.165)[g)]	1.035 (0.141)	−0.749 (0.179)	15	0.996	0.051	450.8
12	A15	0.661			−0.053	15	0.957	0.142	139.9
		0.796 (0.062)	0.244 (0.177)[h)]	0.502 (0.152)	−0.766 (0.193)	15	0.995	0.055	341.7
13	A30	0.597			−0.438	15	0.952	0.135	126.3
		0.599 (0.058)	0.612 (0.210)		−0.608 (0.075)	15	0.989	0.067	274.0
14	D5	0.750			0.179	15	0.945	0.183	109.4
		0.960 (0.048)		0.786 (0.115)	−0.831 (0.151)	15	0.997	0.043	1092.0
15	D10	0.768			−0.070	15	0.969	0.137	202.6
		0.921 (0.052)		0.573 (0.125)	−0.807 (0.164)	15	0.997	0.047	919.5
16	P5	0.758			0.072	15	0.953	0.170	128.5
		0.867 (0.061)	−0.811 (0.174)	0.421 (0.149)	−0.244 (0.189)	15	0.996	0.053	474.9
17	P15	0.836			−0.502	15	0.976	0.133	257.2
		0.834 (0.089)	−0.452 (0.323)[i)]		−0.376 (0.115)	15	0.986	0.103	215.3

a) In 0.01 M phosphate buffer (pH 7.4). The figures after the symbol of organic modifier (A: acetonitrile, D: dioxane, P: 1-propanol) represent the % volume of the modifier. *b*—*e*) See the footnotes in Table 3. *f*) Figures in parentheses are 95% confidence intervals. The terms except the constant term are justified >99.9% unless otherwise noted. *g*) Justified at the 99.8% by *t*-test. *h*) Justified at the 98.8% by *t*-test. *i*) Justified at the 98.9% by *t*-test.

Table 5. Analyses of $\log k'$ for Pyrazines with Various Mobile Phases on the Ph Column by Using Eq. 2

Set	Mobile phase[a)]	Coefficients			Const.	$n^{b)}$	$r^{c)}$	$s^{d)}$	$F^{e)}$
		$\log P$	σ_I	S_{HA}					
				Ph column					
18	A5	0.501			0.119	15	0.879	0.192	44.1
		0.713	−0.497	0.802	−0.775	15	0.991	0.060	191.2
		(0.068)[f)]	(0.195)	(0.167)	(0.212)				
19	A15	0.452			−0.245	15	0.950	0.106	118.9
		0.566		0.427	−0.794	15	0.992	0.044	366.8
		(0.049)		(0.119)	(0.155)				
20	A30	0.396			−0.577	15	0.962	0.079	163.1
		0.398	0.338		−0.671	15	0.988	0.046	254.1
		(0.039)	(0.143)		(0.051)				
21	D5	0.549			−0.017	15	0.911	0.176	
		0.743	−0.446	0.734	−0.837	15	0.993	0.056	247.2
		(0.064)	(0.182)	(0.155)	(0.198)				
22	D10	0.536			−0.206	15	0.947	0.128	
		0.681	−0.243	0.547	−0.842	15	0.995	0.044	357.6
		(0.050)	(0.142)[g)]	(0.122)	(0.155)				
23	D15	0.524			−0.346	15	0.968	0.095	195.6
		0.626		0.384	−0.840	15	0.995	0.041	562.3
		(0.045)		(0.109)	(0.143)				
24	P5	0.555			−0.061	15	0.911	0.178	63.3
		0.702	−0.746	0.562	−0.576	15	0.991	0.063	201.7
		(0.071)	(0.203)	(0.174)	(0.221)				
25	P15	0.586			−0.427	15	0.976	0.092	264.6
		0.635	−0.434	0.190	−0.551	15	0.997	0.038	531.2
		(0.043)	(0.124)	(0.106)[h)]	(0.135)				
26	TMG5	0.524			0.196	15	0.903	0.176	57.7
		0.650	−0.771	0.480	−0.206	15	0.986	0.074	129.8
		(0.084)	(0.240)	(0.205)	(0.261)				
27	TMG10	0.527			0.051	15	0.934	0.143	88.2
		0.630	−0.623	0.395	−0.283	15	0.990	0.060	187.8
		(0.068)	(0.195)	(0.167)	(0.212)				

a) The figures after the symbol of organic modifier (A: acetonitrile, D: dioxane, P: 1-propanol, TMG: trimethylene glycol) represent the % volume of the modifier. *b*—*e*) See the footnotes in Table 3. *f*) Figures in parentheses are 95% confidence intervals. The terms except the constant term are justified >99.9% unless otherwise noted. *g*) Justified at the 99.6% by *t*-test. *h*) Justified at the 98.7% by *t*-test.

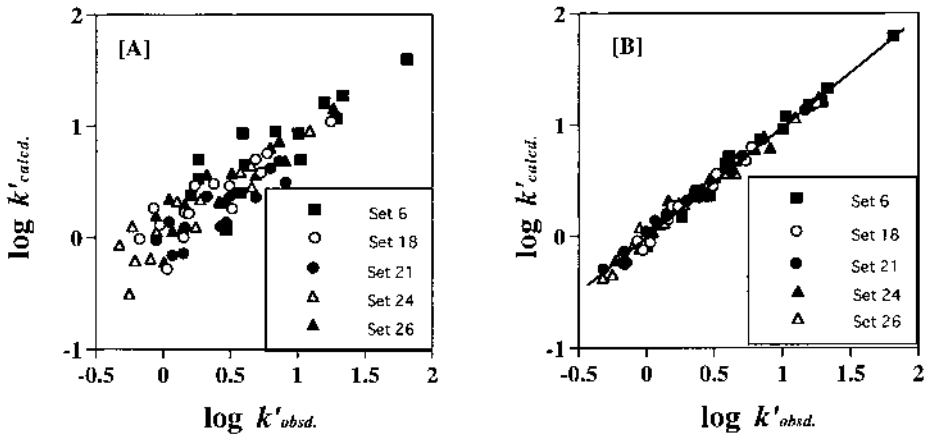

Fig. 1. The Predictive Power of Eq. 2 for $\log k'$ Values of Monosubstituted Pyrazines with Eluents Containing 5% Organic Modifiers on the Ph Column
[A] $\log k'$ values calculated by the uncorrected equation: $\log k = a \log P + \text{const.}$ [B] $\log k'$ values calculated by Eq. 2.

(Eqs. 3 and 4). Addition of aprotic acetonitrile to water by changing the volume fraction will exert a more drastic effect on properties of the resulting eluent compared to addition of protic methanol to water. As pyrazines have polar aza functions in the parent nucleus, their $\log k'$ should be more dependent on the characteristics of the mobile phase and hence vary more widely in aqueous acetonitrile than in aqueous methanol. This concept seems to be justified by finding that preliminary study of a series of less polar monosubstituted benzenes showed much smaller and closer dependence of a on f with gradients of −0.60 and −0.66 for the M- and A-eluent series, respectively.[19)]

In the preceding discussion, we have shown that our S_{HA} parameter can effectively work as an H-acceptor scale for substituents so as to separate the overall $\log k'$ value into hydrophobic and H-bonding components. Recently, we have

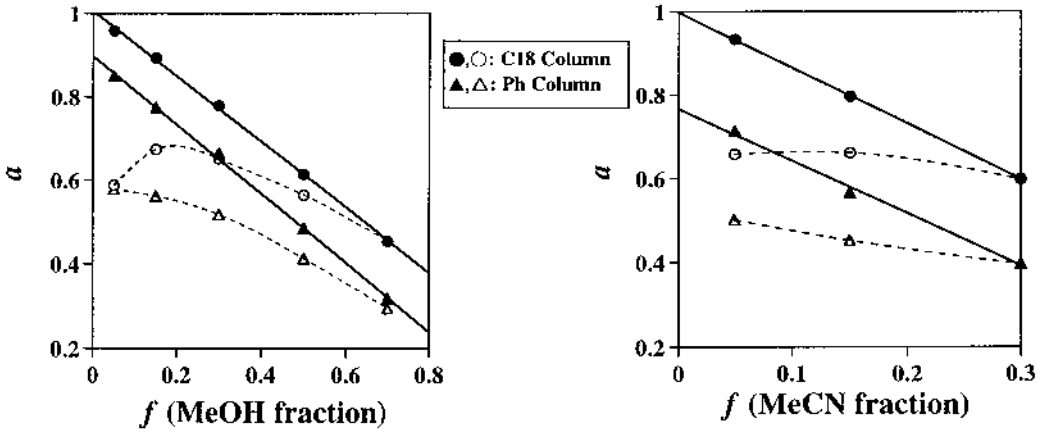

Fig. 2. Plots of *a* Values against Volume Fraction, *f*, of Methanol and Acetonitrile in Eluents

The solid symbols represent *a* values for the best correlations. The hollow symbols represent *a* values derived from the equaion; $\log k = a \log P + \text{const.}$

also succeeded in utilizing S_{HA} to correlate $\log P_{oct}$ (P_{oct}: octanol/water partition coefficient) and $\log P_{CL}$ (P_{CL}: chloroform/water partition coefficient) for monosubstituted (di)azines and presented the rationale as an H-bond acceptor scale.[20] For each series of pyridines, pyrazines and pyrimidines, the values for $\log P_{CL}$ are formulated with high precision by Eq. 7.[20]

$$\log P_{CL} = a \log P_{oct} + sS_{HA} + \text{const.} \quad (7)$$

In an earlier study, we had analyzed the same data set by using the indicator variable *HB* according to Eq. 8,[21]

$$\log P_{CL} = a \log P_{oct} + hHB + \text{const.} \quad (8)$$

where $HB = 0$, 1 or 2 depending on the H-bonding ability of the substituent. Although analysis according to Eq. 8 yielded very good statistical correlations, and the physical interpretation of the discrete H-bond parameter, *HB*, could be explained rationally,[21] classification of compounds into their appropriate groups could still be achieved only on the basis of trial and error. In contrast, use of the S_{HA} parameter removes the necessity to employ this intellectually unsatisfactory and arbitrary procedure.

The present results, demonstrating that the use of the S_{HA} parameter can be applied to a variety of solvent systems, may provide confirmation that S_{HA} can be universally utilized to represent the difference in H-bonding effects involved in various combinations of two different partitioning systems. The solutes so far studied were limited to within a series of congeners of similar structure. A study of S_{HA} for a wide range of aromatic H-accepting solutes is now under way.

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