Solubility Measurements. Saturated solutions of compounds 1–22 in H_2O and pH 7.4 phosphate buffer were prepared by magnetically stirring the initial suspensions for 3 h at 25 °C. The resulting solutions were clarified by passage through an MF Millipore HA 45 μm filter. Concentrations were measured by HPLC on a Waters C-18 column (H_2O–acetonitrile 5–99% gradient, 210 nm detection) and were calibrated versus a water–acetonitrile solution (1:1) of known concentration.

Partition coefficients were determined by agitating the test compounds at 25 °C with a 1:1 mixture of 1-octanol/sodium phosphate–citric acid buffer, pH 7.4, on a flatbed shaker for 45 min. The resulting suspensions were clarified by centrifugation at 2000 rpm for 5 min. Concentrations of aliquots from both phases were measured by HPLC (vide supra).

Biological Methods. Human and dog plasma renin assays were performed by radioimmunoassay for angiotensin I as described by Haber et al.[15] with use of a commercial kit (Clinical Assays), at pH 7.4 (phosphate buffer), 37 °C. Pooled plasma samples were collected on ice in EDTA from mongrel dogs. Lyophilized human plasma was obtained from Ortho Diagnostics (low renin) or Clinical Assays (high renin). IC_{50} values were obtained as previously described.[16]

Porcine kidney renin was assayed as described by Rich et al.[17] by using [^{14}C]Leu-labeled decapeptide substrate, except the pH was raised to 7.3 by use of 0.05 M citrate–phosphate buffer, 30 °C. IC_{50} values were determined by linear regression of logit vs log concentration over a 15–90% inhibition range, with use of three to six concentrations, and were found to be reproducible within ±20%.

The oral dog studies were carried out as follows: Conditioned female mongrel dogs (14–17 kg) were prepared with chronically indwelling catheters in the femoral artery. The catheters were tunneled subcutaneously to exit between the shoulders. When not in use, the catheters were filled with heparinized (40 units/mL) 5% dextrose solution. The dogs were maintained on a low sodium diet (10 mmol/day) and received furosemide (5 mg/kg, po) twice during the first week of low sodium intake and another 5 mg/kg po the evening prior to an experiment. Water was provided ad libitum except during the experiment, but food was removed 18 h prior to any measurements being made. The arterial catheter was used to monitor mean pressure and for collection of 3-mL blood samples for determination of plasma renin activity (PRA). Blood samples were collected in chilled tubes containing EDTA (final concentration 4 mM), centrifuged at 4 °C, and stored frozen until assayed for PRA by radioimmunoassay of generated angiotensin 1.

The conscious dogs were trained to stand quietly in a modified Pavlov sling during the course of the experiment. Compound **12** was administered by gavage in olive oil or as a suspension in 5% acetic acid, 5% dextrose solution. Blood pressure was recorded continuously except during collection of blood samples.

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Registry No. **1**, 87063-27-2; **2**, 105192-85-6; **3**, 109585-11-7; **4**, 87691-49-4; **5**, 87691-50-7; 6, 105192-86-7; **7**, 100902-03-2; **8**, 100901-99-3; 9, 105192-87-8; **10**, 105192-89-0; **10** (free base), 105192-88-9; **11**, 100901-98-2; **12**, 100902-07-6; **13**, 105228-98-6; **13** (free base), 98858-48-1; **14**, 105228-99-7; **14** (free base), 100902-08-7; **15** (diastereomer 1), 115511-03-0; **15** (diastereomer 2), 115511-04-1; **16**, 115511-05-2; **18**, 115511-06-3; 19, 100902-05-4; **20**, 100902-09-8; **21**, 100902-06-5; **22**, 87691-52-9; AMBA, 1477-55-0; BOC-Phe-His-(3*S*,4*S*)-Sta-Leu-OCH_3, 87700-33-2; BOC-Phe-His-(3*S*,4*S*)-ACHPA-Leu-OCH_3, 115464-34-1; $PhCH_2NH_2$, 100-46-9; $H_2N(CH_2)_2NH_2$, 107-15-3; (±)-$H_2NCH(CO_2H)CH_2C_6H_4CH_2NH_2$, 115511-07-4; $H_2N(CH_2)_2CH(NH_2)CO_2H$, 305-62-4; *m*-$H_2NCH_2C_6H_4OCH_2CO_2H$, 115464-36-3; $H_2N(CH_2)_2SO_3H$, 107-35-7; $H_2N(CH_2)_3N(CH_2CH_2OH)_2$, 4985-85-7; renin, 9015-94-5; 2-pyridinecarboxaldehyde, 1121-60-4; diethyl phosphorocyanidate, 2942-58-7; 3,5-dimethylpyrazole-1-carboxyamidine nitrate, 38184-47-3; D-phenylglycine, 875-74-1; benzylpiperazine, 2759-28-6; 2-(aminomethyl)pyridine, 3731-51-9; 4-(aminomethyl)pyridine, 3731-53-1; 2-(1,2-diaminoethyl)pyridine, 115464-35-2.

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A Comprehensive Method for Determining Hydrophobicity Constants by Reversed-Phase High-Performance Liquid Chromatography

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The development of a method for determining hydrophobicity constants for small, organic molecules by reversed-phase liquid chromatography (RPLC) is presented. The method uses capacity ratios measured at a number of different compositions of methanol to obtain derived values, denoted log k'_w, upon which a new scale of hydrophobicity constants can be developed. This scale eliminates potential problems such as peak inversion that hamper RPLC methods using isocratic data to estimate hydrophobicity. The differential hydrogen bond effect observed in most correlations of RPLC data with logarithms of octanol–water partition coefficients (log $P_{o/w}$) for compounds of opposite net hydrogen bonding capabilities (noncongeners) was minimized by adding trace quantities of *n*-decylamine and 1-octanol to the eluent and using an octyl-modified silica gel stationary phase. Values of log k'_w are shown to be largely column-independent as long as the hydrophobic properties of columns are similar. The correlation of log k'_w values with the logarithms of bovine serum albumin binding constants (log $1/C$) is shown to be statistically indistinguishable from the correlation of log $1/C$ with log $P_{o/w}$, indicating that this data models log $1/C$ as well as log $P_{o/w}$ for these compounds. Additionally, the chromatographic system is automatable and thus capable of higher sample throughput than measurements of log $P_{o/w}$ by the shake-flask method.

Reversed-phase liquid chromatography (RPLC) has been touted widely in recent years as a rapid, precise means for estimating physicochemical properties of organic molecules, particularly octanol–water partition coefficients (log $P_{o/w}$), which are used extensively in correlations with other properties such as biological activity (log $1/C$). Many of the early RPLC methods used capacity ratios measured by monocratic methods to estimate log $P_{o/w}$. According

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to these methods, capacity ratios (denoted k'_ϕ, where ϕ = volume fraction of organic modifier in the eluent) are determined at a single eluent composition for all of the compounds in a series. Log k'_ϕ values are then correlated with log $P_{o/w}$ or log $1/C$ by a Collander-type equation:[1]

$$\log P_{o/w} = a \log k' + b \qquad (1)$$

However, an increasing number of papers report the use of retention data measured by polycratic methods to derive parameters that are correlated with log $P_{o/w}$ or log $1/C$.[1,2] According to these methods, capacity ratios are measured for each compound at a number of different eluent compositions and regression analysis of the linear portion of the log k'_ϕ–ϕ data used to obtain values of the slope (S) and intercept (log k'_w) describing each log k'_ϕ–ϕ curve. Either or both of these variables are then correlated with log $P_{o/w}$ or log $1/C$ by using eq 1. References to methods based upon these approaches can be found in three recent reviews.[2-4]

For many RPLC methods, correlations with log $P_{o/w}$ were improved by separating compounds into classes according to hydrogen bonding properties (neutrals, hydrogen bond acceptors, and hydrogen bond donors).[5-9] Members of each class are referred to as congeners. These results demonstrate that most of these methods are more sensitive to hydrogen-bonding differences between noncongeners than the octanol–water system (this behavior is completely analogous to that observed when octanol–water partition coefficients are correlated with alkane–water partition coefficients[10]). As most RPLC methods use alkyl-bonded stationary phases, this type of discrimination is not unexpected. Indeed, chromatographic systems that best modeled octanol–water partitioning employed octanol-saturated alkyl stationary phases and 100% aqueous eluents saturated with octanol.[7,11] These methods have been critized though because of problems associated with the use of octanol and a pure aqueous eluent[12] and thus have not been widely adopted.

Another attempt has utilized multiple regression to resolve the problem of modeling the octanol–water system by RPLC.[13] This polycratic method uses acetonitrile as the organic modifier instead of methanol, and S and log k'_w are both correlated with log $P_{o/w}$ according to eq 2.

$$\log P_{o/w} = a \log k'_w + bS + c \qquad (2)$$

When acetonitrile is used, S and log k'_w have been shown to vary independently with analyte structure,[13,14] and neither correlates independently with log $P_{o/w}$. A potential weakness in methods using acetonitrile as the organic modifier is found in the log k'_ϕ–ϕ_{ACN} data used to derive log k'_w and S. The curvature is usually severe, making assignment of the linear portion of these graphs more subjective and increasing the uncertainty in S and log k'_w. In contrast, when methanol is used as the modifier S and log k'_w have been shown to be highly dependent variables,[1,14-17] allowing either of these parameters in principle to be used to predict log $P_{o/w}$. Also, the curvature in log k'_ϕ–ϕ_{MeOH} data is significantly less and should allow values of log k'_w and S to be determined more accurately.

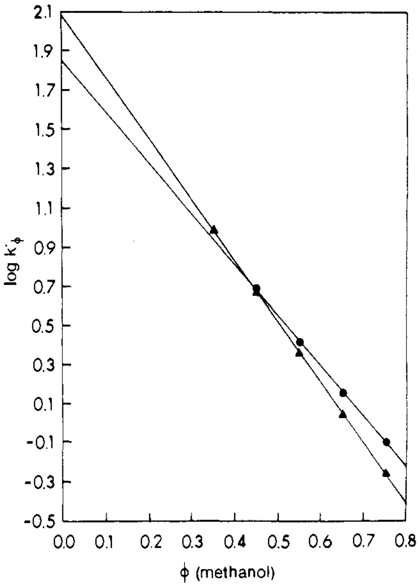

Figure 1. Graph illustrating peak inversion. The logarithm of the capacity ratios (log $k'(\phi)$) measured at various volume fractions of methanol (ϕ) are shown for anisole (●) and 3-(trifluoromethyl)phenol (▲). This data illustrates that the order of hydrophobicities defined by the octanol–water partition coefficients for these compounds would have been incorrectly predicted if they had been estimated by a monocratic method at a volume fraction of methanol greater than approximately 0.40.

In 1983, Brent et al. published a monocratic method for estimating hydrophobicity constants.[12] In practice, a number of problems were encountered with this method that are typical of monocratic methods. First, the intrinsic range of hydrophobicities present in a series was often found to be wider than the dynamic range capability of the column at the methanol composition chosen for the analysis. Compounds unretained or retained too long under the selected set of conditions had to be measured with different column lengths and/or eluent conditions, and this retention data cross-correlated to the original set of conditions. The retention times of many hydrophobic compounds were often found to be excessively long, requiring extended analysis times. In addition to the work required to overcome these problems, the impracticality of this type of method was exacerbated by two additional problems. The first problem involves the possibility of peak inversion, which arises when modifier conditions are changed. Problems associated with peak inversion have been reported,[1,18,19] and may lead to misassignments in

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relative hydrophobicities between compounds. This behavior is illustrated in Figure 1, where the elution order for two compounds are shown to invert at eluent compositions below $\phi_{\mathrm{MeOH}} \sim 0.40$; the order of relative hydrophobicities for these compounds as predicted from their octanol–water partition coefficients is correctly predicted on the basis of retention at $\phi_{\mathrm{MeOH}} < 0.4$. Albeit the errors arising from peak inversion are in all likelihood small, this factor nonetheless contributes to the overall uncertainty in this methodology. The second problem involved the inability to compare directly values of log k'_ϕ. This problem arose because values for different series were measured under different eluent conditions. Although the data measured under the same eluent composition represented a self-consistent data set, the comparison of different data sets was of limited use. Without the ability to directly compare log k' results, the establishment of general data bases of hydrophobicity constants and RPLC fragment constants similar to those developed from log $P_{\mathrm{o/w}}$ data was greatly complicated. In light of these problems, the utility of this method was considered too limited, and the development of an alternative method undertaken.

An investigation of the latest methods reported in the literature indicated that many researchers were turning to polycratic methods to predict log $P_{\mathrm{o/w}}$ values from RPLC retention data. This approach has been justified theoretically by Martire and Boehm in their analysis of retention in RPLC.[20] Other researchers have reported better correlations with log $P_{\mathrm{o/w}}$ than capacity ratios measured by monocratic methods.[17,21–23] Additionally, for solutes that are partially or fully ionized under mobile phase conditions, polycratic methods offer the possibility of correcting log k'_{w} values for ionization by using the pH measured for the aqueous component of the eluent and pK_{a} values measured in water, eliminating problems associated with nonaqueous pH measurements.[12,24] Polycratic methods also allow the dynamic range of the column to be utilized more effectively than monocratic experiments, which can become tedious when a series of solutes with a wide range of hydrophobicities is analyzed. Finally, polycratic methods provide a scale of hydrophobicity constants normalized to one set of conditions (100% aqueous), affording easier establishment of data bases. The potential for establishing general data bases that could be shared between laboratories was demonstrated by Braumann.[2] In Table IV of that article, log k'_{w} values were reported for approximately 70 compounds from seven references in the literature. In all cases, polycratic methods based upon similar conditions (methanol/aqueous eluents, low ionic strength, pH ~neutral) were used to derive the values of log k'_{w}. The standard deviations of the averaged values were remarkably low (the largest was ±0.21 for *n*-butylbenzene (log $k'_{\mathrm{w}} = 4.60$)), leading Braumann to conclude that the small variations in log k'_{w} values were evidence that these constants could be used as a priori hydrophobicity parameters.

Recently, Minick et al. reported the results of correlations of RPLC retention data from monocratic and polycratic methods with the logarithms of octanol–water partition coefficients (log $P_{\mathrm{o/w}}$) and the logarithms of bovine serum albumin binding constants (log $1/C_{\mathrm{BSA}}$) for a set of 34 model compounds.[1] That set of solutes was selected because log $P_{\mathrm{o/w}}$ and log $1/C_{\mathrm{BSA}}$ values were measured by one laboratory and were considered to be reliable data. Also, the compounds form a set of noncongeners. The findings from that study indicated that all of the RPLC conditions examined should be rejected on statistical grounds as models for BSA binding when compared to log $P_{\mathrm{o/w}}$.

Table I. List of Model Compounds Classified According to Hydrogen-Bonding Capability

donors[a]	acceptors[a]	miscellaneous[b]
phenol	3-nitrobenzonitrile	3-fluoroaniline
3-fluorophenol	anisole	4-chloroaniline
4-fluorophenol	benzonitrile	4-methoxyaniline
3-chlorophenol	acetophenone	4-bromoaniline
4-chlorophenol	nitrobenzene	4-methylaniline
4-bromophenol	camphorquinone	1-naphthylamine
4-iodophenol	4-nitroanisole	indole
4-methylphenol	4-chloronitrobenzene	4-methoxybenzyl alcohol
3-ethylphenol	naphthalene	
3-(trifluoromethyl)phenol	azobenzene	
3-cyanophenol		
3-hydroxyphenol		
3-methoxyphenol		
4-methoxyphenol		
thymol		
4-bromoacetanilide		

[a] Compounds were classified as hydrogen-bond donors or acceptors on the basis of the classifications proposed by Leo.[10] [b] The anilines (including naphthylamine and indole) were not classified according to net hydrogen bonding because they behaved as either acceptors or donors depending upon the type and position of the additional functional group present in the structure; 4-methoxybenzyl alcohol was included here because it contains both a donor and an acceptor functional group and was generally observed between the lines defined by the phenols (donors) and pure acceptors.

The results of further investigations in this area are reported here. The same set of compounds used in the earier study were also used here and are listed in Table I. All methods reported in this paper are polycratic. Mobile and stationary phase conditions were varied to optimize the correlation of log k'_{w} with log $P_{\mathrm{o/w}}$. The spectroscopic properties of Reichardt's dye ($E_{\mathrm{T}}(30)$, a solvent polarity probe) were used to characterize changes in eluent properties that accompany changes in composition. These solvent polarity values were used as an alternate variable in correlations with log k'_ϕ. Different procedures for estimating the column dead-time were examined, and the impact of these estimates upon values of log k'_{w} and S are reported. The slope parameter obtained from the linear model is discussed as an alternative to log k'_{w}. Evidence is presented suggesting that log k'_{w} values represent an a priori hydrophobicity scale and therefore do not rely upon a secondary reference system such as log $P_{\mathrm{o/w}}$ for predicting hydrophobicity constants of nonionic solutes. Additionally, a quadratic model used to obtain values of log k'_{w} is examined and rejected as an alternative to linear extrapolation of log k'_ϕ–ϕ_{MeOH} data. The need to cross-correlate log k'_{w} values obtained from different columns is discussed. Finally, log k'_{w} values obtained from the best set of experimental conditions described here are shown to predict log $1/C_{\mathrm{BSA}}$ as well as log $P_{\mathrm{o/w}}$ does. On the basis of these considerations, an RPLC method is proposed that minimizes the differential hydrogen bond effect present in most polycratic methods reported in the

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literature and thus accurately models the octanol–water system.

Theoretical Section

Extrapolation Approach. Unlike monocratic methods, where measured capacity ratios are used in correlations with log $P_{o/w}$ and/or drug activity, the extrapolation approach yields parameters derived by regression of polycratic measurements. Two extrapolation procedures have been discussed in the literature and were examined. The first is an empirical relationship using linear extrapolation to obtain hydrophobicity constants and is shown in eq 3. where ϕ_{MeOH} is the volume fraction of methanol

$$\log k'_{\phi} = \log k'_{w} - S\phi_{MeOH} \qquad (3)$$

in the eluent and log $k_{\prime\phi}$ is the logarithm of the capacity ratio measured at that modifier composition. The intercept (log k'_{w}) represents the retention of the solute under hypothetical chromatographic conditions.[2] While less attention in the literature has focused on the physical interpretation and use of S in eq 3, some researchers have postulated that this parameter is proportional to the number of mobile phase solvent molecules in the solvation sphere of the solute,[25,26] making S a function of the size of the solute and the type(s) and total number of polar substituents present in the solute structure. As mentioned earlier, S and log k'_{w} are highly correlatable for solutes of diverse structural types in methanolic eluents, suggesting that these parameters represent similar properties of solutes under these conditions.

When methanol is used as the organic modifier in a polycratic method, values of log k'_{ϕ} for many eluites are found to vary linearly with ϕ_{MeOH} over the range $0.2 \leq \phi_{MeOH} \leq 0.8$. Although no complete explanation has yet been presented to describe the exact nature of the variation of log k'_{ϕ} with ϕ_{MeOH}, the variation of solute retention with ϕ_{MeOH} within this range would be expected to be approximately linear since many of the properties of the mobile phase and stationary phase that are relevant to retention in RPLC also vary linearly within this region. According to the solvophobic theory,[27] the surface tension of the eluent was shown to be a primary factor in retention in RPLC. For binary solutions of methanol–water, the variation of surface tension with ϕ_{MeOH} within the region $0.2 \leq \phi_{MeOH} \leq 0.8$ is approximately linear.[28] Also, adsorption isotherms reported for RPLC systems using binary mixtures of methanol and water indicate that the compositions of alkyl-bonded stationary phases remain fairly constant within this range.[29-31]

The second extrapolation approach involves fitting a quadratic equation to log k'_{ϕ} vs ϕ_{MeOH} data. This expression is shown in eq 4. Unlike the linear approach,

$$\log k'_{\phi} = a_1(\phi_{MeOH})^2 + a_2(\phi_{MeOH}) + \log k'_{w} \qquad (4)$$

which is empirical, attempts have been made to justify the use of a quadratic equation on theoretical grounds. Martire and Boehm[20] and Dill[32] using lattice models, and Schoenmaker et al.[33] using an extension of Hildebrand's solubility parameter theory derived equations suggesting that the relationship between log $k_{\prime\phi}$ and ϕ_{MeOH} was quadratic. In the work reported here, both eq 3 and 4 are treated as phenomenological models that can be used to estimate values of log k'_{w} by extrapolating beyond the range of measured data.

Determination of the Column Dead-Time. The capacity ratio as a function of either retention time or retention volume is defined in eq 5 where $t_R(\phi)$ is the re-

$$k'(\phi) = t_R(\phi)/t_o - 1 \qquad (5)$$

tention time of a solute at volume fraction ϕ of methanol in the eluent and t_o is the column dead-time. A number of methods have been proposed for measuring t_o, and this subject has been discussed extensively in the literature.[3,30,34-38] Three procedures were investigated to determine the impact of different estimates of this parameter upon the linearity of log k'_{ϕ}–ϕ_{MeOH} data and also upon the values of log k'_{w} derived from this data.

The first procedure was an isotope method based upon the retention time of deuterium oxide (D_2O).[30] The retention time for this probe was measured with a pure aqueous eluent, and capacity ratios are calculated according to eq 5. The retention volume of D_2O measured

$$k'_{\phi} = t_R(\phi)/t_{urea}(\phi) - 1 \qquad (6)$$

under these conditions has been shown to be equivalent to the maximum column volume determined from density measurements.[30,37] In the second procedure, the column dead-time was estimated at each eluent composition from the retention time of urea (denoted $t_{urea}(\phi)$, where ϕ = volume fraction of methanol). Urea has been suggested as a simple eluite for probing the minimum species retention volume observed at various mobile phase compositions.[37] Column dead-times estimated by this probe may reflect more precisely properties of the chromatographic system, which can change as the composition of the eluent is varied. These properties include the mobile phase space within the column, which can vary as a result of solvation/desolvation or partial collapse of the stationary phase, and changes in the bulk properties of the eluent that might affect the movement of bands through the column. The dead-time measured at each ϕ_{MeOH} was then to calculate capacity ratios according to eq 6. The third method used to estimate the column dead-time was the simplified procedure recommended by Knox and Kaliszan.[34] Here, the retention volume of CD_3OD was measured with pure methanol as the eluent; this dead-time has been shown to be porportional to the maximum column dead-volume determined from density measurements.[34,37] This approach should yield more reliable estimates of the maximum mobile phase space within different columns than the D_2O method, where collapse of the stationary phase under pure aqueous conditions could result in the exclusion

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Table II. Experimental Conditions Examined[a]

Experiment	Columns Investigated					Mobile phase components					
	Waters	Brownlee		ES Ind.		ORGANIC PHASE	AQUEOUS PHASE				
	C18	C18	C8	C18	C8	octanol (0.25%)	MOPS (0.02 M)	phosphate (0.005 M)	decylamine (0.15%)	diethylamine (0.075%)	OctOH sat'd water
1	✓						✓				
2	✓							✓			
3		✓				✓	✓				
4			✓			✓	✓				
5		✓				✓	✓		✓		
6			✓			✓	✓		✓		
7		✓				✓	✓			✓	
8			✓			✓	✓			✓	
9				✓		✓	✓		✓		
10					✓	✓	✓		✓		
11					✓	✓	✓		✓		✓
12					✓	✓	✓		✓		✓

[a] For all experiments, flow rate = 2.0 mL/min; wavelength = 220 nm; absorbance = 0.2 aufs; the pH of all aqueous phases were adjusted to 7.4.

Table III. Correlation of Octanol–Water Partition Coefficients with Linearly Derived Capacity Ratios (log k'_w)

$$\log P_{o/w} = a \log k'_w + b$$

eq no.	a^a	b^a	n	r^a	SD[a]
1	1.060 (0.058)	0.105 (0.111)	34	0.955	0.212
2	1.040 (0.054)	0.098 (0.104)	34	0.960	0.200
3	0.791 (0.049)	0.970 (0.077)	33[b]	0.946	0.224
4	0.854 (0.034)	0.729 (0.057)	34	0.976	0.155
5	0.863 (0.034)	0.743 (0056)	34	0.976	0.155
6	0.845 (0.027)	0.783 (0.045)	34	0.984	0.128
7	0.829 (0.041)	0.900 (0.065)	33[b]	0.962	0.194
8	0.837 (0.033)	0.755 (0.057)	34	0.975	0.158
9	1.031 (0.046)	0.467 (0.075)	34	0.970	0.175
10	0.852 (0.025)	0.374 (0.053)	34	0.986	0.119

[a] a = slope, b = intercept, r = correlation coefficient, SD = overal standard deviation of regression; the standard deviations in the slopes and interecepts at the 95% confidence limit are shown in parentheses. [b] 3-Hydroxyphenol was unretained under experimental conditions.

Table IV. Correlation of Octanol–Water Partition Coefficients with the Slope Parameter (S)

$$\log P_{o/w} = aS + b$$

eq no.	a^a	b^a	n	r^a	SD[a]
1	1.332 (0.076)	−1.906 (0.226)	34	0.952	0.219
2	1.177 (0.082)	−1.505 (0.251)	34	0.930	0.263
3	0.893 (0.040)	0.042 (0.095)	33[b]	0.970	0.167
4	0.811 (0.030)	0.029 (0.078)	34	0.979	0.147
5	0.962 (0.050)	−0.525 (0.136)	34	0.960	0.201
6	0.844 (0.035)	−0.131 (0.094)	34	0.973	0.164
7	1.081 (0.080)	−1.068 (0.235)	33[b]	0.924	0.264
8	0.827 (0.035)	−0.183 (0.096)	34	0.973	0.164
9	1.018 (0.032)	−0.167 (0.073)	34	0.984	0.127
10	0.815 (0.016)	0.014 (0.042)	34	0.994	0.080

[a] a = slope, b = intercept, r = correlation coefficient, SD = overall standard deviation of regression; the standard deviations in the slopes and intercepts at the 95% confidence limit are shown in parentheses. [b] 3-Hydroxyphenol was unretained under experimental conditions.

of D_2O from some of the pore volume.

$E_T(30)$ Experiments. Reichardt's dye, or $E_T(30)$, exhibits a large solvatochromic shift and is reported to be quite sensitive to both solvent dipolarity/polarizability and hydrogen bonding interactions.[42] The $E_T(30)$ polarity values for a solution are calculated in energy units according to eq 7 where the constant is the product of

$$E_T(30)(\text{kcal/mol}) = 28593/\lambda_{\max} \qquad (7)$$

Avogadro's number, the speed of light, and Planck's constant, and $\lambda_{\max}$ is the wavelength in the visible spectrum at which maximum absorption by the charge transfer complex is observed. $E_T(30)$ polarity values measured for mobile phases of methanol–water and acetonitrile–water have been used to improve the linearity in log k'_ϕ–ϕ graphs by correlating log k'_ϕ with $E_T(30)$ polarity values rather than the volume fraction of organic modifier in the mobile phase.[40]

Results and Discussion

The RPLC conditions for the experiments performed during the development of this method are shown in Table II. Linear extrapolation of log k'_ϕ vs ϕ_{MeOH} data from experiments 1–10 was used to derive values of log k'_w and S, which were correlated independently with log $P_{o/w}$. The results of these correlations for experiments 1–10 are shown in Tables III and IV for log k'_w and S, respectively. The differential hydrogen bond effect was observed in these correlations for most of the RPLC conditions in Table II and is illustrated in Figure 2 for octyl- and octadecyl-modified silica gels (C_8 and C_{18}, respectively). Variations in the chromatographic conditions in Table II were made in order to minimize this effect. The slope parameter was examined in correlations with log $P_{o/w}$ because this parameter had been shown to distribute more randomly than

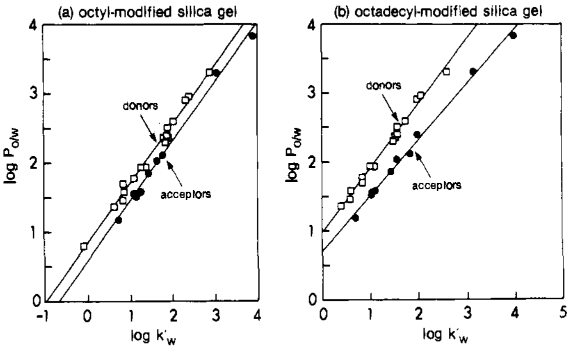

Figure 2. The differential hydrogen bonding effect is illustrated here for (a) octyl-modified silica gel and (b) octadecyl-modified silica gel. The donors and acceptors are the same compounds shown in Table I. The values of log k'_w were obtained from experiments 3 and 4 (Table II).

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(39) Dorsey, J. G.; Johnson, B. P. *J. Liq. Chromatogr.* **1987**, *10*(12), 2695.

(40) Johnson, B. P.; Khaledi, M. G.; Dorsey, J. G. *Anal. Chem.* **1986**, *58*(12), 2354.

(41) Karch, K.; Sebestian, I.; Halâsz, I. *J. Chromatogr.* **1976**, *122*, 3.

(42) Melander, W.; Stoveken, J.; Horvâth, C. *J. Chromatogr.* **1980**, *199*, 35.

log k'_w in correlations with log $P_{o/w}$ and log $1/C_{BSA}$ for a set of noncongeners.[1]

As noted, the development of this method involved modifications in RPLC conditions in order to minimize the differential hydrogen bond effect. Modifications to the mobile phase included variations of buffer salts, amine modifiers, and inclusion of octanol in the organic portion of the eluent. In the aqueous portion of the eluent, two buffer salts and two amine modifiers were examined. In experiments 1 and 2 (Table II), the aqueous portions were buffered with MOPS and phosphate, respectively, and the results of correlating log k'_w from these experiments with log $P_{o/w}$ are reported in eq 1 and 2, Table III. These results are identical within standard deviations, indicating that either salt can be used to buffer the mobile phase at pH 7.4. MOPS was selected because it is an organic salt and should be less susceptible to precipitation at higher methanol compositions. In experiments 5–8, two amine modifiers were compared; the amine modifier was included in the eluent to mask silanophilic interactions. The regression results for these experiments are shown in eq 5–8 (Table III) and indicate that log k'_w values obtained when *n*-decylamine was used correlated better with log $P_{o/w}$ than values obtained by using *N*,*N*-diethylamine; this was true for both C_8 and C_{18} stationary phases. On the basis of these results, *n*-decylamine was selected as the amine modifier. In all likelihood, these results reflect the large difference between the lipophilicities of these modifiers. The enhanced lipophilic character of decylamine may enable this modifier to better mask silanophilic interactions and, if concentrated in the stationary phase, may even modify the overall character of the stationary phase in a way that further reduces hydrogen bond discrimination. In experiments 3–12, 1-octanol was added to the organic portion of the mobile phase. Although several volume percentages of octanol in methanol greater than 0.25% were tested, these solutions were too concentrated and resulted in octanol pooling at $\phi_{MeOH} < 0.50$. The inclusion of octanol in the eluent appears to play a major role in minimizing the differential hydrogen bond effect observed in most RPLC methods. Additional experiments are required to demonstrate unequivocally the role of this modifier, but we believe that without this component the hydrogen bond effect could not have been overcome. Octanol is significantly more lipophilic than methanol and would be enriched in the stationary phase relative to the bulk eluent, producing a more octanol-like phase and thus minimizing hydrogen-bond discrimination. This adaptation is analogous to the use of octanol in monocratic methods, which reportedly overcame the differential hydrogen bond effect by using octanol-saturated stationary phases and pure aqueous eluents saturated with octanol to avoid desolvation of this component from the stationary phase.[7,11]

Two stationary phases were compared in these experiments. In experiments 3–10, C_8- and C_{18}-modified silica gels were used under four different sets of eluent conditions to obtain log k'_w values that were correlated with log $P_{o/w}$. The regression results for these sets of experiments are shown in eq 3–10, Table III, and clearly indicate that C_8 is the stationary phase of choice for modeling log $P_{o/w}$. In experiments 10–12, two different C_8 packings were examined, both of 60-Å pore diameter, but one with 5-μm particles (experiments 10 and 11) and one with 10-μm particles (experiment 12). The column packed with 5 μm particles produced unacceptably long retention times for all but the least lipophilic compounds shown in Table I at compositions of $\phi_{MeOH} < 0.45$, while the column packed with 10-μm particles provided adequate retention for all solutes in the range $0.25 \leq \phi_{MeOH} \leq 0.75$ and represents a better choice as a packing. The conditions listed for experiments 10–12 in Table II thus define the best general set of RPLC conditions for estimating octanol–water partition coefficients. However, additional experiments were conducted to study other aspects pertinent to the development of this method.

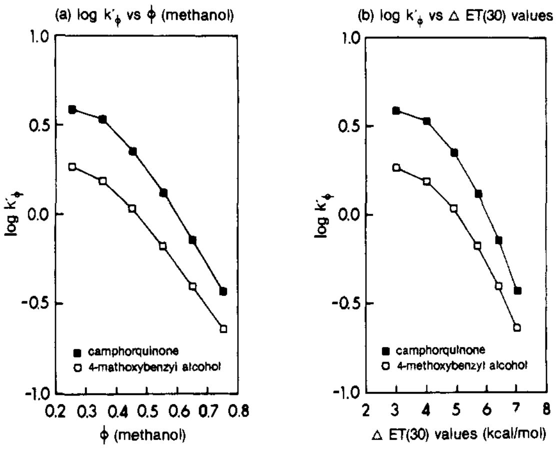

Figure 3. This graph demonstrates that solvent polarity values defined by the $E_T(30)$ probe produced more curvature in correlations with logarithms of measured capacity ratios (log $k'(\phi)$) than the volume fraction of methanol. To illustrate this point, log $k'(\phi)$ are plotted against (a) the volume fraction of methanol in the bulk eluent (ϕ) and (b) solvent polarity values ($E_T(30)$ values) for the two compounds with the most curvature in log k'_ϕ–ϕ_{MeOH} data. Values of log k'_ϕ were measured in experiment 12 (Table II) with $t_0 = 1.005$ min. In a, $r = 0.9877$ and SD = 0.0619 for 4-methoxybenzyl alcohol, and $r = 0.9820$ and SD = 0.0847 for camphorquinone, where r and SD are the correlation coefficients and overall standard deviations of regression obtained by regressing log k'_ϕ with ϕ(methanol). In b, $r = 0.9702$ and SD = 0.959 for 4-methoxybenzyl alcohol, and $r = 0.9616$ and SD = 0.1229 for camphorquinone; here r and SD are the correlation coefficients and overall standard deviations of regression obtained by regressing log k'_ϕ with $E_T(30)$ values.

In an attempt to optimize the linearity of log k'_ϕ–ϕ_{MeOH} data, log k'_ϕ data were correlated with a solvent polarity scale instead of ϕ_{MeOH}. The relationship between the polarity values and the volume percent of methanol in the eluent is quadratic:

$$E_T(30) = 63.4026\ (\pm 0.2038) - 0.1312\ (\pm 0.0068)(\mathrm{OP\%}) + 0.0005\ (\pm 0.0001)(\mathrm{OP\%})^2$$

$$n = 8,\ r = 0.9994,\ \mathrm{SD} = 0.0669 \qquad (8)$$

where OP% = volume percent of methanol in the eluent. The standard deviations in the coefficients are reported in parentheses. Equation 9, reported by Dorsey et al.,[39] was derived for binary mixtures of pure methanol and pure water and is included here for comparison.

$$E_T(30) = 63.1927 - 0.1222(\mathrm{OP\%}) + 0.0005(\mathrm{OP\%})^2$$

$$n = 11,\ r = 0.9994 \qquad (9)$$

A solvent polarity scale calculated from eq 8 was correlated with log k'_ϕ data from experiment 12 (Table II). The results of these correlations revealed that log k'_ϕ generally varied more nonlinearly with $E_T(30)$ value than ϕ_{MeOH}. This is illustrated for two compounds in Figure 3. Since $E_T(30)$ values measure the changing polarity of the mobile phase independently of changes in the stationary phase composition, while log k'_ϕ reflects changes in the properties of both phases, the increased curvature in the log k'_ϕ vs $E_T(30)$ graphs suggests that subtle changes in the stationary phase are occurring as the eluent composition changes. Although further experiments would be needed to substantiate this point, these results demonstrate that $E_T(30)$ values are not a viable alternative to ϕ_{MeOH} in correlations with log k'_ϕ data measured under the conditions reported for experiment 12.

Correlations of S with log $P_{o/w}$ were also examined, and the results are shown in Table IV for experiments 1–10. The best regression results reported in both Tables III and IV involved S values measured under experiment 10 conditions, suggesting that the S parameter may be a better measure of octanol–water hydrophobicity than log k'_w. However, when additional experiments were performed to study the extent to which log k'_w and S values varied with values of the column dead-time estimated by different procedures, log k'_w emerged as the clear choice for developing a scale of relative hydrophobicities.

To study how log k'_w and S varied with t_0, three procedurs for measuring this parameter were examined. In experiments 1–10 in Table II, the D_2O isotope procedure was used to estimate t_0. This procedure was used initially to establish the general stationary and mobile phase conditions for this method, and results shown in Tables III and IV are based upon values of log k'_w and S derived from capacity ratios (k'_ϕ) calculated from t_0 values estimated by this procedure. However, as the development of this method progressed, there was growing concern that values of log k'_w and S may depend upon the value of the column dead-time estimated by a specific procedure and that the D_2O method was not the best for estimating t_0. Evidence in the literature indicated that dead-times measured under these eluent conditions (pure water) might be erroneously low for packings with 60-Å pore diameters due to collapse of the stationary phase.[41] These findings raised questions about how best to estimate t_0, and consequently two other procedures were investigated in experiments 11 and 12.

In experiment 11, the D_2O isotope method was again used. Additionally, column dead-times were estimated at each eluent composition ($0.25 \le \phi_{MeOH} \le 0.75$) from the retention time of urea (denoted $t_0(\phi)$). When the two sets of log k'_w and S values derived from these procedures were compared, log k'_w was found to be largely independent of the exact value of t_0 used, while S was found to be dependent upon the exact value of t_0. This behavior is illustrated in Figure 4 for several solutes. These unexpected results suggested that variations in log k'_w would be small even if the column dead-time was incorrectly evaluated and that a standard procedure for estimating t_0 was not required, allowing researchers to estimate this parameter by their preferred method. Conversely, these results also indicated that S is a function of t_0 and would require the adoption of a standard procedure for estimating t_0 if this parameter became widely accepted as a measure of hydrophobicity.

The relationships between log k'_w, S, and column dead-time(s) were investigated further in experiment 12. Here, values of $t_0(\phi)$ were again used to calculate capacity ratios from which a set of log k'_w and S values was derived. In addition, a second set of log k'_w and S values was derived from the maximum column dead-time estimated by the simplified method proposed by Knox and Kaliszan.[34] According to this procedure, the maximum t_0 for any column can be estimated from the retention time of CD_3OD measured with pure methanol as eluent. As applied here, this parameter was estimated from the retention time of CD_3OD measured with methanol + octanol (0.25%, v/v) as eluent. The amount of octanol in the eluent was low, and the effect of this component upon the column dead-time was considered slight and neglected. Although this approximation does not yield the true maximum dead-time, values of log k'_w were again found to be largely independent of the exact value estimated for the dead-time, particularly when a constant value of t_0 was used. When these sets of log k'_w and S values were compared, both constants were found to vary with t_0, but log k'_w to a lesser extent than S. This behavior is illustrated in Figure 5 for several solutes, where lines representing different S values no longer converge to the same value of log k'_w (see Figure 4). Nonconvergence was observed for all of the solutes in the test series, although the variations between values of log k'_w were generally small (Δ log k'_w = 0.1–0.15). These results appeared to contradict the findings from experiment 11, where log k'_w was shown to be largely independent of the dead-time, whether estimated as a constant (t_0) or as a variable ($t_0(\phi)$). However, when values of log k'_w and S were derived for several of the test compounds from capacity ratios based upon artificial values of t_0 (0.6, 0.8, and 1.0 min), only S varied significantly. These results are reported in Table V and displayed graphically in Figure 6 and clearly demonstrate that as t_0 is varied between 0.6 and 1.0 min., log k'_w changes little, while S progressively increases. This behavior suggests that any constant value for the dead-time (even if incorrectly evaluated, as in the case here) will yield the same value of log k'_w and is thus superior to methods based upon variable values of dead-time estimated at each eluent composition.

Table V. Variations of log k'_w and S with t_0 Using Artificial Values for the Column Dead-Time

	t_0 = 0.6 min		t_0 = 0.8 min		t_0 = 1.0 min	
compound	log k'_w	S	log k'_w	S	log k'_w	S
1, 3-methoxyphenol	1.26	1.73	1.21	2.03	1.25	2.54
2, 4-methoxybenzyl alcohol	0.900	1.17	0.813	1.42	0.814	1.85
3, 4-chlorophenol	2.30	3.02	2.32	3.42	2.35	3.75
4, phenol	1.26	1.74	1.21	2.04	1.26	2.57
5, camphorquinone	1.43	1.76	1.39	2.03	1.42	2.41
6, 1-naphthylamine	2.20	2.78	2.17	3.04	2.20	3.39

Table VI. Comparison of log k'_w Constants Reported in the Literature with Values Estimated by the Best Method

compound	log k'_w (mean ± SD)[a]	log k'_w	log $P_{o/w}$ (method 12)[b]
4-chloroaniline	1.84	1.86	1.83
naphthalene	3.31 ± 0.10	3.19	3.30
phenol	1.30 ± 0.05	1.25	1.46
3-chlorophenol	2.35	2.39	2.50
4-chlorophenol	2.24 ± 0.08	2.31	2.39
4-methylphenol	1.83	1.76	1.94
nitrobenzene	1.92 ± 0.07	1.63	1.85
4-chloronitrobenzene	2.35 ± 0.05	2.17	2.39
acetophenone	1.85	1.36	1.58
benzonitrile	1.80	1.28	1.56

[a] Values taken from ref 1; compounds with no reported standard deviation are averaged from only two values. [b] See Table II for general experimental conditions for method 12; a dead-time of 1.005 min was used to caculate capacity ratios from which these log k'_w were derived.

The independent relationship between log k'_w and t_0 is supported by the data reported by Braumann,[2] who reported only small variances in values of log k'_w for approximately 70 compounds taken from seven references in the literature; this observation is possible only if log k'_w is largely independent of t_0. This is further substantiated in Table VI where log k'_w values obtained by using the constant value of t_0 estimated by Knox and Kaliszan's

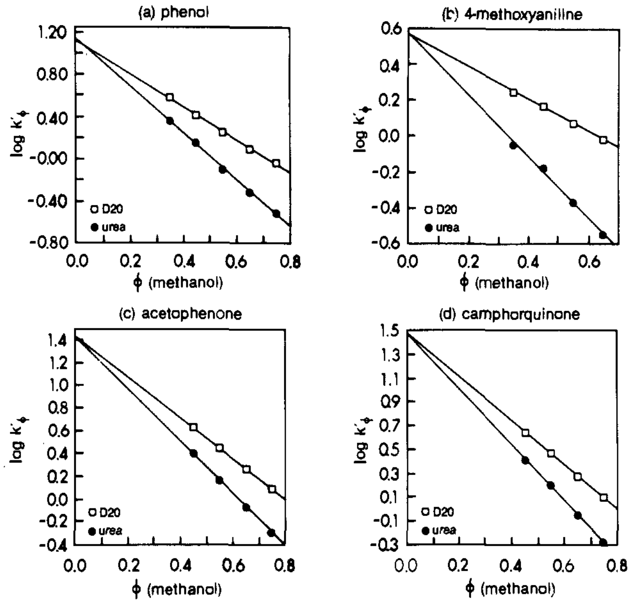

Figure 4. In this figure, the relationships between log k'_w and S (slope) parameter (eq 3) and values of the column dead-times estimated by different procedures in experiment 11 are illustrated for four representative compounds from Table I. Values of log k'_w derived from different sets of log k'_ϕ data are shown to be largely independent of the procedure used to estimate different values of the dead-time. In contrast, S is shown to vary with different estimates of dead-time. The symbol in each graph corresponding to D2O indicates that measured capacity ratios from which log k'_w and S were derived were calculated by using the column dead-time estimated by the D_2O isotope method (t_o ~0.651 min). The symbol corresponding to urea indicates values of log k_w and S which were derived by using a dead-time estimated at each mobile phase composition (for ϕ = 0.75, 0.65, 0.55, 0.45, 0.35, and 0.25, t_o(urea) = 0.956, 0.995, 1.000, 0.973, 0.949, and 0.907 min, respectively). In a, log $k'_w(D_2O)$ = 1.12, log k'_w(urea) = 1.14, $S(D_2O)$ = 1.55, and S(urea) = 2.22; in b, log $k'_w(D_2O)$ = 0.57, log k'_w(urea) = 0.57, $S(D_2O)$ = 0.89, and S(urea) = 1.70; in c, log $k'_w(D_2O)$ = 1.43, log k'_w(urea) = 1.43, $S(D_2O)$ = 1.80, and S(urea) = 2.31; in d, log $k'_w(D_2O)$ = 1.47, log k'_w(urea) = 1.46, $S(D_2O)$ = 1.82, and S(urea) = 2.30.

simplified procedure are compared with the mean values of log k'_w reported by Braumann for the same compounds. Values of log k'_w reported for this method are essentially identical with the mean values reported by Braumann for hydrogen-bond donors. However, the mean values of log k'_w reported for hydrogen bond acceptors are uniformly higher than the corresponding values reported for this method. This systematic deviation can be attributed to the differential hydrogen bond effect and confirms that, unlike the method reported here, most RPLC methods in the literature have not adequately addressed this problem.

As a final point before leaving the topic of dead-time procedures, the urea method was employed in an attempt to improve the linearity in log k'_ϕ–ϕ_{MeOH} data, and thus reduce the uncertainty in estimates of log k'_w. However, when the correlation coefficients from the sets of log k'_ϕ–ϕ_{MeOH} data from experiments 11 and 12 were compared, improvements in linearity were negligible, indicating that there was no advantage to estimating values of t_o at each eluent composition. Thus, the dead-time can be estimated once from the retention of CD_3OD under pure organic conditions, and this value used for the lifetime of the column.

The results discussed thus far have dealt exclusively with linear extrapolation. However, quadratic extrapolation has been proposed as an alternate procedure for obtaining log k'_w (denoted log k'_w(quad)) and is examined next. log k'_w(quad) values were obtained from regression analysis by using eq 4 and the log k'_ϕ–ϕ_{MeOH} data from experiment 12. In that experiment, retention data were acquired at ϕ_{MeOH} = 0.25 for 21 of the solutes studied, making it possible to compare the extents to which log k'_w(quad) and log k'_w depend upon the eluent range over which retention data is acquired. In Table VII, sets of log k'_w and log k'_w(quad) values derived from log k'_ϕ data for $0.25 \leq \phi_{MeOH} \leq 0.75$ and $0.35 \leq \phi_{MeOH} \leq 0.75$ are shown for 11 compounds. These results demonstrate that differences between log k'_w values were smaller than those observed between log k'_w(quad) values and that the latter are more dependent upon the eluent range over which retention data is acquired. This is further demonstrated in Table VIII,

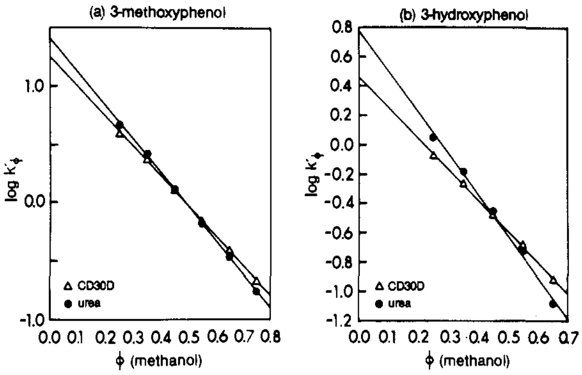

Figure 5. In this figure, the relationships between log k'_w and S (slope) parameter (eq 3), and values of the column dead-times estimated by different procedures in experiment 12 are shown. These results indicate that both log k'_w and S depend on the method used to estimate the column dead-time. The symbol in each graph corresponding to CD3OD indicates that measured capacity ratios from which log k'_w and S were derived by using the column dead-time estimated by the simplified method of Knox and Kaliszan[34] (t_o = 1.005 min). The symbol corresponding to urea indicates values of log k'_w and S were derived using a dead-time estimated at each mobile phase composition (for ϕ = 0.75, 0.65, 0.55, 0.45, 0.35, and 0.25, t_o(urea) = 1.038, 1.040, 1.020, 0.993, 0.939, and 0.873 min, respectively). In a, log $k'_w(CD_3OD)$ = 1.25, log k'_w(urea) = 1.41, $S(CD_3OD)$ = 2.56, and S(urea) = 2.89; in b, log $k'_w(CD_3OD)$ = 0.47, log k'_w(urea) = 0.79, $S(CD_3OD)$ = 2.12, and S(urea) = 2.82.

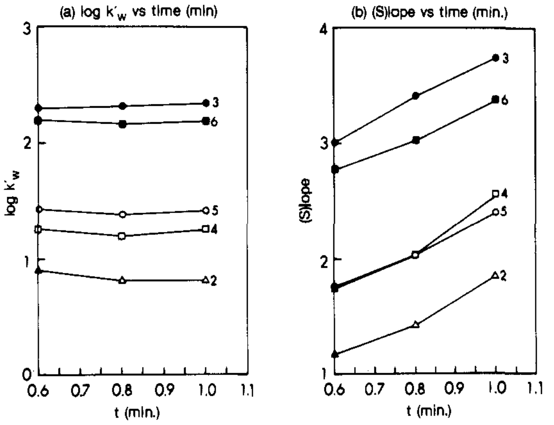

Figure 6. In this figure, the variations in values of log k'_w and S (slope) parameter (eq 4) reported in Table V are illustrated graphically. Compound numbers correspond to those reported in that table. In a, log k'_w values remain essentially unchanged, regardless of the value used for the dead-time. These results indicate that this constant is independent of the exact value estimated for the dead-time, as long as a constant value is used. In b, S values increase as the value of t_o increases. These results demonstrate the independent and dependent relationships between log k'_w and t_o, and S and t_o, respectively.

Table VII. Comparison of log k'_w Constants Derived by Linear and Quadratic Extrapolation Using either All ϕ_{MeOH} Data or $\phi_{MeOH} \geq 0.35$ Data

compound	log k'_w(linear)			log k'_w(quad)		
	1[a]	2[b]	Δ[c]	1[a]	2[b]	Δ[c]
phenol	1.251	1.253	−0.002	1.249	1.262	−0.013
3-fluorophenol	1.749	1.750	−0.001	1.806	1.951	−0.145
4-fluorophenol	1.575	1.588	−0.013	1.591	1.696	−0.105
3-methoxyphenol	1.254	1.295	−0.041	1.307	1.420	−0.113
3∞nitrobenzonitrile	1.014	1.163	−0.149	0.588	0.750	−0.162
benzonitrile	1.279	1.403	−0.124	0.929	1.158	−0.229
acetophenone	1.359	1.477	−0.118	1.037	1.273	−0.236
4-methoxybenzyl alcohol	0.815	0.958	−0.143	0.383	0.574	−0.191
nitrobenzene	1.63	1.729	−0.096	1.376	1.658	−0.282
3-fluoroaniline	1.225	1.301	−0.076	1.040	1.253	−0.213
4-methoxyaniline	0.603	.683	−0.080	0.387	0.558	−0.171

[a] All data was included in extrapolation. [b] Only data for $\phi_{MeOH} \geq$ 0.35 was included in extrapolation; (c) $\Delta = \log k'_w(1) - \log k'_w(2)$.

Table VIII. Regression Equations for log $P_{o/w}$ vs log K'_w(quad) and log k'_w Showing Dependence of log k'_w(quad) upon Range of Eluent Compositions Used To Derive log k'_w(quad)

(i) all lo k'_ϕ–ϕ_{MeOH} data vailable used to derive log k'_w(quad):
$\log P_{o/w} = 0.596\ (0.022) \log k'_w(\text{quad}) + 0.845\ (0.050)$
$n = 34,\ r = 0.979,\ \text{SD} = 0.146$
(ii) log k'_ϕ–ϕ_{MeOH} data for $\phi_{MeOH} \geq 0.35$ used to derive log k'_w(quad):
$\log P_{o/w} = 0.624\ (0.021) \log k'_w(\text{quad}) + 0.723\ (0.049)$
$n = 34,\ r = 0.982,\ \text{SD} = 0.314$
(iii) log k_w derived from all log k'_ϕ–ϕ_{MeOH} data:
$\log P_{o/w} = 0.909\ (0.019) \log k'_w + 0.328\ (0.039)$
$n = 34,\ r = 0.993,\ \text{SD} = 0.086$
(iv) log k'_w derived from log k'_ϕ–ϕ_{MeOH} data for $\phi_{MeOH} \geq 0.35$:
$\log P_{o/w} = 0.950\ (0.022) \log k'_w + 0.211\ (0.045)$
$n = 34,\ r = 0.9928,\ \text{SD} = 0.0926$

where regression results are presented for correlations of these sets of log k'_w(quad) values with log $P_{o/w}$. The correlation with log $P_{o/w}$ was poorest when all of the chromatographic data were used to derive log k'_w(quad) (see eq i, Table VIII). This correlation was improved by restricting the eluent range to $\phi_{MeOH} \geq 0.35$ (eq ii, Table VIII) but was still poorer than either of the correlations using log k'_w values derived by linear extrapolation (eqs iii and iv, Table VIII).

These results point to several general problems associated with the use of quadratic extrapolation. If this procedure is used to obtain hydrophobicity constants, then compounds must be analyzed under the same mobile phase conditions, resulting in a net reduction in the dynamic range of hydrophobicities which can be measured. Also, the equations in this table represent linear free-energy relationships in which the magnitudes of the slopes reflect how well the systems represented by the independent variables (log k'_w and log k'_w(quad)) model the octanol–water system. For the system utilizing log k'_w, the slope is close to unity in both cases, indicating that log k'_w closely models log $P_{o/w}$ for this set of compounds. However, in the case of log k'_w(quad), the slopes are approximately 0.6 in both correlations, indicating that the quadratic approach provides a poorer model for octanol–water partition coefficients. Poorer modeling of log $P_{o/w}$ by log k'_w(quad) is illustrated in Figure 7, where the solutes used in this study are graphed according to the hydrogen bond classifications shown in Table I. Hydrogen-bond discrimination is seen only in the graph of log $P_{o/w}$ vs log k'_w(quad). These results demonstrate that linear extrapolation provides a better model for estimating octanol–water partition coefficients than quadratic extrapolation.

Before examining the prediction of biological data by using log k'_w, the extent to which values of log k'_w are column-dependent is examined. Differences in the column properties that can arise during manufacturing may produce variations in values of log k'_w obtained from different columns. In order to study the extent to which log k'_w depended upon the specific column used to obtain data, values from three different C_8 packings were compared. In the first comparison, log k'_w data acquired from the 5 μ/60 Å nominal pore diameter packing used in experiments 10 and 11 (Table II) were correlated with data from the 10 μ/60 Å nominal pore diameter packing used in experiment 12 (Table II). The result of this correlation is shown in eq 10 where the numbers in parentheses indicate the

$$\log k'_w(11) = 0.966 \log k'_w(12) - 0.036$$
$$n = 34,\ r = 0.983,\ \text{SD} = 0.126 \qquad (10)$$

experiment number from Table II. The slope in this correlation is very near unity and the intercept zero, indicating that the energetics of retention and the phase ratios of these packings are very similar.[42] In the second comparison, log k'_w data acquired from a 10 μ/100 Å nominal pore diameter packing were again correlated with data from the 10 μ/60 Å nominal pore diameter packing useed in experiment 12. The result of this correlation is shown in eq 11 where log $k'_w(12)$ are the constants measured in experiment 12. The slope of this line is again near unity, indicating that the energetics of retention between these columns are equivalent. However, the variation observed in the intercept indicates a substantial difference in the phase ratios of these two packings. These results demonstrate that fairly large variations in log k'_w can arise from different packings and indicate the need for a set of test compounds to assure that the bulk properties of the columns are quite similar. Several papers have recently appeared in the literature in which schemes were described for comparing the hydrophobic and silanophilic characteristics of C_{18} packings between columns.[43,44] Although

$$\log k'_w = 1.072 \log k'_w(12) - 0.540$$
$$n = 34,\ r = 0.992,\ \text{SD} = 0.093 \qquad (11)$$

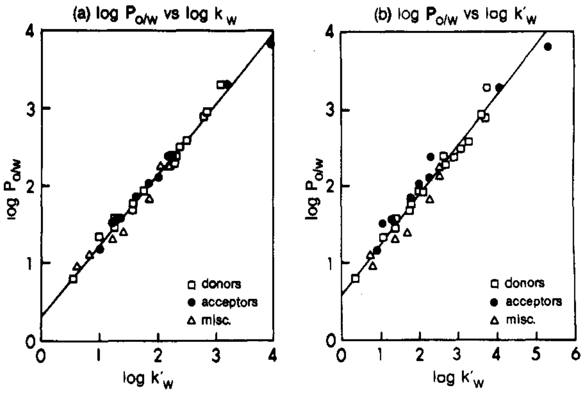

Figure 7. The correlations between log $P_{o/w}$ and (a) log k'_w values derived by linear extrapolation (eq 4) and (b) log k'_w values derived by quadratic extrapolation (eq 4) are illustrated in this figure. Compounds were graphed according to the classifications reported in Table I. These results indicate that log k'_w values derived linearly provide a better model for predicting octanol–water partition coefficients than similar values derived by a quadratic model. Equations ii and iv in Table VIII describe mathematically the correlations shown in a and b, respectively.

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(44) Delaney, M. F.; Papas, A. N.; Walters, M. J. *J. Chromatogr.* **1987**, *410*, 31.

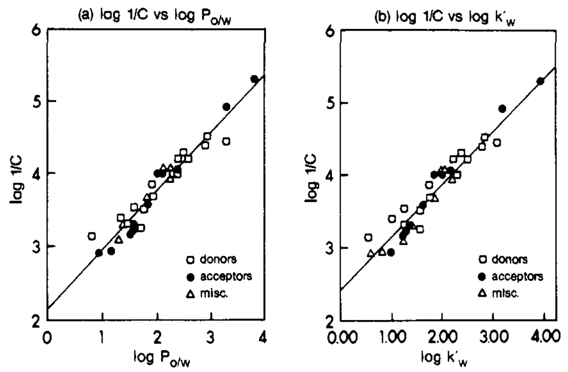

Figure 8. The correlations between log $1/C_{\mathrm{BSA}}$ and (a) log $P_{o/w}$ and (b) log k'_w derived by linear extrapolation of retention data from experiment 12 are shown in this figure. These graphs illustrate that log k'_w obtained under the RPLC conditions described for this experiment model the binding of small, nonionic solutes to BSA as well as log $P_{o/w}$ does. Values of log $1/C_{\mathrm{BSA}}$, log $P_{o/w}$, and log k'_w used in these graphs are reported in Table IX. Note that the differential hydrogen bond effect reported by Minick et al.[1] for correlations similar to that shown in b is minimal in this figure, resulting in a model capable of predicting this biological data as well as log $P_{o/w}$. The regression results describing the relationships shown here are reported in eq 12 and 13, respectively.

these authors have not evaluated those tests in the context of the methodology discussed here, they appear to address problems relevant to comparing columns and monitoring the condition of columns being used to measure log k'_w values. At present, the following compounds are being used to monitor the condition of aging columns and to compare the hydrophobic and silanophilic properties of new columns: phenol, 4-methoxyphenol, naphthalene, acetophenone, camphorquinone, 4-methoxyaniline, and 4-bromoaniline. log k'_w values for these solutes are determined at regular intervals and compared with previous values. Due to the presence of an amine modifier in the eluent, these probes should be most sensitive to changes in the hydrophobic characteristics of the packing. No exact recommendations can be made at this time concerning the magnitude of systematic variations in the values of log k'_w for these compounds that would warrant the purchase of a new column. However, the eluent conditions described for this method are rather harsh and may result in fairly rapid hydrolysis of the stationary phase, particularly in the absence of a silica saturator column.

Tale IX. Values of log k'_w, log $P_{o/w}$, log $1/C_{\mathrm{BsA}}$ Used in Regressions

compound	log k'_w	log $P_{o/w}$ obsd	log $P_{o/w}$ fit	log $1/C_{\mathrm{BSA}}$ obsd	log $1/C_{\mathrm{BSA}}$ fit
1, phenol	1.251	1.46	1.46	3.32	3.34
2, 3-fluorophenol	1.749	1.93	1.92	3.86	3.70
3, 4-fluorophenol	1.575	1.77	1.76	3.52	3.57
4, 3-chlorophenol	2.391	2.50	2.50	4.30	4.17
5, 4-chlorophenol	2.310	2.39	2.43	4.00	4.11
6, 4-bromophenol	2.501	2.59	2.60	4.22	4.25
7, 4-iodophenol	2.795	2.91	2.87	4.40	4.46
8, 4-methylphenol	1.759	1.94	1.93	3.70	3.71
9, 3-ethylphenol	2.228	2.40	2.35	4.22	4.05
10, 3-(trifluoromethyl)phenol	2.847	2.95	2.92	4.52	4.50
11, 3-cyanophenol	1.557	1.70	1.74	3.26	3.56
12, 3-hydroxyphenol	0.544	0.80	0.82	3.15	2.83
13, 3-methoxyphenol	1.254	1.58	1.47	3.54	3.34
4, 4-methoxyphenol	1.004	1.34	1.24	3.40	3.16
15, 3-nitrobenzonitrile	1.014	1.17	1.25	2.94	3.17
16, 4-methoxybenzyl alcohol	0.815	1.10	1.07	2.94	3.02
17, benzonitrile	1.279	1.56	1.49	3.23	3.36
18, acetophenone	1.359	1.58	1.57	3.ou	3.42
19, nitrobenzene	1.633	1.85	1.81	3.58	3.62
20, 4-bromoacetanilide	2.293	2.29	2.41	4.00	4.09
21, 4-nitroanisole	1.857	2.03	2.02	4.00	3.78
22, 4-chloronitrobenzene	2.165	2.30	2.30	4.07	4.00
23, naphthalene	3.191	3.30	3.23	4.91	4.76
24, azobenzene	3.961	3.82	3.93	5.29	5.30
25, anisole	2.006	2.11	2.15	4.00	3.87
26, 3-fluoroaniline	1.225	1.30	1.44	3.09	3.32
27, 4-chloroaniline	1.855	1.83	2.01	3.68	3.78
28, 4-methoxyaniline	0.603	0.95	0.88	2.92	2.87
29, 4-bromoaniline	2.043	2.26	2.18	4.06	3.91
30, 4-methylaniline	1.405	1.39	1.60	3.30	3.45
31, 1-naphthylamine	2.203	2.26	2.33	3.94	4.03
32, indole	2.014	2.14	2.16	4.07	3.89
33, camporquinone	1.225	1.52	1.44	3.17	3.32
34, thymol	3.092	3.30	3.14	4.66	4.67

To examine how well the results from experiment 12 predicted biological data, log k'_w constants for this series were correlated with the logarithm of the BSA binding constants (log $1/C_{\mathrm{BSA}}$) measured by Helmer et al. Observed values of log k'_w, log $P_{o/w}$, and log $1/C_{\mathrm{BSA}}$ are reported in Table IX, and graphs of log $1/C_{\mathrm{BSA}}$ verses log $P_{o/w}$ and log k'_w are shown in Figure 8. This figure illustrates that the differential hydrogen bond effect is minimal in the correlation involving log k'_w. Fitted values of log $P_{o/w}$ and log $1/C_{\mathrm{BSA}}$ were included in Table IX for comparison with measured values. The fitted values of log $P_{o/w}$ were obtained from eq iii, Table VIII, while the fitted values of log $1/C_{\mathrm{BSA}}$ were obtained from eq 12 where

$$\log 1/C_{\mathrm{BSA}} = 0.73\ (0.04) \log k'_w + 2.43\ (0.07)$$

$$n = 34,\ r = 0.963,\ \mathrm{SD} = 0.157 \qquad (12)$$

the standard deviations in the coefficients at the 95% confidence interval are shown in parentheses. When the observed values of log $P_{o/w}$ were correlated with the observed values of log $1/C_{\mathrm{BSA}}$, eq 13 was obtained. Again,

$$\log 1/C_{\mathrm{BSA}} = 0.81\ (0.03) \log P_{o/w} + 2.15\ (0.07)$$

$$n = 34,\ r = 0.973,\ \mathrm{SD} = 0.137 \qquad (13)$$

standard deviations at the 95% confidence interval are included. Although the standard deviation of regression for eq 12 is slightly higher than that for eq 13, this difference is statistically indistinguishable when compared by the null hypothesis of equivalence of variables.[1,12,46] Thus, log k'_w cannot be rejected as a model for predicting log $1/C_{\mathrm{BSA}}$ when compared to log $P_{o/w}$. In contrast, modeling of log $1/C_{\mathrm{BSA}}$ by hydrophobicity constants predicted by another RPLC method published recently in the literature[47] for this same series of solutes can be rejected on this basis. The regression results reported for this correlation are shown in eq 14 where log P_{HPLC} = loga-

$$\log 1/C_{\mathrm{BSA}} = 0.75\ (0.09) \log P_{\mathrm{HPLC}} + 2.25\ (0.20)$$

$$n = 34,\ r = 0.949,\ \mathrm{SD} = 0.189 \qquad (14)$$

rithms of octanol–water partition coefficients predicted by that RPLC method. When the standard deviations for eq 13 and 14 are compared by the null hypothesis, this RPLC method can be rejected as a model for predicting log $1/C_{\mathrm{BSA}}$ values in comparison to log $P_{o/w}$.

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Conclusion

A number of different mobile and stationary phase conditions have been examined to optimize the correlation between log k'_w values and log $P_{o/w}$ by minimizing the differential hydrogen bond effect often observed in RPLC retention data for noncongeners. This investigation has resulted in the development of a rather simple but accurate reversed-phase liquid chromatographic method for estimating hydrophobicity constants (log k'_w) for small, nonionic, organic molecules that are equivalent to octanol–water partition coefficients measured by the classical shake-flask technique.[48] The developed method does not rely on the use of a set of secondary reference standards to produce a relative scale of hydrophobicities. Thus, log k'_w values represent a priori hydrophobicity constants. Evidence was presented that indicated that log k'_w measured by using different columns could be compared directly as long as the properties of the columns are similar. The correlation of log k'_w with the log $1/C_{BSA}$ was statistically indistinguishable from the correlation between log $P_{o/w}$ and log $1/C_{BSA}$, indicating that log k'_w is as effective in predicting biological activity as log $P_{o/w}$ in cases where the latter has been shown to be a relevant variable. On the basis of the results presented here, the following experimental guidelines are proposed for measuring these octanol–water like hydrophobicity constants by reversed-phase chromatography: (i) the method should utilize linear extrapolation of log $k'(\phi)$ vs ϕ_{MeOH} data acquired in the region $0.25 \leq \phi_{MeOH} \leq 0.75$ to derive log k'_w constants; (ii) the organic portion of the mobile phase is composed of methanol containing 0.25% (v/v) 1-octanol; (iii) the aqueous phase is prepared from octanol-saturated water containing 0.02 M MOPS buffer, 0.15% (v/v) of *n*-decylamine, and adjusted to pH 7.4; (iv) octyl-modified silica gel should be used as the stationary phase; (v) a silica saturator column should be included between the pumps and injector to retard column degradation; improvements in column life have reportedly been obtained by including a silica saturator column in the RPLC system;[49] (vi) column dead-times can be estimated by individual researchers preferred methods as long as the value of this parameter is a constant (the simplified method of Knox and Kaliszan described in this text is recommended and will be used in this laboratory to evaluate this parameter); (vi) the following standards are recommended for monitoring column stability and comparing similarities in the hydrophobic properties of different columns: phenol, 4-methoxyphenol, acetophenone, camphorquinone, 4-methoxyaniline, 4-bromoaniline, and naphthalene.

Experimental Section

Materials. HPLC grade methanol, analytical grade 1-octanol, and solutions of sodium hydroxide (1 N) and hydrochloric acid (1 N) were obtained from Fisher Scientific. Analytical grade sodium phosphate (dibasic) as obtained from Mallinkrodt. 4-Morpholinepropanesulfonic acid (MOPS), Reichardt's Dye (E_T(30)), *n*-decylamine (99%), and diethylamine (99%) were obtained from Aldrich Chemical Co. The test solutes were obtained from the Burroughs Wellcome Co. chemical stores or purchased from Aldrich Chemical Co. No attempt was made to purify any of these compounds. All water used in these experiments was obtained from a Millipore Milli-Q Water System. Deuteriated samples of methanol and water used to measure the column dead-volumes were obtained from MSD Isotopes.

Chromatography. A Waters Associates Model 840 Chromatographic System equipped with Model 510 or 590 pumps, a WISP autoinjector, and a Model 450 variable wavelength detector operated at 220 nm was used. A DEC Professional 350 computer was used to control hardware, acquire and store data, and determine retention times. A Cary 118 UV/vis spectrophotometer was used to acquire spectral data from which E_T(30) polarity values were calculated. The following chromatographic columns were used in the experiments reported here: (1) Waters columns (15 cm × 4.6 mm i.d.) prepacked with octadecyl μ-Bondapak packing; (2) Brownlee cartridges (3 cm × 4.6 mm) prepacked with octadecyl- or octyl-modified silica gel; (3) ES Industries columns (15 cm × 4.6 mm i.d.) prepacked with octyl- or octadecyl-modified silica gel. A silica saturator column positioned between the pumps and autoinjector to minimize dissolution of the packing within the analytical column. The saturator column was tap-filled with Reeve Angle Partisil 20/400 silica gel. A Rheodyne column inlet filter containing a 0.45-μm filter element was placed at the inlet of the analytical columns to prevent blockage of the inlet filter. The column dead-times used to calculate the capacity ratios in experiments 1–10 (Table II) were determined by the isotope method by using deuterium oxide (D_2O). In experiment II (Table II), capacity ratios were calculated by using t_o estimated by the D_2O method (see eq 5) and also from the retention times of urea measured at each mobile-phase composition (see eq 6). In experiment 12, capacity ratios were calculated by using the retention times of urea measured at each mobile-phase composition (eq 6) and from the value of t_o estimated by the isotope method with CD_3OD. The D_2O, CD_3OD, and urea bands were detected by using the UV detector (220 nm) as a refractometer; that is, by using the UV detector to detect changes in the refractive index of the eluent as these bands passed through the detector cell. Solute retention times were measured in triplicate initially and later in duplicate at ambient temperature (25 ± 2 °C). Changes in the volume fraction of organic modifier in the mobile phase were accomplished by computer-control with use of two pumps. The requested flow rate was 2.0 mL/min for all experiments. Retention times were obtained by using the data analysis capabilities of the chromatographic system. Regression analyses were preformed by using Prophet statistics.[50]

Preparation of the Mobile Phases. The aqueous portions of the mobile phase were prepared by first dissolving the buffering agent (MOPS (0.02 M) or phosphate salt (0.005 M)) and amino modifier (*n*-decylamine (0.15%, v/v) or *N*,*N*-diethylamine (0.075%, v/v)) in deionized/distilled water and then adjusting the pH of this solution to pH 7.4. In experiments 1 and 2 methanol was the only component in the organic portion of the mobile phase. In experiments 3–11, 1-octanol was added volumetrically to the methanol (0.25%, v/v) prior to filtering. All mobile-phase solvents were filtered through 0.45-μm filters before use and purged continuously with helium during each experiment. In experiments 11 and 12, the deionized/distilled water used to prepare the aqueous portion of the mobile phase was presaturated with 1-octanol.

The E_T(30) Experiment. Several milligrams of the dye were placed in ~10 mL of mobile phase obtained directly from the HPLC pumps. Visible spectra were acquired for solutions with eluent compositions of $0.25 \leq \phi \leq 1.0$, and the positions of λ_{max} located by inspection of the visible spectrum recorded on the strip chart; λ_{max} shifted from ~520 nm in 100% methanol to ~476 nm in 25% methanol. The eluent polarity values were then calculated from eq 7.

Acknowledgment. We wish to acknowledge Julie Sabatka for contributing many useful ideas that were instrumental in the development of this method.

Registry No. MeOH, 67-56-1; octanol, 111-87-5.

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(48) Since the submission of this manuscript, approximately 100 proprietary pharmaceutical research compounds have been analyzed by this method. Series ranged from 5 to 26 members and contained complex structures with multiple functional groups; one set consisted of a heterocyclic fused ring system containing 1 to 5 aromatic nitrogens. The correlation coefficients resulting from regressions of log k'_w values with either measured or calculated log $P_{o/w}$ data for these series varied between 0.91 and 0.99. log k'_w data for a series of six compounds correlated well with biological activity ($r = 0.99$).

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(50) Prophet statistics, National Institutes of Health (US), 1985.