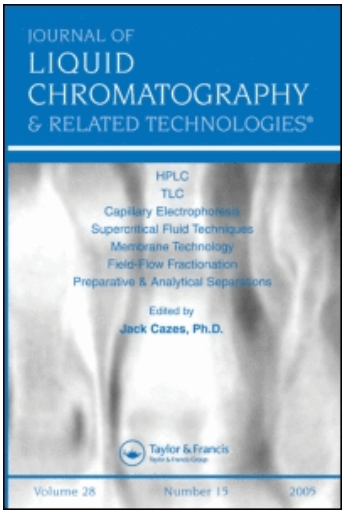

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Quantitative Structure-Activity Relationships Using Hydrophobicity Constants Measured by High-Pressure Liquid Chromatography: A Comparison with Octanol-Water Partition Coefficients

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QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIPS USING HYDROPHOBICITY CONSTANTS MEASURED BY HIGH-PRESSURE LIQUID CHROMATOGRAPHY: A COMPARISON WITH OCTANOL-WATER PARTITION COEFFICIENTS

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ABSTRACT

Capacity ratios (k') for a set of small organic compounds of miscellaneous structure were measured under a variety of reversed-phase liquid chromatoghraphic conditions. The capacity ratios from these experiments were correlated with the binding of these solutes to bovine serum albumin (BSA). Hydrophobic binding of small molecules to BSA is considered to be a nonspecific process (i.e., requiring no special orientation or restriction of movement of the solute molecules) and serves as a model for the hydrophobic binding of small molecules to other macromolecules, such as hemoglobin and ribonuclease. Standard deviations from these correlations were compared using the null hypothesis to determine the set of chromatographic conditions giving the best correlation with the binding constants. The null hypothesis was again applied to compare the correlation of the best chromatographic data with binding to the correlation of the binding data with the octanol-water partition coefficients of these compounds. For the chromatographic parameters varied here, the statistical differences between these methods are generally nonsignificant. However, the correlation involving partition coefficients is shown to be statistically better at the 99% confidence level than those involving capacity ratios.

INTRODUCTION

The goal of this study was to select a set of biological data from the literature that was considered to be highly reliable and had been accurately modeled by measured octanol-water partition coefficients (log P) and to compare this correlation with correlations involving capacity ratios measured from various reversed-phase liquid chromatography (RPLC) experiments. The set of data selected for this study is shown in Table 1.

The binding of small molecules to BSA was chosen because it is considered to exemplify nonspecific binding of small molecules to other macromolecules such as hemoglobin and ribonucleases (1). The good correlation between the binding constants of the solutes shown in Table 1 and their partition coefficients was taken as an indication that the linear free energy changes in binding and those associated with the transference of small molecules from water to octanol in the partitioning system were similar and that binding of small organic molecules to BSA was a nonspecific process involving only hydrophobic interactions; as in the partitioning process, the movement of the molecules was essentially unrestricted and required no special orientation when binding to BSA.

Although log $P_{O/W}$ has become the standard method for quantifying the hydrophobicity of organic compounds and modeling biological membranes (2), the conventional shake-flask method is problematic, and numerous studies have been undertaken in the past ten years to use thin-layer chromatography (3) or RPLC (4-6) to predict octanol-water partition coefficients. The chromatographic methods are not without problems of their own. Some of the advantages and disadvantages of log P and RPLC methods are outlined in Table 2.

TABLE 1

Octanol-Water Partition Coefficients and Bovine Serum Albumin (BSA) Binding Constants for a Set of Small Organic Molecules.[a]

	Compound	Log $P_{O/W}$	Log (1/C)
1)	phenol	1.46	3.32
2)	3-fluorophenol	1.93	3.86
3)	4-fluorophenol	1.77	3.52
4)	3-chlorophenol	2.50	4.30
5)	4-chlorophenol	2.39	4.00
6)	4-bromophenol	2.59	4.22
7)	4-iodophenol	2.91	4.40
8)	4-methylphenol	1.94	3.70
9)	3-ethylphenol	2.40	4.22
10)	3-trifluoromethylphenol	2.95	4.52
11)	3-cyanophenol	1.70	3.26
12)	3-hydroxyphenol	0.80	3.15
13)	3-methoxyphenol	1.58	3.54
14)	4-methoxyphenol	1.34	3.40
15)	3-nitrobenzonitrile	1.17	2.94
16)	4-methoxybenzyl alcohol	1.10	2.94
17)	benzonitrile	1.56	3.23
18)	acetophenone	1.58	3.31
19)	nitrobenzene	1.85	3.58
20)	4-bromoacetanilide	2.29	4.00
21)	4-nitroanisole	2.03	4.00
22)	4-chloronitrobenzene	2.39	4.07
23)	naphthalene	3.30	4.91
24)	azobenzene	3.82	5.29
25)	anisole	2.11	4.00
26)	3-fluoroaniline	1.30	3.09
27)	4-chloroaniline	1.83	3.68
28)	4-methoxyaniline	0.95	2.92
29)	4-bromoaniline	2.26	4.06
30)	4-methylaniline	1.39	3.30
31)	1-naphthylamine	2.26	3.94
32)	indole	2.14	4.07
33)	camphorquinone	1.52	3.17
34)	thymol	3.30	4.66

a) Reference 1.

TABLE 2

Comparison of HPLC and Log P For Measuring Hydrophobicity

	Advantages	Disadvantages
Log P	o Good reflection of characteristics of some biological lipid phases. o Highly sensitive to differences in hydrophobicity. o Values available for ≈10,000 substances. o Many studies available on structure-activity relationships and bioaccumulation. o Calculation methods available. o Measuring range: -2 to 4.	o Not applicable to surface-active or organometallic substances. o Applicable to a limited extent to ionic cmpds and cmpds. o Errors caused by the presence of impurities. o Sample loss due to instability of solute. o Lack of a generally accepted method resulting in neglect of buffer species, temperature, ionic strength, and pH; e.g. log P of butyl amine varies from 0.68 to 1.02. o Formation of emulsions. o Method is time-consuming, resulting in low sample throughput. o Unreliable calculations for novel or complex structures.
HPLC	o Fast method; high sample throughput; readily automatable. o Suitable for substances containing impurities and mixtures. o Requires no quantitation. o Applicable to volatile substances. o Well suited to hydrophobic substances. o Highly reproducible. o Measuring range of ca. -2 to 8.	o Not applicable to organometallic substances. o Capacity ratios reflect mixed mechanism of retention; adsorption and partition vs pure liquid-liquid partitioning in log P. o Capacity ratios represent a dynamic equilibrium; log P represents a static equilibrium. o Hydrogen bonding and steric effects are less attenuated than in octanol-water partitions. o Column instability.

In 1951, Collander (7) observed that partition coefficients measured in one organic phase could be used to calculate those for another organic phase according to equation 1:

$$\log P_{1/w} = a \log P_{2/w} + b \qquad (1)$$

where 1 and 2 denote the different organic phases, and w = water. Leo (8) later demonstrated that this type of relationship was valid only if the solutes being compared were members of a congeneric series or if the properties of the organic phases were sufficiently similar, e.g. octanol vs pentanol. Most of the correlations between log P and the logarithm of capacity ratios (log k') have assumed that the relationship between these variables is linear and based upon a Collander-type relationship:

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

This type of relationship has proven moderately successful in predicting partition coefficients. However, as Valko demonstrated (9), this equation is invalid when comparing log P values with log k' values for a noncongeneric series of compounds measured using acetonitrile as the organic modifier in the mobile phase. Instead an extended version of this equation (equation 3) must be used:

$$\log P_{o/w} = a \log k'_w + b\,(S) + c \qquad (3)$$

Here, $\log k'_w$ = the logarithm of the capacity ratio measured in a totally aqueous eluent, and S = the slope obtained from log k' vs ϕ

graphs (discussed below). This general equation reduces to the simpler Collander-type equation (eq. 2) when log k' is correlated with log P for congeneric series or when methanol is used as the organic modifier in the mobile phase (10).

Log k'_W cannot generally be measured and is usually obtained by linear extrapolation according to the relationship described by Snyder et al. (11):

$$\log k'_\phi = \log k'_W - S \times \phi \qquad (4)$$

where ϕ = volume fraction of organic modifier in the mobile phase, S is the slope of the linear portion of this plot, (in general, $0.20 \leq \phi \leq 0.80$), and log k'_W is the intercept obtained by extrapolating the linear portion to 100% water; see Figure 2 for examples of this type of graph. When acetonitrile-water is used as the eluent, values of the slope (S) and log k'_W for structurally dissimilar compounds are highly independent, as shown in Fig. 1-a. However, when methanol-water is used to obtain these parameters, they are highly dependent, as can be seen in Figure 1-b. Log k'_W is generally considered to be proportional to the hydrophobic surface area of a molecule (2,13) and the slope parameter a function of solute-solvent and solute-stationary phase interactions, as well as the size and shape of the solute (13). Nonetheless, a precise explanation of what molecular properties these parameters represent is at present unavailable. We believe that the differences shown in Figure 1 indicate that the slope and log k'_W must be proportional to similar molecular properties in methanol-water but not in acetonitrile-water. Because of the high degree of correlation between log k'_W and S for noncongeneric

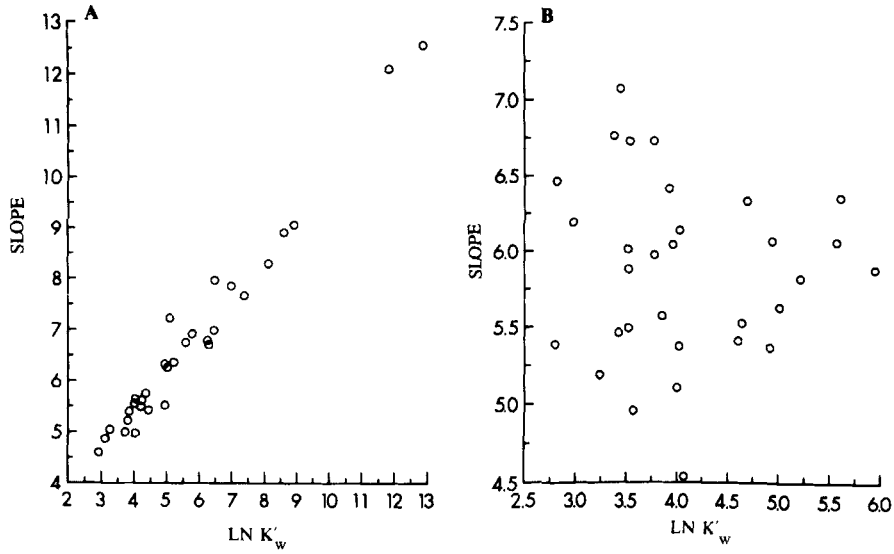

Figure 1. Correlation between the slope and intercept (ln k'_w) derived from the straight-line approximations of ln k' versus ϕ for different organic modifier systems: (a) for methanol-water, $S = 2.27 + 0.79 \ln k'_w$, correlation coefficient = 0.98; (b) for acetonitrile-water, no correlation, (coef. = -0.06). P.J. Schoenmaker, H.A. Billet and L. de Galan, *J. Chrom.* 185 (1979) 179-195.

compounds, equation 2 has proven to be adequate in most log $P_{O/W}$ vs log k'_w correlations when methanol-water is used as the eluent. However, the use of any Collander-type equation for correlating log k' with log P has been criticized. Wells and Clark (14) pointed out that log k' and log P would be linearly proportional only if log k' resulted from a pure liquid-liquid interaction and the stationary phase behaved like a monolytic alcohol, neither of which is entirely true. Although both log P and log k' involve hydrophobic expulsion of the solute from the aqueous phase, the mechanism of retention in RPLC is undoubtedly mixed, involving features of both adsorption and partitioning. Additionally, most RPLC measurements are made using

alkyl-bonded phases, resulting in nonequivalent hydrogen bonding effects when compared to log P. The stationary phase cannot be considered to be purely alkyl in composition due to the enrichment of this liquid phase with the organic component from the mobile phase. In this respect, capacity ratios measured using methanol-water as eluent should give better correlations with octanol-water partition coefficients than those measured using acetonitrile-water since a methanol enriched alkyl-bonded surface would be expected to have solvation properties more similar to a monolytic alcohol (i.e., more octanol-like) than an alkyl stationary phase enriched with acetonitrile. In addition to these basic differences between log P and log k', Wells and Clark (14) pointed out that log P describes a static equilibrium, while log k' describes a dynamic equilibrium. In view of these problems and the difficulties inherent in the shake-flask technique, we decided to measure capacity ratios for the solutes shown in Table 1 under a variety of chromatographic conditions. These sets of data were correlated directly with the binding constants shown in Table 1 in order to determine a set of RPLC conditions that best modeled non-specific hydrophobic binding to BSA. The regression results were compared quantitatively using the null hypothesis (15) to determine the RPLC conditions giving the best correlation. The standard deviation of the best RPLC method was then compared by the null hypothesis to that obtained by correlating log P with log (1/C).

The selection of various chromatographic conditions was based upon experiments in the literature that reported good correlations between log k' and log $P_{o/w}$. Evaluation of the stationary phase was based upon the work of Thus and Kraak (16), who reported better correlations with log P using capacity ratios measured on phenyl-

modified silica instead of octadecyl-modified silica. Additionally, they correlated log P with log k' values obtained under three mobile phase conditions: 1) isocratic methanol/water mixtures (unbuffered); 2) isocratic mixtures of methanol/water, but buffering the aqueous phase with 0.005-M phosphate and adjusting the pH to 7.4 prior to mixing with methanol; 3) extrapolation of log k'_{ϕ} vs ϕ plots to 100% water using mixtures of methanol and water (unbuffered). Log k'_{w}, extrapolated from solutions of methanol-water (unbuffered), gave the best regression results (the lowest standard deviation) when correlated with log P. As we were interested in examining data obtained isocratically and by extrapolation, we decided to include these mobile phases in our study. We also decided to include an extrapolation procedure utilizing a buffered aqueous phase that was pH adjusted to 7.4 prior to mixing with methanol. The mobile phase selected for this experiment was reported by El Tayar et al. (17). The aqueous portion of this mobile phase (pH 7.4) contained the zwitterionic buffer 3-(N-morpholino) propanesulphonic acid (MOPS) and n-decyl amine (to mask silanophilic interactions). MOPS is an organic buffer and should be more soluble in mobile phases of high methanol content than an inorganic buffer, avoiding problems that may arise from precipitation of buffer salts. Log k'_{ϕ} values, used to derive log k'_{w}, were measured using mobile phases with methanol compositions up to 75% in our study and have been used up to 80% by El Tayar et al. (17,18). Also, unlike many organic buffers, MOPS has been reported not to form ion-pairs (19). Extrapolation of log k' to a buffered aqueous solution rather then pure water may prove necessary if reproducible retention times are to be obtained for compounds that are ionizable under chromatographic conditions or if corrections to log k'_{w} for ionization are to be made, see ref. 18.

EXPERIMENTAL

Materials

HPLC grade methanol was purchased from Fisher Scientific. Analytical grade sodium phosphate (dibasic) was purchased from Mallinkrodt, 3-(N-morpholino)propanesulphonic acid (MOPS) from ICN Biochemicals, and n-decyl amine from Aldrich.

Chromatography

A Waters 840 Chromatographic System equipped with two Model 510 pumps, a Model 590 pump, a WISP auto-injector, and a Model 441 monochromatic uv detector (wavelength: 254 nm) was used. Columns (15 cm x 3.9 mm I. D.) were prepacked with Waters Phenyl u-BONDAPAK and Waters Octadecyl u-BONDAPAK packings, (10 um particle size). A guard column (25 cm x 4.6 mm I. D.) tap-filled with Porasil A (37-75 um particle size) was placed between the pumps and auto-injector to protect the analytical columns from damage by buffer salts. A DEC Pro 350 data station, which controls the 840 system, was used for data acquisition and determination of retention times. The void volumes for the columns were determined by the isotope method (20) using D2O as the unretained solute and a mobile phase of 100% water. Retention times were measured at ambient temperature (22 $\pm$ 2°C) and the capacity ratios calculated according to equation 5:

$$k' = (t_r - t_o)/t_o \qquad (5)$$

where t_r = the retention time of the solute, and t_o = the column void volumn. The flow rate was 2.0 ml/min for all retention measurements. The pH of the phosphate and MOPS buffers were adjusted to 7.4 in the aqueous solutions (i.e., prior to mixing with methanol) using HCl

and NaOH. n-Decyl amine (0.2% V/V) was used in the MOPS buffer as a masking agent for silanophilic interactions. The preparation of the MOPS buffer has been described elsewhere (18).

RESULTS AND DISCUSSION

The results of measuring capacity ratios under the four mobile phase conditions for the phenyl support are shown in Table 3 and those under the same conditions for the octadecyl support in Table 4. Log k'_w values were calculated using equation 3; in almost all cases $r > 0.99$. Typical straight-line approximations of log k'_ϕ vs ϕ for the phenyl and octadecyl columns are shown in Figure 2. These graphs demonstrate one of the advantages of using log k'_w values instead of log k' values measured isocratically; the problem of peak inversion is averted. In Figure 2-a, the retention time of nitrobenzene is greater than that of 4-bromoaniline above approximately 35% methanol ($\phi \simeq 0.35$) in the mobile phase, but less than that of 4-bromoaniline below that percentage. Examination of the four log k' values reported for these compounds in Table 3 (nos. 19 and 29, respectively) indicates that only the extrapolated values correctly predicted the order of relative hydrophobicities reflected by the partition coefficients and binding constants reported in Table 1. Similarly, for the octadecyl column (Figure 2-b), the retention times of these solutes inverted near 40% methanol ($\phi \simeq 0.40$) and the order of relative hydrophobicities was again correctly predicted only by the extrapolated log k' values shown in Table 4. In our study, the highly dependent nature of log k'_w and slope (S) when using methanol as the organic modifier is shown in Table 5. The log k'_w vs S correlations are better for the octadecyl support than the phenyl support, but good linearity was

TABLE 3

Log k' Terms Used in Regressions With Binding Constants; Phenyl Support.

Cmpd	log k'[a]	log k'[b]	log k'_w[c]	slope[c]	r[e]	log k'_w[d]	slope[d]	r[e]
1	0.093	0.12	0.99	2.52	0.999	0.94	2.30	1.000
2	0.29	0.29	1.30	2.85	0.999	1.28	2.71	1.000
3	0.22	0.23	1.20	2.77	0.999	1.17	2.60	1.000
4	0.57	0.55	1.68	3.16	0.999	1.70	3.11	1.000
5	0.54	0.54	1.65	3.13	1.000	1.64	3.03	1.000
6	0.65	0.64	1.80	3.28	0.999	1.83	3.24	1.000
7	0.81	0.79	2.02	3.47	1.000	2.08	3.50	1.000
8	0.37	0.38	1.37	2.86	1.000	1.35	2.71	1.000
9	0.64	0.65	1.77	3.23	1.000	1.77	3.14	1.000
10	0.77	0.75	2.06	3.67	1.000	2.11	3.67	1.000
11	0.28	0.26	1.35	3.04	1.000	1.39	2.98	1.000
12	-0.35	-0.27	0.60*	2.72*	1.000	0.52	2.31	1.000
13	0.25	0.28	1.23	2.80	1.000	1.15	2.56	1.000
14	0.15	0.18	1.12	2.78	1.000	0.98	2.38	1.000
15	0.43	0.45	1.33	2.56	1.000	1.23	2.31	1.000
16	0.23	0.26	1.18	2.72	1.000	1.04	2.36	1.000
17	0.50	0.53	1.50	2.85	1.000	1.42	2.61	1.000
18	0.56	0.58	1.57	2.91	1.000	1.46	2.65	1.000
19	0.58	0.60	1.53	2.71	1.000	1.50	2.59	1.000
20	0.73	0.76	1.90	3.36	1.000	1.86	3.21	1.000
21	0.81	0.82	1.89	3.12	1.000	1.85	2.99	1.000
22	0.84	0.84	1.93	3.07	1.000	1.87	2.93	1.000
23	1.22	1.20	2.54	3.79	1.000	2.50	3.65	1.000
24	n.d.	1.73	3.48	4.78	1.000	3.37	4.53	0.999
25	0.62	0.62	1.61	2.81	1.000	1.54	2.63	1.000
26	0.19	0.20	1.11	2.60	0.998	1.00	2.31	1.000
27	0.47	0.47	1.50	2.93	1.000	1.42	2.72	1.000
28	0.32	0.14	0.90*	1.69*	0.989	0.77	2.06	1.000
29	0.58	0.57	1.65	3.06	1.000	1.58	2.89	1.000
30	0.45	0.33	1.35	2.55	0.990	1.14	2.39	1.000
31	0.75	0.73	1.99	3.34	1.000	1.83	3.17	1.000
32	0.55	0.55	1.60	2.98	1.000	1.55	2.83	1.000
33	0.67	0.69	1.74	3.06	1.000	1.61	2.77	1.000
34	1.16	1.13	2.55	3.98	1.000	2.48	3.80	1.000

a) methanol/water, 35% (V/V), isocratic.
b) methanol/water (phos. buffered), 35% (V/V).
c) methanol/water, extrapolation method.
d) methanol/water (MOPS buffered), extrapolation method.
e) r = correlation coefficient;
* 3 points only.
n.d.= not determined.

TABLE 4

Log k′ Terms Used in Regressions With Binding Constants; Octadecyl Support.

Cmpd	log k′[a]	log k′[b]	log k'_w[c]	slope[c]	r[e]	log k'_w[d]	slope[d]	r[e]
1	0.12	0.062	1.24	2.48	1.000	1.21	2.47	1.000
2	0.35	0.28	1.63	2.85	1.000	1.64	2.90	1.000
3	0.25	0.19	1.49	2.74	1.000	1.49	2.79	1.000
4	0.65	0.58	2.08	3.19	1.000	2.10	3.26	1.000
5	0.62	0.55	2.04	3.17	1.000	2.04	3.21	1.000
6	0.72	0.65	2.19	3.28	1.000	2.18	3.30	1.000
7	0.86	0.79	2.43	3.50	1.000	2.43	3.55	1.000
8	0.42	0.36	1.71	2.89	1.000	1.67	2.83	1.000
9	0.72	0.66	2.20	3.32	1.000	2.15	3.28	1.000
10	0.90	0.82	2.62	3.82	1.000	2.60	3.83	1.000
11	0.20	0.054	1.52	2.92	1.000	1.59	3.04	0.999
12	-0.43	-0.53	0.68	2.44	1.000	0.71	2.52	1.000
13	0.17	0.11	1.36*	2.66*	1.000	1.33*	2.64*	1.000
14	0.039	-0.021	1.15	2.48	1.000	1.10	2.44	1.000
15	0.30	0.23	1.47	2.61	1.000	1.37	2.49	1.000
16	0.15	0.092	1.30	2.59	1.000	1.19	2.44	1.000
15	0.41	0.34	1.67	2.81	1.000	1.55	2.67	1.000
18	0.47	0.41	1.72	2.81	0.999	1.59	2.65	1.000
19	0.58	0.51	1.83	2.79	1.000	1.74	2.69	1.000
20	0.71	0.66	2.18	3.28	1.000	2.10	3.19	0.999
21	0.75	0.68	2.15	3.13	1.000	2.06	3.04	1.000
22	0.86	0.80	2.29	3.20	1.000	2.20	3.10	1.000
23	1.32	1.27	3.06	3.89	1.000	3.00	3.84	1.000
24	n.d.	1.95	4.00	4.70	1.000	3.93*	4.66*	1.000
25	0.69	0.64	1.97	2.86	1.000	1.89	2.78	1.000
26	0.14	0.098	1.29	2.56	1.000	1.24	2.51	1.000
27	0.42	0.38	1.72	2.88	1.000	1.69	2.87	1.000
28	0.027	-0.15	0.85	1.87	0.998	0.81	2.16	1.000
29	0.52	0.49	1.87*	3.00*	1.000	1.84	3.00	1.000
30	0.32	0.24	1.38	2.36	1.000	1.35	2.48	1.000
31	0.62	0.58	2.10	3.29	1.000	2.02	3.20	1.000
32	0.53	0.48	1.87	2.98	1.000	1.83	2.94	1.000
33	0.53	0.50	1.87	2.98	1.000	1.77	2.87	1.000
34	1.29	1.23	3.13	4.10	1.000	3.03	4.00	1.000

a) methanol/water, 45% (V/V), isocratic.
b) methanol/water (phos. buffered), 45% (V/V).
c) methanol/water, extrapolation method.
d) methanol/water (MOPS buffered), extrapolation method.
e) r = correlation coefficient.
* 3 points only.
n.d.= not determined.

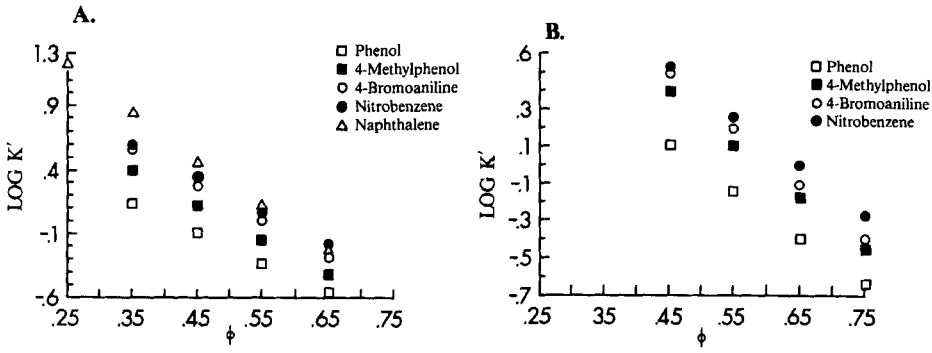

Figure 2. Straight-line approximations of log k' vs ϕ for (a) phenyl-modified silica, and (b) octadecyl-modified silica; $r \geq 0.998$ for all lines.

TABLE 5

Linear Regression Data for the Correlation of Slope Parameter(S) With the Intercept (log k'_w).

Calculated according to: $S = a \log k'_w + b$

Support	Conditions	a	b	n	r	F	sd
Phenyl	methanol/water (unbuffered)	0.98 (0.10)	0.81 (0.31)	34	0.865	95	0.299
	methanol/water containing MOPS	0.91 (0.05)	1.45 (0.08)	34	0.958	355	0.152
ODS	methanol/water (unbuffered)	0.80 (0.04)	1.50 (0.07)	34	0.970	514	0.133
	methanol/water containing MOPS	0.78 (0.04)	1.55 (0.07)	34	0.968	475	0.134

n = no data points; r = correlation coef.; F = F-test; sd = standard deviation.

observed between these parameters for the phenyl support using the methanol/MOPS buffered mobile phase.

The log k' data from Tables 3 and 4 was correlated with the binding data in Table 1 according to equation 6-a for isocratic data and equation 6-b for extrapolated data:

$$\log (1/C) = a \log k'_{\phi} + b \qquad (6\text{-a})$$

$$\log (1/C) = a \log k'_{w} + b \qquad (6\text{-b})$$

where $\phi = 0.35$ for the phenyl support and $\phi = 0.45$ for the octadecyl support. The data used in these correlations are shown graphically in Figure 3 for the phenyl support and in Figure 4 for the octadecyl support. The results of the linear regressions are presented in Table 6. The null hypothesis of the equality of variances had been used previously to quantitatively compare regression results from different methods utilizing the same dependent variable (21). According to this hypothesis, values of F are calculated from a table of distributions at the 95% and 99% confidence levels depending on the number of degrees of freedom (df, where df = no. data points -1). The observed distribution of F (F_o) is derived according to equation 7:

$$F_o = \frac{sd(1)^2}{sd(2)^2} \qquad (7)$$

where sd = standard deviation of regression, and $sd(1) > sd(2)$. If $F_o > F$, then the method with the lowest standard deviation is considered to be statistically better at the confidence level evaluated. For our experiments, 34 data points were used in the regressions with

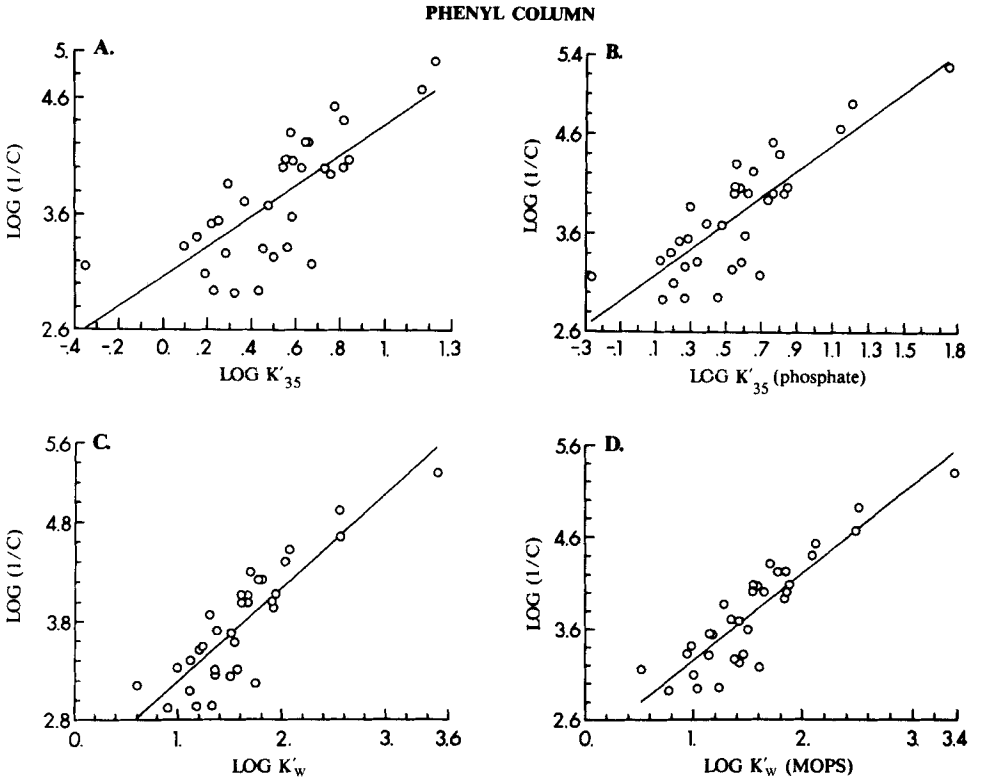

Figure 3. Log-log relationships between bovine serum albumin binding constants (1/C) and capacity ratios (k') for the 34 test solutes shown in Table 1: (a) mobile phase, methanol-water 35:65 (V/V), isocratic log k'; (b) mobile phase, methanol-water, 35:65 (V/V) containing 0.005-M phosphate (pH 7.4), isocratic log k'; (c) mobile phase, methanol-water, extrapolated log k'; (d) mobile phase, methanol-water containing 0.02-M morpholinopropane sulfonic acid and 0.2% (V/V) n-decyl amine (pH 7.4), extrapolated log k'.

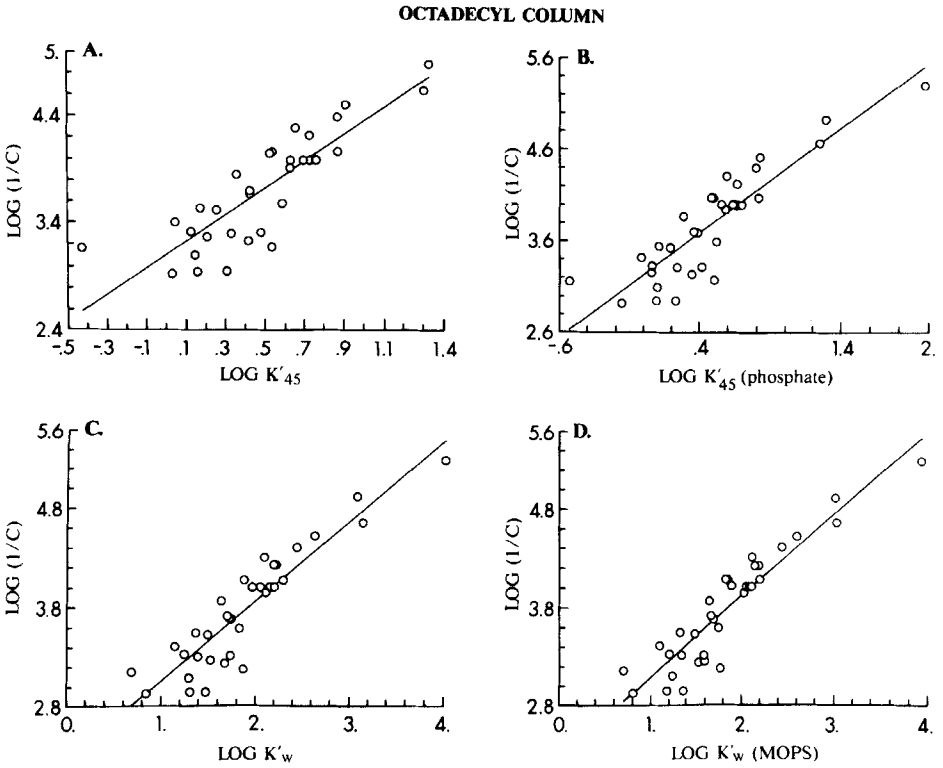

Figure 4. Log-log relationships between bovine serum albumin binding constants (1/C) and capacity ratios (k') for the 34 test solutes shown in Table 1: (a) mobile phase, methanol-water 45:55 (V/V), isocratic log k'; (b) mobile phase, methanol-water, 45:55 (V/V) containing 0.005-M phosphate (pH 7.4), isocratic log k'; (c) mobile phase, methanol-water, extrapolated log k'; (d) mobile phase, methanol-water containing 0.02-M morpholinopropane sulfonic acid and 0.2% (V/V) n-decyl amine (pH 7.4), extrapolated log k'.

TABLE 6

Linear Regression Data for the Correlation of Bovine Serum Albumin (BSA) Binding Constants (1/C) with Capacity Ratios (k') Measured Under Various Chromatographic Conditions.

Calculated according to: $\log (1/C) = a \log k' + b$

Method	Conditions	a	b	n	r	F	sd
1)	phenyl support, methanol/water 35:65 (V/V) isocratic.	1.30 (0.20)	3.07 (0.12)	33	0.753	40.5	0.355
2)	phenyl support, methanol/water 35:65 (V/V), phosphate buffer, isocratic.	1.33 (0.16)	3.07 (0.11)	34	0.818	64.8	0.342
3)	phenyl support, methanol/water extrapolated.	0.95 (0.10)	2.24 (0.17)	34	0.864	94.2	0.300
4)	phenyl support, methanol/water containing MOPS, extrapolated.	0.95 (0.09)	2.30 (0.14)	34	0.891	124	0.270
5)	ODS Support, same as (1) above but 45:55 (V/V).	1.27 (0.14)	3.11 (0.08)	34	0.853	83.2	0.281
6)	ODS Support, same as (2) above but 45:55 (V/V).	1.16 (0.11)	3.24 (0.07)	34	0.881	111	0.281
7)	ODS Support, same as (3) above	0.81 (0.07)	2.25 (0.13)	34	0.909	152	0.248
8)	ODS Support, same as (4) above	0.83 (0.06)	2.25 (0.12)	34	0.925	189	0.226

n = no data points; r = correlation coef.; F = F-test; sd = standard deviation.

one exception; see method 1, Table 6. The F values corresponding to 33 x 33 degrees of freedom were calculated from Table IV of reference 15. At the 95% confidence level F = 1.79, and at the 99% confidence level F = 2.29. Comparison of the observed F distributions derived from the standard deviations in Table 6 and equation 7 indicate that extrapolated capacity ratios from Method 8 (Table 6) correlated better with binding than either Methods 1 or 2 (Table 6) at the 99% confidence level and better than Method 3 (Table 6) at slightly less than the 95% confidence level. These findings directly contrast those of Thus and Kraak (16), who reported their best correlations between log $P_{O/W}$ and log k' using a phenyl support instead of an octadecyl support. Comparison of the remaining standard deviations in Table 6 for Methods 4-8 indicate that improvements in the standard deviations are statistically nonsignificant at the confidence levels evaluated.

The result of correlating the partition coefficients and binding constants reported in Table 1 is shown in equation 8:

$$\log (1/C) = 0.81(\ 0.03)\ \log P_{O/W} + 2.15(0.07)$$

n	r	F	sd	
34	0.973	572	0.137	(8)

where the standard deviations are shown in parentheses. The observed distribution of F derived from the standard deviation in equation 8 (sd = 0.137) and the RPLC method wth the lowest standard deviation (Method 8, Table 6) indicates that the correlation between log P and log (1/C) is better than any of the correlations involving log k' and log (1/C) at the 99% confidence level ($F_O > 2.72$).

To understand why log P modeled the binding so much better than log k', log (1/C) vs log P and log (1/C) vs log k'_W were plotted

according to functional group classes. These graphs are shown in Figures 5-a and 5-b, respectively; the data used for log k'_w was taken from column 7, Table 4. In the graph of log (1/C) vs log P, the functional group classes appear scattered fairly randomly about the line fit to that data, indicating that binding and partitioning are both nonspecific processes. However, this is not the case for the correlation of RPLC retention with binding for small molecules. In Figure 5-b, thirteen of the fourteen phenols examined lie above the fitted line, while all of the compounds with an unsaturated group exo to the benzene ring lie below the fitted line; the phenol lying below the line is cyanophenol, which is the only phenol containing an unsaturated functional group. Figure 5-b indicates that log k'_w does not represent a nonspecific interaction, as do log P and BSA binding. On the contrary, log k'_w appears to reflect interactions with the stationary phase in addition to simple hydrophobic ones suggesting that this partameter is not simply a function of the hydrophobic surface area of a compound, but also of molecular properties involving polar and hydrogen bonding interactions with the surface of the stationary phase (adsorptive interactions). The specificity of adsorptive interactions would depend on the type and location of substituents in a molecule, introducing orientation effects on retention that are absent from pure liquid-liquid partitioning. Such orientation effects have been reported for a series of biphenyl acids (22). These conclusions lend support to the criticisms by Wells and Clark that linear equations do not accurately describe log P vs log k' correlations.

In Table 5, log k'_w and slopes were shown to be highly dependent variables, and consequently, the correlation of the slope parameter

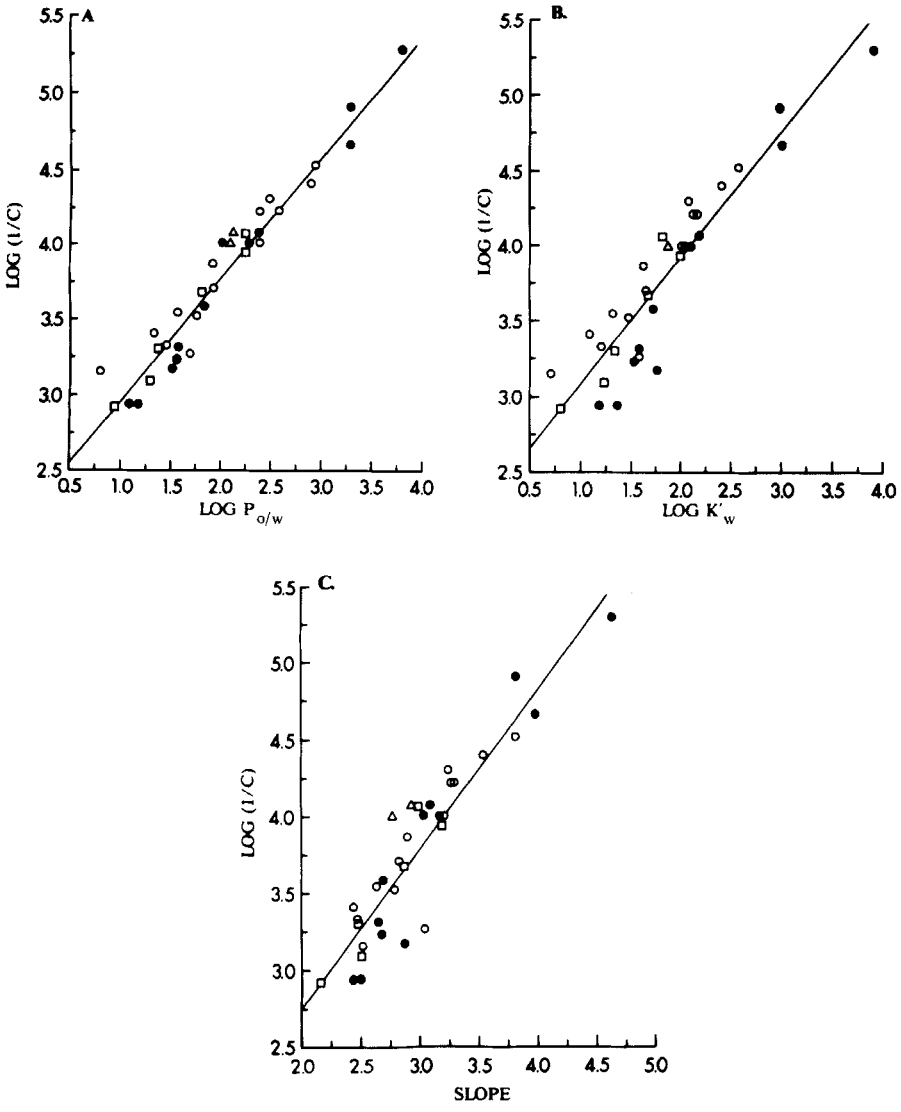

Figure 5. Log-log relationships between bovine serum albumin binding constants (1/C) and (a) octanol-water partition coefficients ($P_{O/W}$), (b) extrapolated capacity rations (k'_W) using octadecyl-modified silica and a mobile phase of methanol-water containing 0.02-M morpholinopropane sulfonic acid and 0.2% (V/V) n-decyl amine, and (c) the slope values under the same conditions as (b); o phenols, Δ anilines, ● unsaturated compounds (nitrobenzene, benzonitrile, etc.), □ miscellaneous.

with binding was examined. The slope values used in this correlation were taken from column 8, Table 4. The result of this regression is shown in equation 9:

$$\log (1/C) = 1.03(0.08)\ S + 0.69(0.23)$$

n	r	F	sd	
34	0.924	186	0.228	(9)

where the standard deviations are shown in parentheses. Although the standard deviation is essentially equal to that obtained from log (1/C) vs log k'_w, a graph of the data indicated that the functional group types were scattered more randomly about the fitted line. This is shown in Figure 5-c. These results suggest that 1) the slope parameter is more nonspecific in nature then log k'_w and may more accurately reflect the hydrophobic properties of a molecule than the intercept (log k'_w), and 2) the slope parameter should be evaluated in the development of quantitative structure-retention relationships between chromatographic retention data of either liquid-liquid partition coefficients or biological activity known to involve nonspecific hydrophobic binding.

CONCLUSION

The main results from this study can be summarized as follows:

1) Capacity ratios obtained using an ODS support gave statistically significant improvements in correlations with the binding of a series of small organic molecules to bovine serum albumin over those obtained using a phenyl-modified support.

2) Log k'_w values obtained by extrapolation to 100% aqueous gave only qualitatively better correlations with binding than

isocratically measured retention data, but have the advantage of being able to predict more accurately the order or relative hydrophobicities.

3) The correlation of octanol-water partition coefficients was statistically better at the 99% confidence level than correlations of RPLC retention data with binding.

4) Liquid-liquid partitioning and binding of small organic molecules to BSA are both nonspecific processes while log k'_w appears to be more specific in nature, resulting in poorer modeling of binding to BSA than log $P_{o/w}$.

5) Slope parameters appear to be less specific than the intercept parameters (log k'_w), possibly reflecting more accurately the hydrophobic properties of molecules as modeled by octanol-water partition coefficients and should be considered in quantitative structure-retention relationships with processes such as log $P_{o/w}$ and BSA binding, both of which are nonspecific.

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