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STUDY OF THE RELATIONSHIP BETWEEN DYNAMIC AND STATIC EQUILIBRIUM METHODS FOR THE MEASUREMENT OF HYDROPHOBICITY

COMPARISON OF CAPACITY FACTORS AND PARTITION COEFFICIENTS FOR SOME 5,5-DISUBSTITUTED BARBITURIC ACIDS

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SUMMARY

The capacity factors for twelve 5,5-disubstituted barbituric acid derivatives in a reversed-phase chromatographic system are reported. The solvophobic theory was used to describe the relationship between the capacity factor and eluent composition in order to determine log k'_w (the hypothetical capacity factor in totally aqueous eluent) for each solute. The dynamically derived descriptor of hydrophobicity, log k', was compared with log P, a static equilibrium descriptor. Comparisons were made between log k' values determined at various solvent compositions and log P values measured using solvents of differing polarities as the organic component of the binary mixture.

INTRODUCTION

Hydrophobic parameters are useful predictors for those molecular behaviors which depend upon the relative distribution of molecules between hydrophilic and lipophilic regions. The distribution of solutes between water and a variety of water-immiscible solvents is often used as an index of hydrophobicity, *i.e.*, the partition coefficient, P. Many of these values have been measured and tabulated or can be calculated[1,2].

With the present increase in the use of reversed-phase liquid chromatography (RPLC) has come a proliferation of articles which attempt correlation of the chromatographic capacity factor, k', with the partition coefficient[3–10]. There is no question of the potential utility of using RPLC to predict hydrophobicity in cases when traditional shake-flask methods are difficult to use or in the limited availability of pure

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compound. The most appropriate RPLC parameter for quantitative correlation with hydrophobicity is the capacity factor determined in a totally aqueous eluent (k'_w or k'_0). However, many reports have demonstrated the correlation between partition coefficients and the reversed-phase capacity factor obtained at various solvent compositions other than pure water[3,4,6,8,9]. Even when the capacity factor is measured at an aqueous–organic solvent mixture other than 100% aqueous, correlations are generally observed between log k' and log P for selected series of congeners.

D'Amboise and Hanai[7] have shown that different families of compounds are described by separate lines on a log k' *versus* log P plot when measured in water–acetonitrile (50:50) on an octadecyl packing. This observation is not unexpected according to hydrophobic theory. When an organic component is added to water (as with the methanol– or acetonitrile–water eluents used in RPLC), the hydrophobic effect decreases because the hydrogen bonds can no longer form an isotropic network throughout the solvent as in pure water. The organic component exerts its own solvophobic effect, but water exhibits the strongest solvophobic effect, so that the overall solvophobic effect is diminished as the organic component increases. However, the rate of decrease is solute dependent and this can cause a family dependence in log k' *versus* log P plots measured in a binary solvent system. If a column dependent continuum exists for a diversity of compounds in a log k' *versus* log P plot it is most likely to be obtained from capacity factor measurements derived in a totally aqueous eluent in which the only solvophobic effect is the hydrophobic effect.

However, k'_w can only be directly obtained for a relatively small number of solutes. Therefore, some means of predicting this value must be utilized. Historically, linear extrapolation to the intercept of a capacity factor *versus* organic modifier concentration plot has been used to predict log k'_w. Butte *et al.*[5] and Hammers *et al.*[10] have used linear extrapolation to derive k'_w values and compared these numbers with log P values determined in octanol–water. However, results in this laboratory[11,12] and others[13] suggest that linear extrapolation may not be an adequate predictor of log k'_w values. In this paper, the theoretical model[14,15] of Horváth and Melander, commonly known as the solvophobic theory, has been used to derive the log k'_w values for twelve barbiturates. The results of this mathematical modelling are described along with the observed relationships between chromatographic capacity factors and partition coefficients.

EXPERIMENTAL

All chemicals were of reagent-grade quality or better and were used as purchased. Spectrophotometric grade acetonitrile was obtained from Fisher Scientific (Fair Lawn, NJ, U.S.A.) and sodium nitrate was purchased from Allied Chemicals (Morristown, NJ, U.S.A.).

The liquid chromatograph consisted of a Waters (Milford, MA, U.S.A.) 6000A solvent pump, U6K injector, 440 UV-absorbance detector and a Linear (Sunnyvale, CA, U.S.A.) recorder. The column (15 cm × 4.6 mm I.D.) was packed with Supelcosil (Supelco, Bellefonte, PA, U.S.A.) LC-18, a 5-μm spherical octadecylsilane stationary phase. The analytical column was preceded by a guard column (7 cm × 2.1 mm I.D.) dry-packed with Whatman (Clifton, NJ, U.S.A.) CO:PELL ODS (30–38 μm). The mobile phase consisted of various portions of acetonitrile and water (chro-

matographic grade) mixed on a volume–volume basis and pumped at 1.5 ml/min. Chromatographic grade water was prepared by pumping double distilled water through a 7 cm × 2.1 mm I.D. column packed with Whatman CO:PELL ODS. The void volume was determined by injecting sodium nitrate in the presence of background electrolyte.

The UV detector was operated at 254 nm and 0.01 a.u.f.s. Capacity factors were calculated in the usual manner and based on the average of at least two determinations.

Regression analyses were performed using the Statistical Analysis System (SAS Institute, Cary, NC, U.S.A.) on an IBM 3031, Computer Services, Auburn University.

RESULTS AND DISCUSSION

The RPLC retention properties of twelve barbiturates were investigated. The measured and extrapolated log k' data are plotted as a function of mobile phase composition in Fig. 1. The solvophobic theory[14,15] was used to predict the extrapolated portions of these plots and consequently derive the hypothetical capacity factor

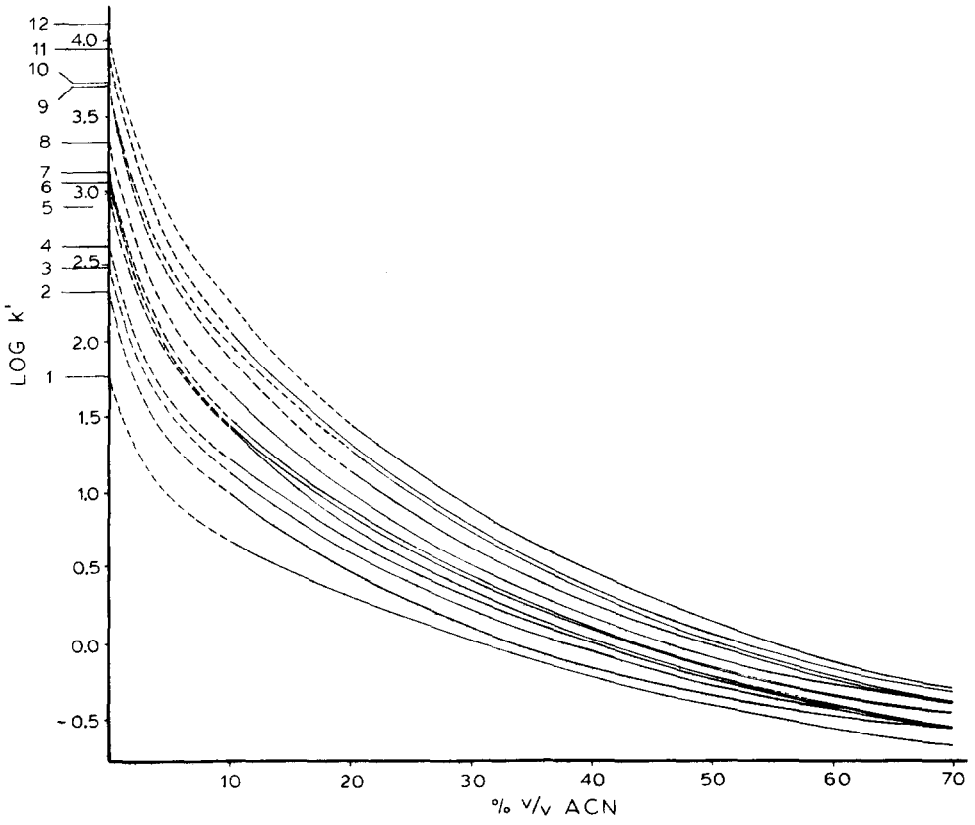
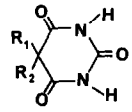


Fig. 1. Measured (——) and extrapolated (----) log k' data as a function of mobile phase composition (ACN = acetonitrile) for twelve 5,5-distributed barbiturates identified according to Table I.

TABLE I

REGRESSION COEFFICIENTS AND CALCULATED SOLVOPHOBIC PARAMETERS FOR THE 5,5-DI-SUBSTITUTED BARBITURATES INCLUDED IN FIG. 1



$X = (A + E) + B\mathscr{D} + C\gamma$*

Compound No.	R_1	R_2	*B*	*C*	*(A + E)*	*r*	*log* k'_w**	$\Delta A(Å^2)$***
1	Ethyl	Ethyl	−269.59	182,309,863	246.145	0.9713	1.782	74.1
2	Ethyl	Allyl	−281.09	210,333,559	256.711	0.9907	2.337	85.4
3	Ethyl	Isopropyl	−246.12	210,521,335	222.763	0.9891	2.499	85.5
4	Allyl	Allyl	−242.13	215,348,777	218.811	0.9890	2.633	87.5
5	Propyl	Allyl	−230.82	228,759,795	207.596	0.9900	2.997	92.9
6	Propyl	Isopropyl	−199.63	223,824,751	177.477	0.9931	3.050	91.0
7	Ethyl	Phenyl	−245.97	242,822,069	221.738	0.9843	3.118	98.6
8	Propyl	Propyl	−216.19	241,007,097	193.152	0.9956	3.331	97.9
9	Butyl	Allyl	−197.84	255,758,102	174.990	0.9997	3.718	103.9
10	Ethyl	Isopentyl	−155.41	244,802,960	134.176	0.9991	3.730	99.4
11	Ethyl	*sec.*-Pentyl	−187.88	263,146,403	165.234	0.9998	3.952	106.9
12	Allyl	*sec.*-pentyl	−141.10	258,251,785	120.074	0.9951	4.119	104.9

* See eqn. 8, ref. 12.
** See eqn. 1, ref. 12.
*** See eqn. 4, ref. 12.

for the compounds in pure water (k'_w). Table I gives the solvophobic regression coefficients B, C, and (A + E) determined for the barbiturates.

The variables in the solvophobic regression equation in Table I are the surface tension of the solvent, γ, and $\mathscr{D}$ which is a function of the static dielectric constant of the solvent. To avoid the repetition of all the equations involved in these calculations, the reader is referred to references[12,16,17] in which the procedure is described in detail.

The contact surface area (ΔA) of the associated solute-bonded ligand complex is derived from regression coefficient *C*. The smallest predicted contact surface area is for the diethyl substituted barbiturate (74.1 Å^2) and the largest for the ethyl, *sec.*-pentyl substitution (106.9 Å^2). In comparing the ΔA values for the ethyl, ethyl (74.1 Å^2), the ethyl, allyl (85.4 Å^2) and the allyl, allyl (87.5 Å^2) derivatives, it is obvious that the replacement of the first ethyl group by allyl had a greater effect upon the difference in contact surface area ($\Delta\Delta A$) than the replacement of the second ethyl group. As observed previously in a series of N-alkylbenzamides[12], the branched-chain barbiturate substituents generally elute earlier (*i.e.* smaller log k'_w) and have lower contact surface areas with the bonded phase than do their isomeric, straight-chain analogues. The correlation between log k'_w and ΔA (eqn. 1) indicates, as shown previously[12], that the contact surface area between the solute and stationary phase is not the sole contributing factor to retention.

$$\log k'_w = 0.071\Delta A - 3.54 \qquad r = 0.973 \tag{1}$$

TABLE II

PREDICTED AND EXPERIMENTAL log k' VALUES OF SOME 5,5-DISUBSTITUTED BARBITURATES

For compounds , see Table I.

Compound No.	*log* k'_w*	*log* k'_{20}*	*log* k'_{50}*
1	1.782	0.182	−0.401
2	2.337	0.391	−0.336
3	2.499	0.530	−0.274
4	2.633	0.560	−0.249
5	2.997	0.751	−0.181
6	3.050	0.877	−0.158
7	3.118	0.773	−0.249
8	3.331	0.951	−0.118
9	3.718	1.155	−0.032
10	3.730	1.285	0.053
11	3.952	1.303	0.041
12	4.119	1.481	0.146

* Subscripts w, 20, and 50 refer to 0%, 20%, and 50% acetonitrile in water, respectively.

The plots in Fig. 1 were extrapolated from 10% to 0% acetonitrile for nine of the barbiturates and from 20% for three of the more lipophilic compounds. This region of low organic modifier concentration is responsible for a major portion of the deviation from linearity of log k' *versus* solvent composition plots. However, the non-linearity of these plots can be seen in the data measured at 20% and higher proportions of acetonitrile. The difficulty in measuring retention data at low organic modifier concentration is obvious. However, previous work[12,16,17] has shown that

TABLE III

PARTITION COEFFICIENTS REPORTED FOR SOME 5,5-DISUBSTITUTED BARBITURATES

For compounds, see Table I.

Compound No.	*log* P_{oct}*	*log* P_{CHCl_3}	*log* P_{CCl_4}
1	0.65**	−0.14***	−1.456***
2	0.85	0.12	−1.201
3	0.95	0.20	−1.215
4	1.05	0.33	−0.962
5	1.35	–	–
6	1.45	–	–
7	1.42	0.65	−0.633
8	1.65	–	–
9	1.85	–	–
10	2.07	1.53	−0.025
11	2.07	1.38	−0.033
12	2.15	1.72	0.334

* oct = Octanol.
** From refs. 18 and 19.
*** From ref. 20.

the curvature in this type of plot is real and that calculations based on the solvophobic theory adequately predict the curvature and the log k'_w values. The capacity factors at 0% organic modifer (log k'_w) were calculated as described above and are reported in Table II with the measured log k' values at 20 and 50% acetonitrile. The partition coefficients for the barbiturates in this study[18–20] are reported in Table III.

The relationship between log P and log k'

Most work to date has assumed the linearity of plots of log k' *versus* log P to be theoretically based on a relationship known as the Collander[21] equation. Collander[21] reported that "The partition in one alcohol/water system can be calculated approximately from that in another such system, using the equation log $k_1 = a \log k_2 + b$ where k_1 and k_2 denote the partition coefficients in the two solvent systems, while a and b are constants" (Here in the original form k refers to the partition coefficients, not capacity factor.) Simply put, this means that plotting the partition coefficients of solutes determined with two different monohydric alcohols (*i.e.*, log P_{pentanol} *versus* log P_{octanol}) against each other should yield a linear relationship. As for extending this concept to establish the linearity of log k' *versus* log P_{octanol} plots, this would hold true only if (1) log k' results from a true partition mechanism and (2) the stationary phases behaves like a monohydric alcohol (*i.e.*, 1-octanol). The 1-octanol–water partition coefficient "inherently includes the effect of hydrogen bonding"[22], and it is unlikely that equivalent hydrogen-bonding effects are reflected in measurements on alkyl bonded phases. Numerous theories concerning the mechanism of retention in RPLC have been reported and span the range from pure partition to pure adsorption. It is highly probable that a mixed retention mechanism is the best approach. Furthermore, k' values are determined in a dynamic process whereas partition coefficients are the result of an equilibrium experiment. Concerning the second point, Collander[21] suggested only that the log P results from one monohydric alcohol should linearly correlate with those from a second monohydric alcohol. Extending this analogy to expect linear relationships between log P results obtained from alcohols and hydrocarbons appears somewhat dangerous. Indeed, Leo *et al.*[23] have shown clear evidence that the Collander equation does not apply when the non-polar phases of the partitioning systems differ widely, and especially when the solute sets contain both hydrogen-bonding and non-hydrogen-bonding solutes.

If, for example, the log P_{CCl_4} values are regressed against the log P_{oct} values (Table III) for some of the barbiturates in this study (a closely related set of congeners all having the same hydrogen-bonding ability in the barbituric acid backbone), the linear regression is highly correlated (eqn. 2). If the linear log P_{alkane} *versus* log P_{oct} regression is considered for more diverse groups of solutes (of varying hydrogen-bonding abilities) as in refs. 5 and 10, the regression is not very well correlated. Log P_{alk} represents the partitioning of solutes in a *n*-alkane–water system obtained as mean values for data in hexane, heptane, and octane–water (see eqns. 3 and 4).

This study, Table III ($n = 8$):

$$\log P_{CCl_4} = 1.072 \log P_{\text{oct}} - 2.151 \qquad r = 0.99 \tag{2}$$

Data from ref. 5 (n = 22):

$$\log P_{alk} = 1.774 \log P_{oct} - 3.213 \qquad r = 0.84 \tag{3}$$

Data from ref. 10 (n = 26):

$$\log P_{alk} = 1.814 \log P_{oct} - 2.596 \qquad r = 0.86 \tag{4}$$

It is informative to compare and contrast the data of Butte *et al.*[5] and Hammers *et al.*[10] with the data presented here. The data of refs. 5 and 10 is somewhat unique in the literature because they recognize the need to use the capacity factor in water as the estimator of hydrophobicity. However, in each of these references, the log k'_w is obtained by linear extrapolation of log k' *versus* solvent composition plots. In their paper, Butte *et al.*[5] found that for a plot of log k'_w (linearly extrapolated) *versus* log P_{oct}, the slope was near unity and the intercept not far from zero. Hammers *et al.*[10] showed a similar relationship for non-polar solutes in a log k'_w (linearly extrapolated) *versus* log P_{alkane} plot. The implication of this information is that RPLC retention is controlled by a partition mechanism in which the octadecyl phase behaves toward solutes like octanol does. In Figs. 2 and 3, their data of log k'_w (obtained from linear extrapolation of log k' *versus* solvent composition plots) is graphed as a function of log P obtained with two different lipid phases. These log P values were obtained from refs. 1, 5 and 10. The data base of Butte *et al.*[5] consisted entirely of substituted phenols, therefore all solutes contain at least the hydroxyl group which is capable of hydrogen-bonding with octanol, and some solutes also contained other hydrogen-bonding groups. As expected, these curves do not cross (Fig. 2). The data of Hammers *et al.*[10] included some non-hydrogen bonding solutes, such as methyl-

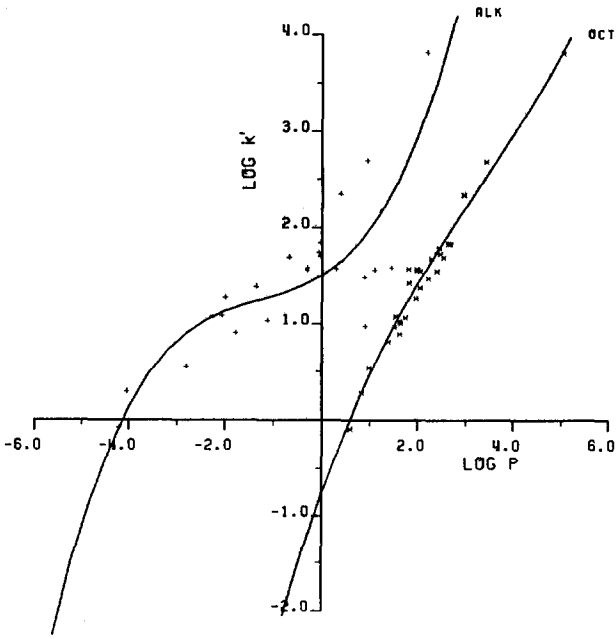


Fig. 2. log k'_w *versus* log P [ALK (+) = n-alkane–water system; OCT(×) = 1-octanol–water system] plots for substituted phenols fitted by cubic equation. Data from ref. 5.

TABLE IV

REGRESSION EQUATIONS FOR FIGS. 2–8

$\log k' = a(\log P)^3 + b(\log P)^2 + c(\log P) + d$

Source	*Curve identification*	*a*	*b*	*c*	*d*	*n*	*r*	*s*
Fig. 2	ALK	0.035241 (0.017998)*	0.132805 (0.067605)	0.310558 (0.109080)	1.503518 (0.141008)	22	0.8402	0.4730
	OCT	0.020165 (0.018330)	−0.203338 (0.149985)	1.423042 (0.350821)	−0.770990 (0.246901)	29	0.9831	0.1404
Fig. 3	ALK	0.043828 (0.014842)	−0.023482 (0.034780)	0.074447 (0.084119)	1.757977 (0.096588)	26	0.9379	0.2728
	OCT	−0.000409 (0.013899)	0.066985 (0.119799)	0.620990 (0.316288)	0.543574 (0.253547)	51	0.9927	0.1444
Fig. 4	SOLV	0.758975 (0.364853)	−3.437196 (1.551030)	6.230939 (2.066248)	−0.991892 (0.854163)	12	0.9937	0.0930
	EXTRAP	0.557407 (0.446625)	−2.589838 (1.898652)	4.525383 (2.529343)	−1.281011 (1.045601)	12	0.9712	0.1725
Fig. 5a	0% ACN	–	–	1.366661 (0.067560)	1.111314 (0.103986)	12	0.9880	0.1146
	20% ACN	–	–	0.775391 (0.029350)	−0.278175 (0.045175)	12	0.9929	0.0498
	50% ACN	–	–	0.321245 (0.024036)	−0.615250 (0.036996)	12	0.9731	0.0408
Fig. 5b	0% ACN	–	−0.222389 (0.154300)	2.007512 (0.449251)	0.703058 (0.299997)	12	0.9902	0.1089
	20% ACN	–	0.029536 (0.073712)	0.690279 (0.214617)	−0.223954 (0.143315)	12	0.9930	0.0520
	50% ACN	–	0.097725 (0.051458)	0.039633 (0.149823)	−0.435848 (0.100047)	12	0.9809	0.0363
Fig. 5c	0% ACN	0.758975 (0.364853)	−3.437196 (1.551030)	6.230939 (2.066248)	−0.991892 (0.854163)	12	0.9937	0.0930
	20% ACN	0.413578 (0.159474)	−1.722265 (0.677940)	2.991693 (0.903137)	−1.147560 (0.373346)	12	0.9962	0.0407
	50% ACN	0.261609 (0.119410)	−1.010377 (0.507624)	1.495393 (0.676246)	−1.020075 (0.279552)	12	0.9881	0.0304
Fig. 6	cubic	0.758975 (0.364853)	−3.437196 (1.551030)	6.230939 (2.066248)	−0.991892 (0.854163)	12	0.9937	0.0930
	linear	–	–	1.366661 (0.067560)	1.111314 (0.103986)	12	0.9880	0.1146
Fig. 7	0% ACN	0.129339 (0.332878)	−0.103310 (0.599117)	0.935965 (0.262163)	3.836861 (0.090855)	8	0.9921	0.1407
	20% ACN	−0.036006 (0.189545)	−0.086913 (0.341144)	0.675361 (0.149279)	1.286899 (0.051734)	8	0.9921	0.0801
	50% ACN	−0.013349 (0.121625)	0.043533 (0.218902)	0.371982 (0.095788)	0.034574 (0.033196)	8	0.9821	0.0514
Fig. 8	CCl_4	0.129339 (0.332878)	−0.103310 (0.599117)	0.935965 (0.262163)	3.836861 (0.090855)	8	0.9921	0.1407
	$CHCl_3$	0.155286 (0.285684)	−0.781981 (0.653624)	2.027399 (0.391735)	2.092502 (0.073412)	8	0.9938	0.1249
	OCT	0.758975 (0.364853)	−3.437196 (1.551030)	6.230939 (2.066248)	−0.991892 (0.854163)	12	0.9937	0.0930

* Standard error of regression coefficients.

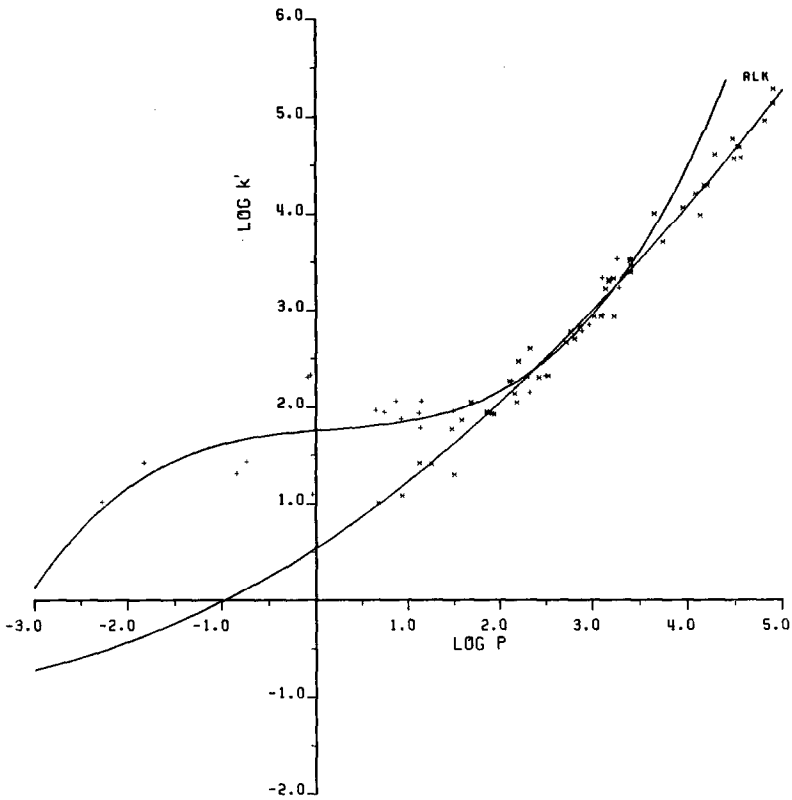


Fig. 3. log k'_w *versus* log P [ALK (+) = *n*-alkane–water system; OCT (×) = 1-octanol–water system] plots for polar and non-polar hydroaromatics fitted by cubic equations. Data from ref. 10.

benzenes, as well as some polar benzenes which were capable of hydrogen bonding. Cubic equations produced adequate descriptions of these data (see Table IV).

The curves in Fig. 3 approach contact in the area of the non-hydrogen-bonding solutes (*i.e.*, no offset due to hydrogen bonding), with intersection of the curves actually occurring at the points (log P, log k'_w):(−3.57, −0.80); (2.38, 2.38); and (3.24, 3.24). The last two points lie perfectly on a line of slope = 1 which passes through the origin. This line, of course, represents log k'_w = log P. It is still the opinion of this laboratory that the log k' value derived from linear extrapolation is not the true log k'_w. However, it would be unwise to ignore such a tendency to linearity with a slope of one found between log P_{oct} and the linearly extrapolated log k'_w data of the Butte[5] and Hammers[10] research groups.

To try to gain a better understanding of the situation, the difference (see Table V) in calculating log k'_w by the solvophobic theory as compared to its estimation by the linear extrapolation of a log k' *versus* solvent composition plot (*i.e.*, obtained by linear extrapolation of each plot in Fig. 1) for the 5,5-disubstituted barbiturates is presented in Fig. 4. The log k'_w points determined by the solvophobic theory demonstrated a higher correlation coefficient and a lower standard deviation (see Table IV) when plotted against log P_{oct} than did the log k'_w values determined by linear extra-

TABLE V

log k'_w VALUES DERIVED FROM DIFFERENT SOURCES

For compounds see Table I.

Compound No.	*log k'_w (solvophobic)*	*log k'_w (linear)*
1	1.782 (0.9713)*	0.740 (0.9617)
2	2.337 (0.9907)	0.991 (0.9516)
3	2.499 (0.9891)	1.173 (0.9623)
4	2.633 (0.9890)	1.258 (0.9613)
5	2.997 (0.9900)	1.488 (0.9596)
6	3.050 (0.9931)	1.611 (0.9689)
7	3.118 (0.9843)	1.453 (0.9505)
8	3.331 (0.9956)	1.702 (0.9602)
9	3.718 (0.9997)	1.643 (0.9834)
10	3.730 (0.9991)	1.775 (0.9808)
11	3.952 (0.9998)	2.154 (0.9713)
12	4.119 (0.9951)	2.000 (0.9802)

* Correlation coefficient (r) in parentheses.

polation. Although fit to a cubic equation in Fig. 4, the points determined by linear extrapolation appear scattered about a line approximately described by log k' = log P. (Points located in the curved region of Fig. 1 were not excluded in the linear extrapolation, and the organic modifier was acetonitrile.)

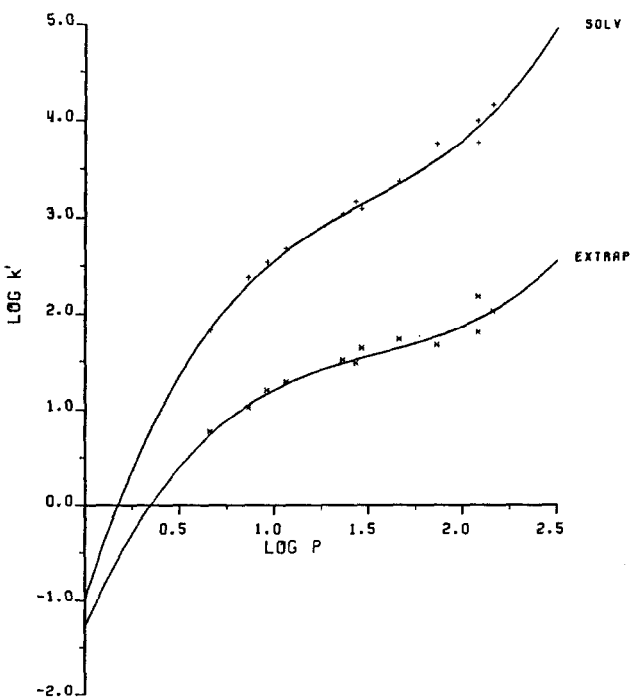


Fig. 4. log k'_w *versus* log P_{oct} plots for 5,5-disubstituted barbiturates fitted by cubic equations. SOLV(+) = log k'_w derived by the solvophobic theory; EXTRAP (×) = log k'_w derived by the linear extrapolation of a log k' *versus* solvent composition plot.

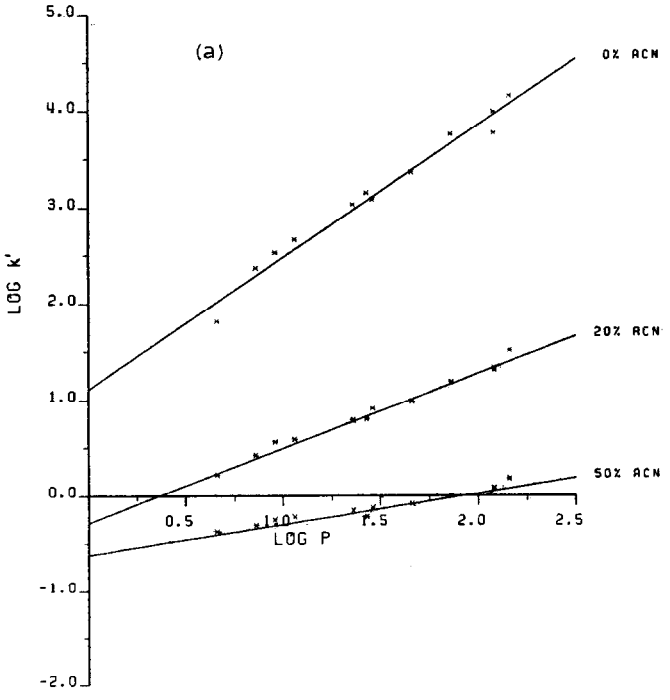
The following general observations can be made from the data of Butte *et al.*[5], Hammers *et al.*[10], and this report. The log P = log k' relationship of slope = 1 seems to be valid for:

(1) Polar solutes whose partition coefficients were measured using octanol and log k'_w determined by linear extrapolation (and not for polar solutes whose partition coefficients were measured in a non-polar organic phase);

(2) Non-polar solutes with partition coefficients measured using either octanol or a non-polar organic phase, and the log k'_w determined by linear extrapolation.

The data presented here points to the conclusion that it is in the more linear region of a log k' *versus* solvent composition plot where the mechanism of retention most nearly approaches partition. This can be rationalized on the basis that the stationary phase is enriched with the organic modifier[24–26]. The organic modifier is believed to undergo hydrophobic expulsion from the mobile phase similarly to the solute[27,28]. The amount of modifier extracted will depend upon its solvent strength as well as the percentage of water present[28]. In aqueous methanol eluents, the solvation layer reaches its maximum thickness in pure methanol[29], whereas there is virtually no solvation layer in pure water[30].

The solvophobic theory quite adequately describes retention over the entire solvent composition range, whereas partition theory is only compatible with the linear portion of a log k' *versus* solvent composition plot. The true log k'_w does not lie on a log k' = log P relationship (see Fig. 4).



(*Continued on p. 330*)

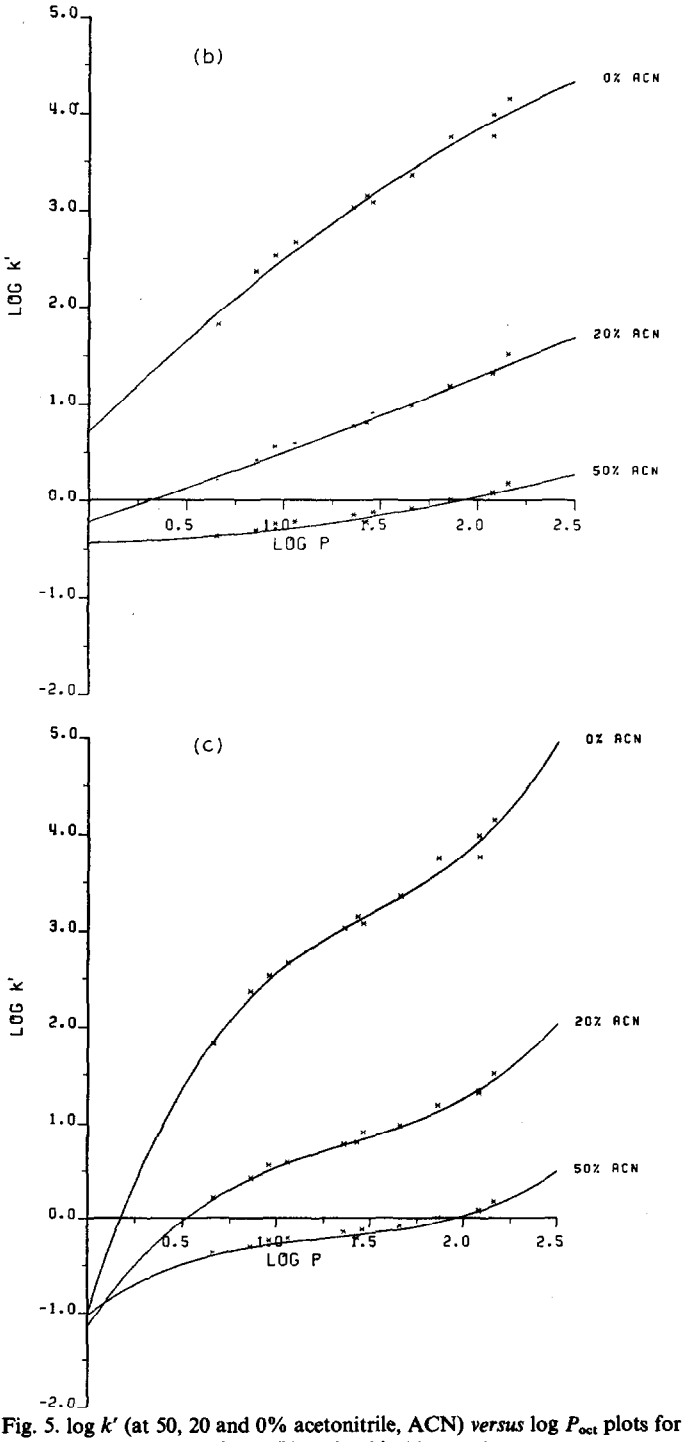


Fig. 5. log k' (at 50, 20 and 0% acetonitrile, ACN) *versus* log P_{oct} plots for 5,5-disubstituted barbiturates fitted by linear (a), quadratic (b) and cubic (c) equations.

The relationship between log P and solvophobically derived log k'_w

Linear relationships for plots of log k' *versus* log P values have been established or intimated by other workers[3–5,7–10]. In earlier work from this laboratory it has been shown that a quadratic relationship can give a better description of the log k' *versus* log P plot[6]. The logarithm of the capacity factors obtained at 50, 20, and 0% acetonitrile (Table II) are plotted against their partition coefficients measured in octanol (Table III) in Figs. 5a–c. From regression analysis, the best linear, quadratic, and cubic fits to the data are depicted in Figs. 5a, 5b, and 5c, respectively. (All regression equations are listed in Table IV.) The results appear to show a strongly sigmoidal dependence of the capacity factors of these barbiturates upon their partition coefficients. This tendency is nicely approximated by a cubic equation. The addition of the cubic term is statistically significant for 0% and 50% acetonitrile (significance level $\alpha = 0.10$) and for 20% acetonitrile ($\alpha = 0.05$). For comparing diverse groups of compounds it is best to use the log k'_w value, but in this limited homologous series, a relatively accurate prediction of log P from log k' can be obtained at either of the solvent compositions indicated using a cubic relationship (Fig. 5c).

The cubic and linear fit to the log k'_w *versus* log P_{oct} values are directly compared in Fig. 6. The sigmoidal relationship between log k' and log P suggests that the rate of change in log k' is greater for solutes having low log P values and for those having high log P values than predicted by the linear relationship. Assuming a linear relationship results in overestimation of the magnitude of log P at low k' values and underestimation at high k' values.

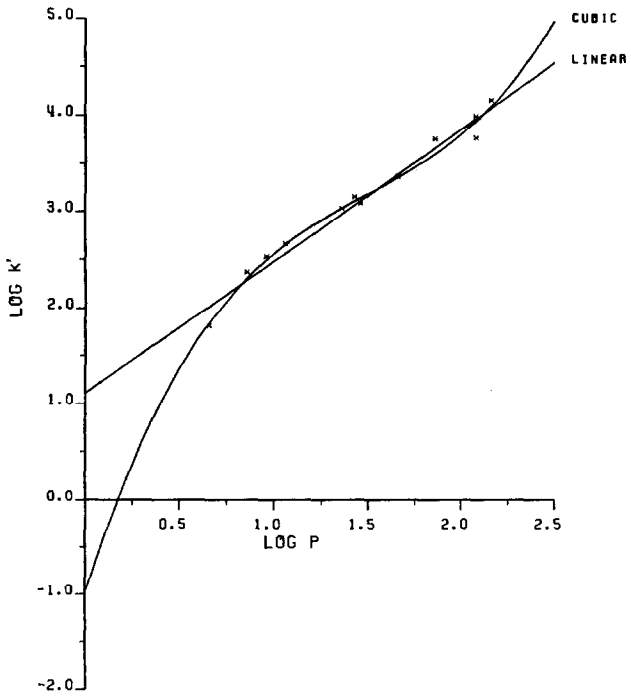


Fig. 6. log k'_w *versus* log P_{oct} plots for 5,5-disubstituted barbiturates fitted by cubic and linear equations.

In the log k' *versus* log P plots, the y-intercept (that is, where log P = 0, P = 1) represents the point at which the compound is equally distributed between the aqueous and organic phases used to measure the partition coefficient. In the graph illustrating the cubic fit to the log P values measured in carbon tetrachloride (Fig. 7), the predicted log k' value of a solute with log P_{CCl_4} = 0 is strongly solvent dependent, while the log k' value for a solute of log P_{oct} = 0 (Fig. 5c) is virtually independent of solvent composition. A compound having a log P_{CCl_4} near zero is very hydrophobic compared to a compound which has a log P_{oct} of zero and would be expected to display greater retention in RPLC.

The partition coefficients for most of the barbiturates are listed in Table II for three different organic phases, *e.g.*, carbon tetrachloride, chloroform, and 1-octanol. In every case the highest log P value for an individual barbiturate occurs in 1-octanol and the lowest in carbon tetrachloride. The trend in the magnitude of the log P values is obviously log P_{CCl_4} < log P_{CHCl_3} < log P_{oct}. When the organic phase is capable of hydrogen bonding with the solute being measured, the value of log P is larger. That is, $P = C_{lipid}/C_{aqueous}$ is larger when hydrogen bonding increases the concentration of the solute in the lipid phase. But if these values are plotted against log k'_w we can visualize their offset in two dimensions (Fig. 8). All of the compounds involved in Fig. 8 are derived from the barbituric acid backbone, and differ from

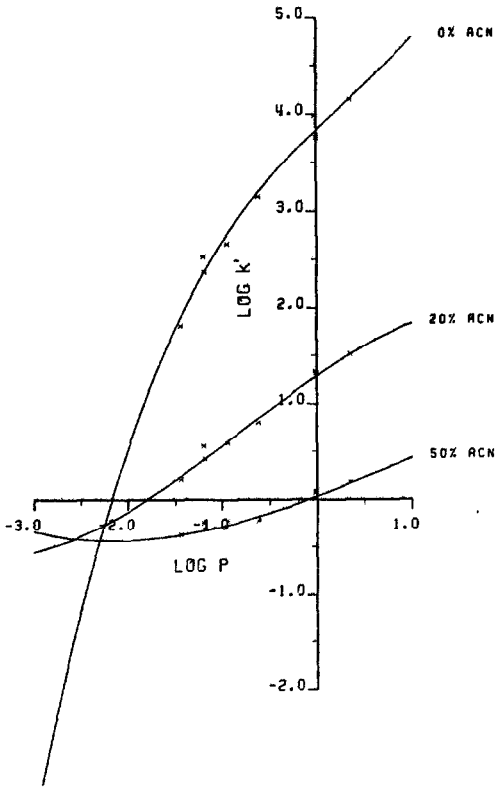


Fig. 7. log k' (at 50, 20 and 0% acetonitrile, ACN (*versus* log P_{CCl_4} plots for 5,5-disubstituted barbiturates fitted by cubic equations.

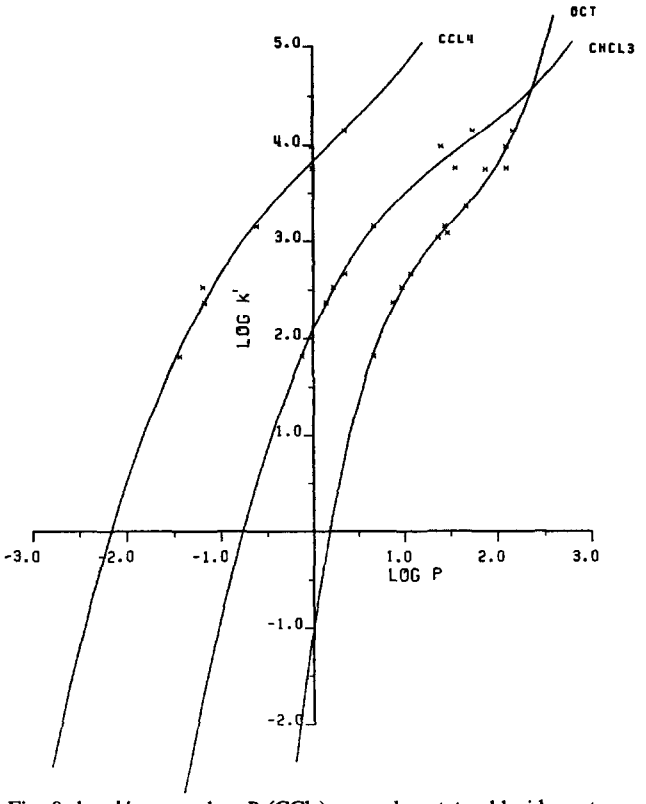


Fig. 8. log k'_w *versus* log P (CCl_4) = carbon tetrachloride–water system; $CHCl_3$ = chloroform–water system; OCT = 1-octanol–water system) plots for 5,5-disubstituted barbiturates fitted by cubic equations.

each other only in the hydrocarbon portion of the disubstitution pattern at the 5-position. The curves are approximately parallel since the hydrogen-bonding ability of these solutes will be fairly constant throughout this homologous series[31]. This parallelism is not observed when log P values measured in different lipid phases are compared for diverse solute groups differing in hydrogen-bonding ability (Fig. 3).

There is probably a large error associated with extending the curves in Fig. 8 so far in the negative y-direction as there may indeed be another twist in the plot. Or, it may simply be invalid to extend these plots since they result from a homologous series and not a diverse group. Now in hindsight it is easy to see the need to measure log k'_w values for solutes having very low log P values in order to anchor the curve and get a more accurate description of the entire spectrum of log k' *versus* log P.

The curves of Fig. 8 were extended in such a manner so that another important concept could be discussed. This figure substantiates the intuitive implication that even solutes used to measure the chromatographic void volume, often water, methanol, or organic salts[32] also demonstrate an offset in the magnitude of their log P values (*i.e.*, water is more soluble in octanol than in carbon tetrachloride[33]), thus, as these curves are extended in the negative y-direction, they should not be expected to converge. In RPLC a marker solute used for measuring the column void volume is (ideally) totally unretained by the stationary phase. The compound that elutes at the

void volume has a k' of zero, with log k' undefined. As solutes are measured having smaller and smaller retention volumes approaching that of the void volume, the log k' becomes more negative. If a compound truly measures the RPLC void volume, its partitioning behavior should be such that it would reside entirely in an aqueous phase in contact with a hydrocarbonaceous lipid phase. The partition coefficient of this compound would be $P = 0/1$ with the log P value undefined since log P = log 0 − log 1 = log 0 − 0 = log 0. As the partition coefficient gets smaller, values of log P become more negative. So, a log k' *versus* log P plot cannot be used to determine the void volume of a column since this does not exist as a discrete point on such a graph. However, the plot of log k' *versus* log P measured in a non-hydrogen bonding solvent should approach such a value. The smallest valid y values plotted are really a function of the accuracy with which log k' values are measured and the smallest x values plotted reflect both the accuracy of measuring partition coefficients as well as the hydrogen-bonding ability of the lipid phase used to measure log P.

CONCLUSION

The results of this study indicate that retention is controlled by a partition process in eluents of high organic modifier content. For a group of closely related homologues, as in the barbiturates, a high degree of correlation exists between log k' and log P. This relationship is sigmoidal and is adequately described by cubic equations. Of the methods used to evaluate log k'_w from measured retention data, the solvophobic theory appears to be the most accurate.

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