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MODELING OCTANOL–WATER PARTITION COEFFICIENTS BY REVERSED-PHASE LIQUID CHROMATOGRAPHY

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SUMMARY

The dynamic range of a polycratic reversed-phase liquid chromatographic (RPLC) method for measuring hydrophobicity constants (log k'_w) for non-ionic, organic molecules was examined. Evidence is presented that indicates that the differential hydrogen bonding effect present in most RPLC methods has been minimized by selection of appropriate stationary and mobile phase conditions. Thus, values of log k'_w determined for compounds of different net hydrogen bonding properties (non-congeners) can be compared directly. Results indicate that non-congeners with hydrophobicities varying between $-0.20 \leqslant \log k'_w \leqslant 5.27$ can be measured by this procedure. Additionally, the net hydrogen bonding property of any solute molecule can be predicted from the chromatographic experiment. Finally, the suppression of hydrogen-bonding discrimination effects in this method is shown to be a result of the low levels of octanol and alkylamine added to the mobile phase.

INTRODUCTION

Octanol–water partition coefficients (log $P_{o/w}$) have been used successfully in correlations with biological activities for a large number of structurally diverse organic compounds[1,2]. However, the shake-flask technique that is used to measure log $P_{o/w}$ is prone to errors and experimental problems[3], and alternative methods for estimating this important physical parameter, including chromatographic ones, have been investigated. Reversed-phase liquid chromatography (RPLC) has been used most often to estimate values of log $P_{o/w}$. Good correlations are generally found between chromatographic retention data and log $P_{o/w}$ for structurally similar compounds[3–5]. Dissimilar compounds (non-congeners) usually yield poorer results when log $P_{o/w}$ constants are correlated with RPLC retention data in comparison to those obtained by subdividing the set into structurally similar classes and regressing the data in each class independently[6–11]. The inability to describe these correlations by a single regression equation is due to a differential hydrogen bonding effect that is qualitatively similar to that observed when octanol–water partition coefficients are correlated with alkane–water partition coefficients for a set of non-congeners[12]. The usual RPLC and alkane–water systems exhibit greater hydrogen bonding selectivity than does the octanol–water system.

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A number of methods have been reported by investigators attempting to suppress hydrogen bonding discrimination in the chromatographic system. As early as 1976, Mirrlees *et al.*[13] obtained a good correlation between log $P_{o/w}$ and RPLC retention for a set of non-congeners. They were able to successfully model octanol–water partitioning by using octanol-saturated Kieselguhr as the stationary phase and octanol-saturated water as the eluent. Later, Unger *et al.*[14] obtained similar results using octanol-saturated octadecyl silica gel as the stationary phase. In more recent studies, Terada[15] and Miyake *et al.*[16] obtained good correlations between log $P_{o/w}$ and capacity factors (log k') for sets of non-congeners by including hydrogen bonding indicator variables in their regression equations. The inclusion of these variables improved regression results by partially compensating for the hydrogen bonding properties inherent in their chromatographic data. RPLC systems with methanol as the organic component of the eluent have been shown to model octanol–water partitioning well[8,17]. Valkó[18] has reported a method for estimating log $P_{o/w}$ when acetonitrile is used as the organic component in the eluent. Under these conditions, values of log $P_{o/w}$ for a set of non-congeners were accurately predicted by a multiple regression equation containing two parameters derived from chromatographic data. The parameters obtained for each compound were the slope and intercept of the linear regression of log k'_{φ} *vs.* φ_{ACN} data (ACN stands for acetonitrile), where log k'_{φ} is the capacity factor measured at a number of different volume fractions of organic modifier (φ_{ACN}). Extrapolation of data measured at several eluent strengths in this manner is termed "polycratic", from the Greek kratos or "strength". The parameters derived by polycratic methods generally yield hydrophobicity constants which correlate better with log $P_{o/w}$ than retention data obtained under monocratic conditions, as discussed by Braumann[17].

Recently, we reported a polycratic procedure[19] for estimating hydrophobicity constants by RPLC that largely eliminates the discrimination arising from differences in hydrogen bonding capacity of non-congeners. This method used mixtures of aqueous buffer, methanol, octanol and *n*-decylamine as eluents and octyl-modified silica as the stationary phase to obtain extrapolated capacity factors (log k'_w). log k'_w is the intercept of the linear regression of log k'_{φ} and φ_{MeOH}, defined analogously to φ_{ACN}, above. Unlike a previously reported method for estimating hydrophilicity with varying mobile phase composition[20], this new approach included trace amounts of octanol and alkylamine in the eluent, and showed little differential hydrogen bonding effects, presumably owing to enrichment of these components in the stationary phase. In addition, log k'_w was shown to be relatively independent of the value measured for the column dead-time (t_0) and was chosen as the chromatographic parameter for constructing a hydrophobicity scale. This has the advantage that log k'_w results from different research groups can be compared without complications which may arise from differences in procedures used to estimate t_0. Finally, this method does not require converting chromatographic data to the octanol–water scale in order to compare compounds of varying hydrophilicity. Instead, values of log k'_w are considered to be *a priori* hydrophobicity constants[17] that reflect the same molecular properties which control partitioning in the octanol–water system.

In this paper, we wish to report additional results obtained by using the recently published method. First, the range of hydrophobicities measurable by this method was evaluated. In this study, log k'_w values were determined for two sets of non-congeners.

The first set contained compounds that were relatively hydrophobic ($0.95 \leqslant \log P_{o/w} \leqslant 5.5$), and was used to evaluate the upper range of the chromatographic method. The second set contained compounds that were relatively hydrophilic ($-0.65 \leqslant \log P_{o/w} \leqslant 2.26$), and was used to assess the lower range. Data from both experiments were correlated with measured ($\log P_{o/w}$) and calculated (CLOGP) octanol–water partition coefficients in order to demonstrate that $\log k'_w$ varied linearly with these parameters over the range examined. In addition, since the compounds comprising these sets are non-congeneric, these results confirm that the hydrogen bonding selectivity present in most chromatographic systems has been effectively suppressed under these RPLC conditions. An octadecyl-derivatized polymeric stationary phase was examined as a possible alternative to octyl-modified silica gel in the analysis of hydrophilic analytes, and should provide greater column stability at the relatively high mobile phase pH employed. The results also show that the net hydrogen bonding properties of solutes can be predicted from the same chromatographic data used to determine hydrophobicity constants ($\log k'_w$) in which this information was suppressed. This capability is demonstrated for the two sets of non-congeners. Finally, chromatographic data are presented which help to explain why the conditions chosen for this method overcome the differential hydrogen bonding effect.

EXPERIMENTAL

Chromatographic equipment

All chromatographic data were obtained using a Waters Assoc. (Milford, MA, U.S.A.) Model 840 chromatographic system equipped with two Model 590 pumps, a WISP 712 autoinjector, and a Model 450 variable-wavelength detector, operated at 220 nm and 0.2 a.u.f.s. This system utilizes a Professional 380 computer (Digital Equipment Corporation, Maynard, MA, U.S.A.) to control hardware, acquire and store data, and estimate retention times.

Stainless-steel columns (15 cm × 4.5 mm, I.D.) packed with 5-μm octyl-modified silicas were purchased from ES Industries (Marlton, NJ, U.S.A.). A stainless-steel column (15 cm × 4.6 mm, I.D.) packed with octadecyl-modified Macrophase MP-1 was supplied by Interaction Chemicals (Mountain View, CA, U.S.A.). A silica saturator column (5 cm × 4.5 mm, I.D.) positioned between the pumps and the autoinjector was filled with Partisil 20/400 silica gel from Whatman (Clifton, NJ, U.S.A.). A Rheodyne (Cotati, CA, U.S.A.) column inlet filter containing a 0.5-μm filter element was positioned between the autoinjector and the analytical columns in order to prevent blockage.

Materials

HPLC-grade methanol, analytical-grade 1-octanol, and solutions of 1 *M* sodium hydroxide and 1 *M* hydrochloric acid were purchased from Fisher Scientific (Pittsburgh, PA, U.S.A.).

4-Morpholinopropanesulfonic acid (MOPS), *n*-decylamine (99%), and all test solutes were obtained from Aldrich (Milwaukee, WI, U.S.A.). The water used to prepare the mobile phases was purified using a Milli-Q Water System from Millipore (Bedford, MA, U.S.A.). Deuterated methanol was obtained from MSD Isotopes (Montreal, Canada).

Procedures

The flow-rate was 2.0 ml/min for all the experiments in which silica-based packings were used. For the experiment with the polymer-based packing, a flow-rate of 1.0 ml/min was used. All eluents were filtered through a 0.45-μm Durapore filter (Millipore) prior to use, and purged continuously with helium during use to prevent problems associated with the build-up of dissolved gases in the eluent.

Capacity factors were measured in duplicate at each eluent composition. Dead-times (t_0) for the silica-based packings were estimated from the retention of deuterated methanol in a pure methanol eluent. For the polymeric packing, the dead-time could not be estimated at $\varphi_{\mathrm{MeOH}} = 1$ due to the restricted eluent ranges recommended for this packing. Consequently, this parameter was approximated by the retention time of the solvent disturbance peak measured from injections of pure methanol at an eluent composition of $\varphi_{\mathrm{MeOH}} = 0.75$. Although it is unlikely that the latter estimate of t_0 rigorously defines the column dead-volume, we have shown previously that the derivation of log k'_w was insensitive to the exact value of t_0 used[19].

For experiments with silica-based packings, mobile phases consisted of different volume fractions of organic and aqueous solutions which were mixed from separate pumps. Variations in the volume fraction of organic modifier in the eluent were accomplished by the pump control capabilities of the chromatographic system. The organic portion contained methanol and 1-octanol (0.25%, v/v), while the aqueous portion contained MOPS buffer (0.02 *M*) and *n*-decylamine (0.15%, v/v) and was adjusted to pH 7.4 by additions of 1 *M* sodium hydroxide or 1 *M* hydrochloric acid. For the experiments wih the polymeric packing, solutions containing 80% (v/v) and 20% (v/v) of the organic portion of the eluent were blended before use. Different volume fractions of organic modifier were then formed by pumping the amounts of these solutions needed to achieve the desired volume fraction. The organic portion contained methanol and 1-octanol (0.25%, v/v), while the aqueous portion contained MOPS buffer (0.02 *M*). The aqueous portion was adjusted to pH 7.4 prior to blending with the organic portion.

Chromatographic retention data were measured at 0.1-increments of φ_{MeOH} for $0.25 \leqslant \varphi_{\mathrm{MeOH}} \leqslant 0.75$ for all eluites except thymine and adenine, and these data used in eqn. 1 to derive values of log k'_w and S (the slope parameter). Thymine and adenine were unretained at $\varphi_{\mathrm{MeOH}} \geqslant 0.45$, and additional retention data were required at $\varphi_{\mathrm{MeOH}} = 0.30$ and $\varphi_{\mathrm{MeOH}} = 0.27$ in order to decrease the uncertainty in estimates of log k'_w and S for these compounds. The correlation coefficients, r, from the log k'_φ *vs.* φ_{MeOH} regressions were >0.990 for all compounds except camphorquinone where $r > 0.983$, indicating that log k'_φ varies linearly with φ_{MeOH} within the range of organic compositions examined. Values of CLOGP were calculated using the MedChem Software, (Pomona, CA, U.S.A.), and the correlations between chromatographic results and octanol–water partition coefficients were analyzed using the linear regression program in the RS/1 statistical package (Cambridge, MA, U.S.A.).

RESULTS AND DISCUSSION

Establishment of the dynamic range

The set of non-congeners used to investigate the upper limit of the dynamic range is shown in Table I. These solutes were selected on the basis of both hydrophobic and

TABLE I

COMPOUNDS AND VALUES OF SLOPE, LOG k'_w, LOG $P_{o/w}$. AND CLOGP USED TO INVESTIGATE THE UPPER LIMIT OF DYNAMIC RANGE

Compound	*Classification**	Slope	log k'_w	*log* $P_{o/w}$**	CLOGP***
Phenol	d	2.46	1.16	1.46	1.48
4-Methoxyphenol	d	2.33	1.02	1.34	1.58
Acetophenone	a	2.34	1.40	1.58	1.58
Naphthalene	a	3.98	3.00	3.30	3.32
2-Hydroxynaphthalene	d	3.35	2.15	2.70	2.65
1,4-Dihydroxynaphthalene	d	2.79	1.32	na	1.98
N-phenyl-1-naphthylamine	d	5.11	3.97	na	4.79
4-Bromoaniline	a	3.16	2.01	2.26	2.08
4-Methoxyaniline	a	1.61	0.62	0.95	1.02
Camphorquinone	a	2.11	1.23	1.52	0.46
1-Phenylhexane	a	6.19	5.27	5.52	5.44
Anthracene	a	4.77	3.94	4.45	4.49
9-Methylanthracene	a	5.01	4.26	5.07	5.14
9-Anthracene carboxaldehyde	a	4.17	3.44	na	3.84
9-Anthracenemethanol	d	4.08	2.99	na	3.45
Anthraquinone	a	3.56	2.91	3.39	2.72
1,8-Dichloroanthraquinone	a	3.96	3.27	na	4.19
9-Acetylanthracene	a	4.40	3.42	na	3.93
1-Aminoanthraquinone	a	3.61	2.93	na	2.15
1-Amino-4-hydroxyanthraquinone	m	3.28	2.50	na	1.80
4-Biphenylmethanol	d	3.89	2.74	na	2.99
2-Biphenylmethanol	d	3.82	2.62	na	2.99
4-Biphenylcarboxaldehyde	a	3.97	2.98	na	3.38
Carbazole	d	4.15	3.03	3.72	3.53
Quinoline	a	2.72	1.93	2.03	2.05
Phenanthadine	a	3.65	3.00	na	3.22
Acridine	a	3.56	2.87	3.40	3.43
Benz(a)anthracene-7,12-dione	a	4.74	4.26	na	3.89
4,5-Diphenylimidazole	d	4.34	3.25	na	4.15
Phenanthrene	a	4.81	3.94	4.46	4.49
Fluoranthene	a	4.96	4.21	na	4.95
Pyrene	a	4.96	4.26	4.88	4.95

* d = net hydrogen bond donor, a = net hydrogen bond acceptor, m = mixed net hydrogen bonding properties.

** Values were taken from ref. 1.

*** Values calculated by using MedChem software.

net hydrogen bonding characteristics. Net hydrogen bonding classifications were made according to the general scheme reported by Leo *et al.*[12], and values of log $P_{o/w}$ were taken from ref. 1. Values of log k'_w and S were obtained by linear regression using

$$\log k'_\varphi = \log k'_w - S\varphi_{\mathrm{MeOH}} \tag{1}$$

where φ_{MeOH} = volume fraction of methanol in the eluent, log k'_φ = the logarithm of the capacity ratio measured at each φ_{MeOH}, S = the slope parameter, and log k'_w = chromatographic hydrophobicity constant. Values of log k'_w were correlated with log $P_{o/w}$ and CLOGP, and the regression results reported in Table II. Graphs of

TABLE II

UPPER LIMIT STUDY: LINEAR REGRESSION EQUATIONS FOR LOG $P_{o/w}$ *vs.* LOG k'_w AND CLOGP *vs.* LOG k'_w

n = Number of data points; r = correlation coefficient, S.D. = overall standard deviation of the regression. The standard deviations in the slope and intercept are shown in parentheses.

(i) $\log P_{o/w} = 1.076\ (0.032) \log k'_w + 0.216\ (0.095)$

$n = 17 \quad r = 0.9933 \quad \text{S.D.} = 0.175$

(ii) $\text{CLOGP} = 1.008\ (0.051) \log k'_w + 0.386\ (0.161)$

$n = 29 \quad r = 0.9673 \quad \text{S.D.} = 0.313$

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the log $P_{o/w}$ *vs.* log k'_w and CLOGP *vs.* log k'_w regressions are shown in Fig. 1a and b, respectively, where data are plotted according to the net hydrogen bonding classifications reported in Table I. In the graph of log $P_{o/w}$ *vs.* log k'_w, the data show only minimal systematic deviations arising from the differential hydrogen bonding effect and demonstrate that hydrophobicity constants for non-ionic non-congeners can be estimated up to at least log $P_{o/w}$ = 5.5 without any serious deviations arising from this effect. In the graph of CLOGP *vs.* log k_w, the calculated values for camphorquinone, 1-aminoanthraquinone and 1-amino-4-hydroxyanthraquinone were considered incor-

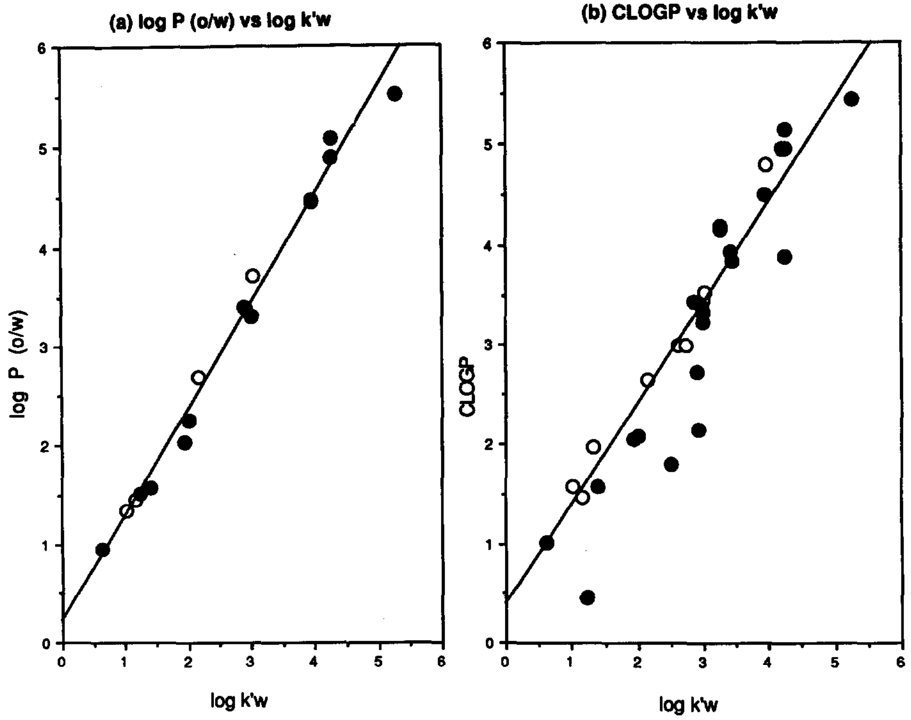

Fig. 1. Relationship between chromatographic hydrophobicity constants (log k'_w) and (a) measured octanol–water partition coefficients (log $P_{o/w}$), and (b) calculated octanol–water partition coefficients (CLOGP) for a relatively hydrophobic series of non-congeners. Solutes have been graphed according to the net hydrogen bonding classifications in Table I. The two solute classes are designated by ○ = donors and ● = acceptors. These log k_w data were determined on C_8-modified silica.

rect and were omitted from the regression reported in Table II for this correlation. A comparison between log $P_{o/w}$ and CLOGP for camphorquinone (1.52 and 0.46, respectively) indicates that the calculated value is incorrect. For the other two compounds, no log $P_{o/w}$ values are available to compare with the CLOGP values. However, these compounds are both capable of forming intramolecular hydrogen bonds, and it has been our experience that this type of effect is not well predicted by CLOGP. The formation of these bonds reduces the availability of acceptor and donor groups to form intermolecular hydrogen bonds with water molecules, resulting in measured log $P_{o/w}$ values which are higher than predicted from a calculation based upon the additive properties of isolated groups. Although log k'_w cannot be completely ruled out as the source of the error observed here, the expected hydrogen bonding characteristics for these solutes were predicted exactly from the slope *vs.* log k'_w data (see discussion below), suggesting that these solutes behaved normally under these RPLC conditions. Regardless of these considerations, the good correlation between log $P_{o/w}$ and log k'_w in contrast to the relatively poor correlation between CLOGP and log k'_w serves to illustrate potential inaccuracies in the calculated data. More importantly, the correlation between log k'_w and CLOGP illustrates that, even for a relatively large set of data, hydrogen bonding discrimination is minimal in this chromatographic system.

The set of non-congeners used to investigate the lower limit of the dynamic range is reported in Table III. Some variations were observed for values of log k'_w for the test compounds measured in both experiments, particularly for phenol. The origin of these variations is unclear and is currently being examined. As before, this set was selected on the basis of both the hydrophilic and net hydrogen bonding properties of these solutes. CLOGP and log $P_{o/w}$ data were both correlated with log k'_w, and the results

TABLE III

COMPOUNDS AND VALUES OF SLOPE, LOG k'_w, LOG $P_{o/w}$, AND CLOGP USED TO INVESTIGATE THE LOWER LIMIT OF DYNAMIC RANGE

Compound	*Classification*	*Slope*	*log k'_w*	*log $P_{o/w}$**	*CLOGP***
Phenol	d	2.59	1.26	1.46	1.48
4-Methoxyphenol	d	2.38	1.07	1.34	1.58
Aniline	a	1.94	0.86	0.98	0.92
4-Methoxyaniline	a	1.67	0.68	0.95	1.02
4-Bromoaniline	a	3.24	2.09	2.26	2.08
Camphorquinone	a	2.20	1.29	1.52	0.46
Acetophenone	a	2.34	1.40	1.58	1.58
Naphthalene	a	4.20	3.14	3.30	3.32
Resorcinol	d	2.12	0.50	0.80	0.81
Hydroquinone	d	1.91	0.19	0.55	0.81
Pyridine	a	1.35	0.74	0.65	0.67
4-Acetylpyridine	a	1.19	0.47	0.54	0.44
Pyrazole	a	1.11	0.088	0.26	0.26
Adenine	a	0.44	−0.20	−0.09	−0.34
Thymine	a	—	—	−0.65	error

* Values taken from ref. 1.

** Values calculated by using MedChem software.

TABLE IV

LOWER LIMIT STUDY: LINEAR REGRESSION EQUATIONS FOR LOG $P_{o/w}$ *vs.* LOG k'_w AND CLOGP *vs.* log k'_w

n = Number of data points; r = correlation coefficient; S.D. = overall standard deviation of regression. The standard deviations in the slope and intercept are shown in parentheses.

(i) $\log P_{o/w} = 1.010\ (0.075) \log k'_w + 0.176\ (0.063)$
$n = 12$ $r = 0.9737$ S.D. = 0.126
(ii) $\text{CLOGP} = 0.982\ (0.141) \log k'_w + 0.237\ (0.113)$
$n = 11$ $r = 0.9180$ S.D. = 0.222

reported in Table IV. As before, camphorquinone was omitted from the CLOGP *vs.* log k'_w regression. Thymine, the most hydrophilic compound in this study, was unretained under these experimental conditions. Also, data for naphthalene and 4-bromoaniline were omitted from the correlation in order not to skew the results away from the hydrophilic region. These results indicate that values of log k'_w can be estimated for solutes with measured log $P_{o/w}$ values below zero. Graphs illustrating

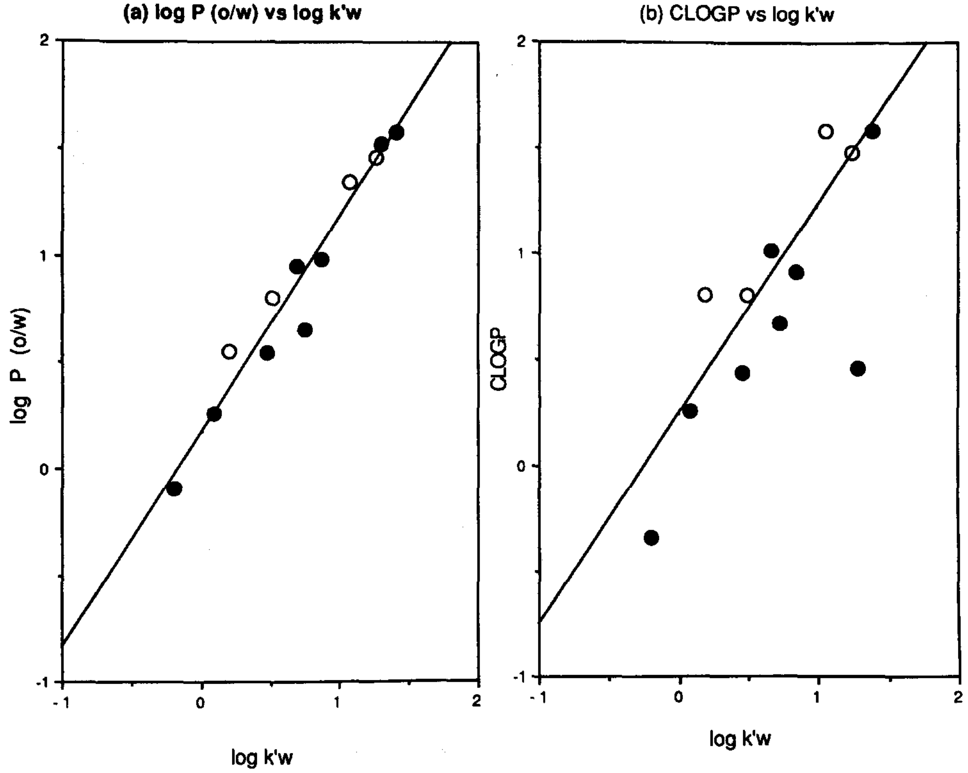

Fig. 2. Relationship between chromatographic hydrophobicity constants (log k'_w) and (a) measured octanol–water partition coefficients (log $P_{o/w}$), and (b) calculated octanol–water partition coefficients (CLOGP) for a relatively hydrophilic series of non-congeners. Solutes have been graphed according to the net hydrogen bonding classifications in Table III. The two solute classes are designated by ○ = donors and ● = acceptors. These log k_w data were determined on C_8-modified silica.

these correlations are shown in Fig. 2. Although the presence of some hydrogen bond discrimination is suggested in this figure, this effect appears to be slight.

In an attempt to lower the dynamic range further, an octadecyl-derivatized polymeric stationary phase was investigated. The increased phase ratio for this packing should allow relatively hydrophilic compounds that are unretained on the C_8 silica-based packing to be measured. Using the polymeric packing, log k'_w for thymine was estimated, indicating that the hydrophobicity index could be extended to include solutes with log $P_{o/w}$ values as low as -0.65. The correlation between log $P_{o/w}$ and log k'_w values measured on this column is illustrated in Fig. 3. The regression statistics are included in the figure legend. The data for thymine were omitted from the regression so that results could be compared directly with those derived from the C_8 packing. The statistical results are slightly poorer than those reported for eqn. 1 in Table IV. The deviation in the slope of this regression from unity indicates that the physical processes involved in retention on this column are not identical to those governing retention on the octyl-silica column. Equations correlating log $P_{o/w}$ and log k'_w data represent linear free-energy relationships in which the slope is an estimate of how closely the free

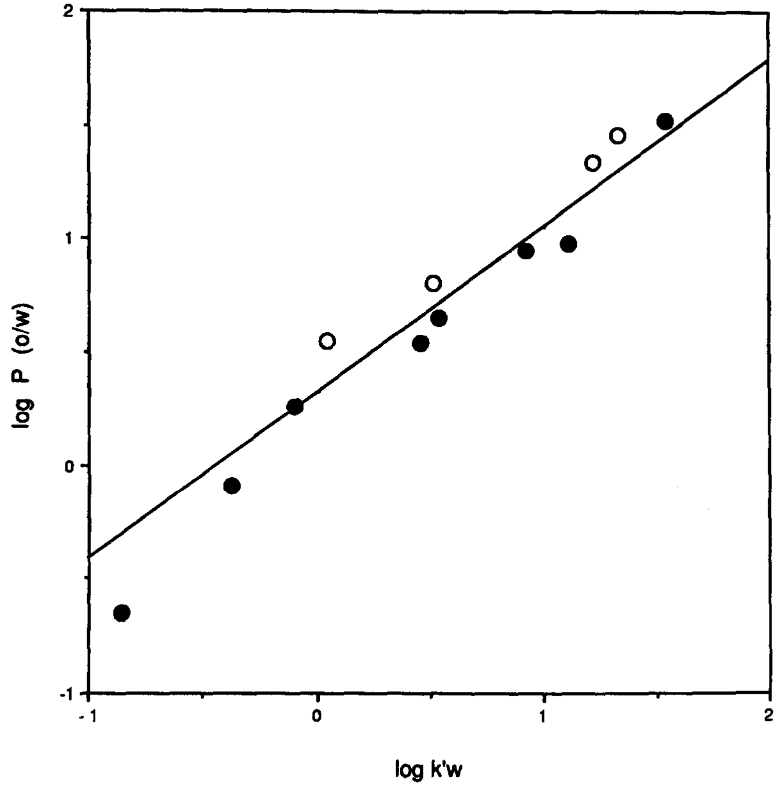

Fig. 3. Relationship between measured octanol–water partition coefficients (log $P_{o/w}$) and chromatographic hydrophobicity constants (log k'_w) determined using an octadecyl-derivatized polymeric stationary phase. The regression equation fit to this data is: log $P_{o/w}$ = 0.730 (0.058) × log k'_w + 0.324 (0.058), where the standard deviations in the slope and intercept are shown in parentheses. For this regression, $n = 11$, $r = 0.9702$ and S.D. = 0.134. Solute classes are designated by ○ = donors and ● = acceptors.

energies of the processes compare[21]. A unit slope in such a plot indicates that the two processes are "homoenergetic"[21], *i.e.*, the free energy changes are identical. However, the slope of the regression equation relating log $P_{o/w}$ with log k'_w values derived from the polymeric C_{18} phase is 0.730, indicating that these two processes are "homeoenergetic"[21], or energetically similar, since there is a linear correlation between the two, but not identical. Although more pronounced hydrogen bonding discrimination has been reported for C_{18} phases than for C_8 phases[19], the relatively large deviation from unity in the slope may also be due to solute interactions with the polymeric backbone of this packing.

The log $P_{o/w}$ and log k'_w data in Tables I and III were combined to produce an overall regression equation. For solutes measured in both experiments, log k'_w values used in this correlation were obtained from averaged k'_w. This correlation is shown in Fig. 4, where the regression results are reported in the legend. As expected, these results indicate that the values of log k'_w for these non-congeners vary linearly with log $P_{o/w}$ data over the entire range and that hydrogen bonding discrimination is minimal.

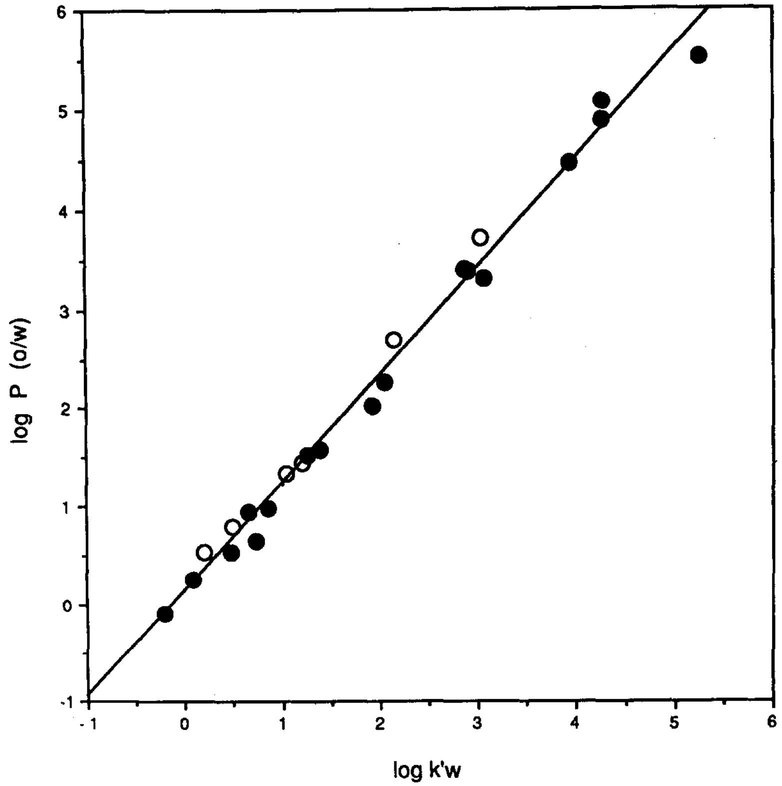

Fig. 4. The overall relationship between all of the chromatographic hydrophobicity constants (log k'_w) and measured octanol–water partition coefficients reported in Tables I and III. The regression equation fit to this data is: log $P_{o/w}$ = 1.090 (0.023) × log k'_w + 0.146 (0.058), where the standard deviations in the slope and intercept are shown in parentheses. For this regression, $n = 24, r = 0.9951$ and S.D. = 0.172. Solute classes are designated by ○ = donors and ● = acceptors.

Determination of hydrogen-bonding tendency

A distinct advantage of this RPLC method over the standard measurement of octanol–water partition coefficients is the ability to derive qualitative hydrogen bonding information from the same chromatographic data used to derive hydrophobicity constants. In the course of this work, the differential hydrogen bonding effect was observed to vary inversely in correlations between log $P_{o/w}$ and either log k'_w or S, where S is the slope constant derived from eqn. 1. Consequently, as chromatographic conditions were varied to minimize this effect between log $P_{o/w}$ and log k'_w data, it became more pronounced between log $P_{o/w}$ and S. This observation indicated that a parameter containing hydrogen bonding information could be obtained simultaneously from the same RPLC experiment designed to obtain hydrophobicity constants in which hydrogen bonding differences were attenuated.

To illustrate this, a graph of the S *vs.* log k'_w data from Tables I and III is shown in Fig. 5, where compounds were graphed according to net hydrogen bonding. In Fig. 5, compounds for which the net hydrogen bonding properties can be unambiguously assigned (*e.g.*, phenols = donors, ketones = acceptors) fall on different but approximately parallel lines. The lines shown here were fit by using a limited number of

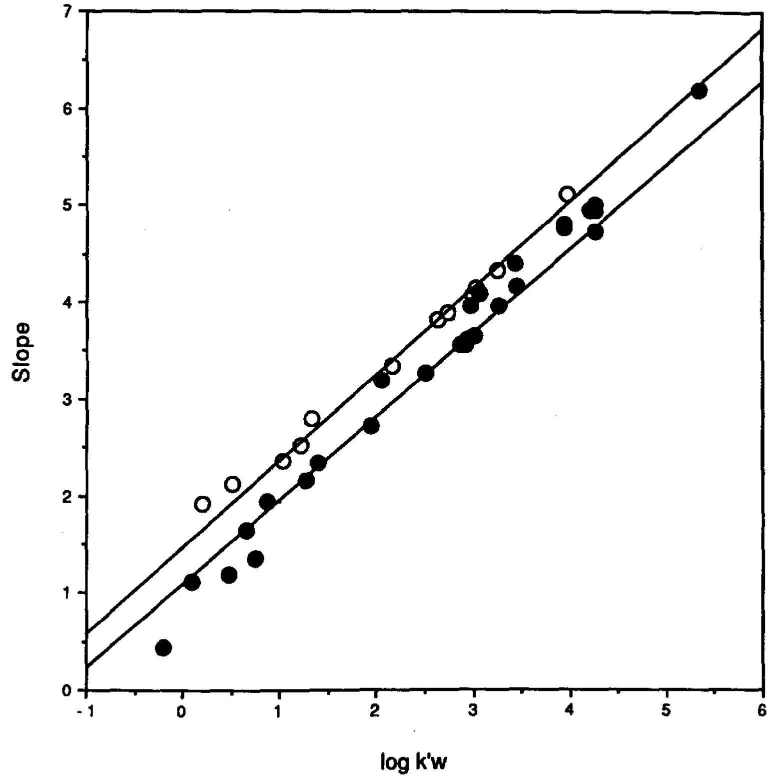

Fig. 5. Relationship between the chromatographic hydrophobicity constants, log k'_w and S, derived from eqn. 1. Data are graphed according to the net hydrogen bonding classifications shown in Tables I and III such that ○ = donors and ● = acceptors. Fitted lines were included for illustrative purposes and do not represent lines fit by regressing all S *vs.* log k'_w data from these tables.

S *vs.* log k'_w data ($n = 6$ data points in both cases), and were included primarily for illustration purposes. The results generally support the classifications reported in Tables I and III, including the classification of di-N-substituted aromatic amines (carbazole, N-phenyl-1-naphthyl amine, and 4,5-diphenylimidazole) as donors, and other aromatic amines (anilines) and ring-N compounds as acceptors, along with ketones and aldehydes. Fused-ring aromatics were uniformly found to lie between the donor and acceptor lines, and although this class of solutes was grouped as acceptors by Leo *et al.*[12], the data here suggest that these solutes might be more accurately classified as neutral compounds. The point corresponding to 1-aminoanthraquinone lies on the acceptor line, while that corresponding to 1-amino-4-hydroxyanthraquinone lies between the donor and acceptor lines. Since aminoanthraquinone contains only acceptor groups, the point for this compound should lie close to the line describing other strong acceptors. In the second compound, the presence of the hydroxyl group would be expected to contribute donor properties to the overall hydrogen bonding characteristics, resulting in a weaker net acceptor. This behavior is predicted exactly, and illustrates how these RPLC results can be used to qualitatively predict the net hydrogen bonding property of a solute containing both donor and acceptor functional groups, and may facilitate structure elucidation of unknown compounds.

The only aromatic amine observed to deviate significantly from the scheme of Leo *et al.*[12] was 4-bromoaniline, which lies between the donor and acceptor lines. This location indicates that this compound acts more like a weak acceptor or neutral than a strong acceptor, in contrast to the behavior of the other two anilines investigated here. The classification of this solute as a weak acceptor may be explained in part by considering the pK_a values of these compounds. The pK_a reported for 4-bromoaniline is low (3.82) in comparison to aniline (4.60) and 4-methoxyaniline (5.31)[22]. Since the pK_a is a measure of the proton accepting strength of a solute, this compound would be predicted to behave as a weaker acceptor than the other compounds. Two additional compounds showing deviations from expected behavior were 9-acetylanthracene and 4-biphenylcarboxaldehyde, which were both located between the donor and acceptor lines, instead of on the acceptor line. It is unclear why this behavior was observed, but the results suggest that these solutes may have undergone chemical reactions, such as the formation of a hemiacetal or hemiketal, which produced a change in their net hydrogen bonding properties.

The results shown in Fig. 5 demonstrate that two different molecular properties are obtainable from a single experiment, when the chromatographic conditions described here are used. Values of log k'_w can be used to estimate hydrophobicity quantitatively without an overlapping effect arising from variations in hydrogen bonding between different classes of solutes. Concomitantly, S appears to reflect both hydrophobic and hydrogen bonding properties and can be correlated with log k'_w to classify solutes qualitatively according to net hydrogen bonding. Since the slope parameter does exhibit hydrogen-bonding discrimination, S may be useful as an alternative estimate of hydrophobicity in correlations with biological activity that are better modeled by alkane–water partition coefficients. The parallel nature of the lines describing strong donors and strong acceptors suggests that this scheme might be useful in quantifying net hydrogen bonding properties of solutes from a general knowledge of the strengths of hydrogen bonds.

TABLE V

CORRELATION OF LOG $P_{o/w}$ WITH LOG k'_w DATA DETERMINED FROM THE MODIFIER COMPONENTS STUDY

n = Number of data points; r = correlation coefficient; S.D. = overall standard deviation of regression. The standard deviations in the slope and intercept are shown in parentheses.

(a) Methanol (no octanol)/MOPS (no amine)
$\log P_{o/w} = 1.094\ (0.227) \log k'_w - 0.157\ (0.423)$
$n = 7$ $r = 0.9069$ S.D. = 0.359
(b) Methanol + octanol (0.25%, v/v)/MOPS (no amine)
$\log P_{o/w} = 1.005\ (0.092) \log k'_w + 0.105\ (0.166)$
$n = 7$ $r = 0.9796$ S.D. = 0.171
(c) Methanol (no octanol)/MOPS + *n*-decylamine (0.15%, v/v)
$\log P_{o/w} = 1.162\ (0.123) \log k'_w - 0.216\ (0.223)$
$n = 7$ $r = 0.9731$ S.D. = 0.196
(d) Methanol + octanol (0.25%, v/v)/MOPS + *n*-decylamine (0.15%, v/v)
$\log P_{o/w} = 0.947\ (0.026) \log k'_w + 0.297\ (0.046)$
$n = 7$ $r = 0.9980$ S.D. = 0.053

Chromatographic conditions influencing the suppression of hydrogen bonding

Trace modifiers in the eluent were varied in order to determine what properties of the mobile phase were responsible for suppressing the differential hydrogen bonding effect. Results from these experiments indicated that the combination of these trace components produces suppression of this effect. The set of test compounds used in this study had been proposed earlier for comparing the hydrophobic properties of different columns[4] and included: phenol and 4-methoxyphenol (strong donors); 4-methoxyaniline, acetophenone, and camphorquinone (strong acceptors); 4-bromoaniline and naphthalene (weak acceptors). The eluent conditions were varied as follows: (a) methanol as the organic component (no octanol) and MOPS buffer as the aqueous phase (no amine); (b) methanol + octanol, but MOPS buffer only in the aqueous phase; (c) methanol only, but MOPS + amine in the aqueous phase; (d) methanol + octanol, and MOPS buffer + amine (the standard mobile-phase operating conditions). Values of log k'_w data derived from each set of eluent conditions were correlated with the values of log $P_{o/w}$ reported in Table I. The results of these correlations are reported in Table V and illustrated in Fig. 6, where log $P_{o/w}$ *vs.* log k'_w data are graphed according to the net hydrogen bonding properties of the solutes. These results show that the differential hydrogen bonding effect is greatest when the eluent contains no octanol or amine, but is still present when either but not both of these trace components is present in the eluent. However, it is clear from Fig. 6d that maximum suppression of this effect is observed only when both of these components are present. These results suggest that octanol and *n*-decylamine in the eluent alter properties of the stationary phase synergistically, producing a net desolvation–solvation process in this system that models closely the desolvation–solvation process occurring in the octanol–water system.

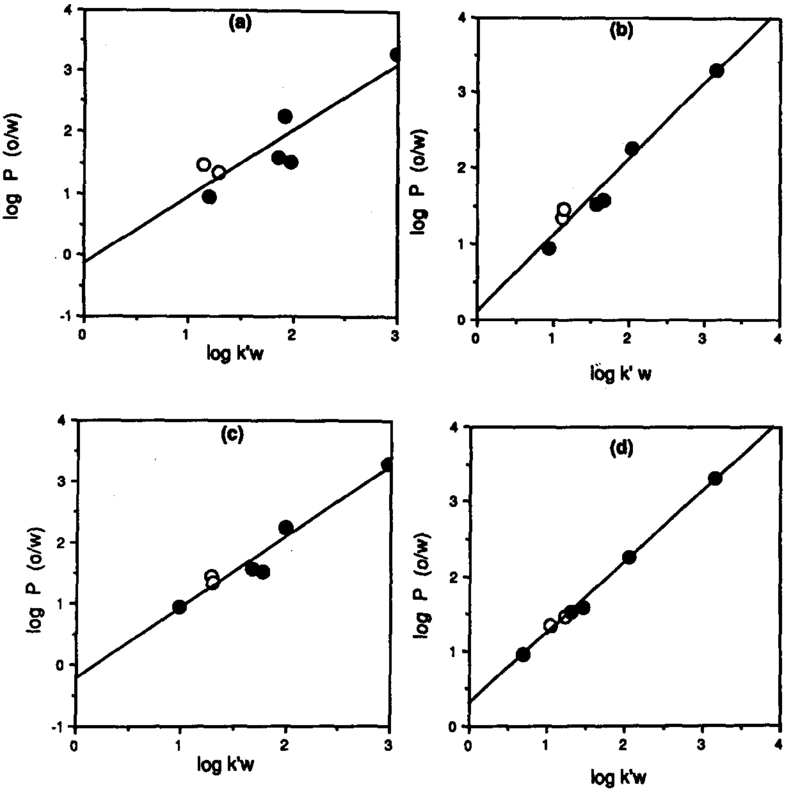

Fig. 6. Graphs illustrating the effect of trace components in the eluent. These graphs indicate the synergistic effect of octanol and decylamine upon the properties of the chromatographic system which result in accurate modeling of the octanol–water liquid–liquid partitioning system. Solutes have been graphed according to net hydrogen bonding: ○ = donors and ● = acceptors.

CONCLUSIONS

The dynamic range of a recently published method for measuring hydrophobicity constants has been investigated. Results indicate that this method is capable of estimating these constants within the range $-0.20 \leqslant \log k'_w \leqslant 5.27$, corresponding to a range $-0.09 \leqslant \log P_{o/w} \leqslant 5.52$ in the log $P_{o/w}$ scale. As discussed by Braumann[17], values of log k'_w determined by extrapolation appear to form an *a priori* scale of hydrophobicities. Comparisons between log k'_w and log $P_{o/w}$ demonstrate that this chromatographic method yields hydrophobicity constants for non-ionic solutes which closely estimate the same molecular properties estimated by octanol–water liquid–liquid partition coefficients. However, the RPLC experiment is more information-rich than the shake-flask experiment used to measure log $P_{o/w}$, for in addition to providing hydrophobicity constants (log k'_w) devoid of hydrogen bonding information, a second hydrophobicity constant (the S constant) containing information about hydrogen bonding tendencies is obtained simultaneously. The RPLC experiment is faster and more readily automated than the shake-flask method used to measure log P, and is not dependent on the purity of the sample, as is the spectrophotometric determination of log *P*.

Finally, the role of octanol and *n*-decylamine in the eluent was examined, and results were presented which suggest that it is the combination of these modifiers in the eluent which is responsible for minimizing the differential hydrogen bonding effect.

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