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Research Article

Development of a Preformulation Lipophilicity Screen Utilizing a C-18-Derivatized Polystyrene-Divinylbenzene High-Performance Liquid Chromatographic (HPLC) Column

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Alkane/water partition coefficients have been predicted from the retention times of solutes using a C-18-derivatized polystyrene-divinylbenzene HPLC column (Act-I). Several classes of compounds, with molecular weights from 78 to 379 and partition coefficients ranging over several orders of magnitude, were included in the present study. A high correlation coefficient (0.953) was obtained from log-log plots of alkane/water partition coefficient versus capacity factor. A poor correlation was observed for octanol/water partition coefficients, presumably due to the hydrogen-bonding capability of octanol. The alkane/water correlation suggests that the system is devoid of significant specific solute-stationary phase interactions which are known to impart anomalous retention behavior to traditional reverse phase columns. Deviations of calculated alkane/water partition coefficients (and Hansch Π_{alkane} coefficients) from observed values could not be explained in terms of solute (or substituent) polarizability, dipole moment, σ_{para}, or pK_{HB} values, further suggesting that specific interactions between the stationary phase and the solute are not significant. A molecular weight dependence that was independent of lipophilicity was observed. Thermodynamic and extrathermodynamic parameters of retention were obtained in order to investigate retention mechanisms for the Act-I column. The molecular weight dependence does not appear to be due to size exclusion or entropic expulsion of the solute from the stationary phase. Hansch Π substituent coefficients calculated from retention times were found to be similar for benzene and steroid derivatives. Thus, the Act-I column may be utilized as a rapid lipophilicity screen for drug candidates of similar molecular weight.

KEY WORDS: high-performance liquid chromatography (HPLC); partition coefficient; distribution coefficient; lipophilicity; enthalpy; entropy; compensation; thermodynamics.

INTRODUCTION

Oil/water partition coefficients (PC) are one of the key physicochemical parameters available to the pharmaceutical scientist. They are often used to predict biomembrane permeability (1–3), pharmacological activity (4), toxicity (5), metabolism (6), and thermodynamic properties (7). The importance of partition coefficients is further demonstrated by the availability of a priori methods of estimating partition coefficients (8–10). Traditionally, partition coefficients have been determined by the "shake flask" method. This method is rather laborious, requires an analytical method and relatively large quantities of drug, and is sensitive to impurities and degradation. These attributes greatly diminish the usefulness of the shake flask method as a screening tool for potential drug candidates. For this reason, reverse-phase HPLC retention times are often used to estimate partition coefficients (11–14). However, traditional reverse-phase HPLC columns are known to have specific solute-stationary phase interactions which may yield a poor correlation between oil/water partition coefficient and capacity factor (k') (12–16).

In a previous report (17), the use of a C-18-derivatized polystyrene-divinylbenzene HPLC column (Act-I) to predict alkane-water partition coefficients was introduced. This system has an advantage over traditional reverse-phase HPLC columns in that specific solute-stationary phase interactions appear to be insignificant (18). Unlike silica-based reverse-phase columns, polymeric columns have relatively no mobile phase pH limitation (18–20). Free silanol groups and trace metals, which are known to impart anomalous retention behavior to silica-based columns (12–16,21), are also absent. In an attempt to avoid these effects, some investigators have attempted to use polystyrene-divinylbenzene HPLC columns (19,20). However, it has been shown that polystyrene-vinylbenzene stationary phases are also capable of specific interactions (19,20), possibly due to the electron-rich pi orbitals which are present (18,22,23). Benson and Woo (18) have suggested that C-18 derivatization virtually eliminates solute interaction with the aromatic portion of the

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polymeric stationary phase through steric hinderance. Preliminary studies using the Act-I column in our laboratory support this hypothesis (17). An excellent correlation of alkane/water partition coefficient and capacity factor was observed for 30 compounds including non-hydrogen-bonding compounds, acids/alcohols, bases, and hydrogen bonding acceptors, with partition coefficients ranging over several orders of magnitude.

The objective of this report was to verify quantitatively the usefulness of the Act-I column as a lipophilicity screen for potential drug candidates. Deviations of calculated alkane/water partition coefficients from observed values were investigated in terms of solute (or substituent) molecular weight, polarizability, dipole moment, σ_{para}, or pK_{HB} values. The enthalpy and relative entropy of retention and Hansch II substituent coefficients were also analyzed to provide insight into the retention mechanisms for the Act-I column.

EXPERIMENTAL

The chromatographic system utilized in the present study has been previously described (17) and is only briefly described here. A specially prepared 5-cm Act-I column (normally available as a 15-cm column) was received from Interaction Chemicals (Mountain View, CA). A Plexiglas column jacket and a constant-temperature circulating water bath (Brinkman RM20, Westbury, NY) were used to control the temperature of the column and the reservoir. Experiments were performed at 25°C unless otherwise stated. A methanol/water (60:40) mobile phase was utilized at a flow rate of 1 ml/min. The pH of the mobile phase was adjusted for acidic and basic compounds. The compounds used in the present study and their sources are listed in Table I. Ibuprofen, alprazolam, and the various steroids were obtained from The Upjohn Company, Kalamazoo, MI. Samples were prepared in the mobile phase at a concentration of approximately 1 mg/ml, with an injection volume of 20 μl and a detection wavelength of 230 nm. Capacity factor was calculated as $k' = (t_r - t_o)/t_o$, where t_r and t_o are the retention times of the sample (in duplicate) and an unretained solute (methanol), respectively.

Hexane/water partition coefficients were determined at 25°C (in duplicate) by the traditional "shake flask" method, as described previously (17). The volume ratio of hexane to water was 1, with the exception of ibuprofen, where a volume ratio of 0.1 was utilized. In general, low concentrations (less than 2×10^{-4} *M*) were utilized to minimize aggregation. Carboxylic acids are known to dimerize in oil phases due to intermolecular hydrogen bonding (24). Therefore, several concentrations were studied for ibuprofen. The intrinsic partition coefficient was determined by Eq. (1):

$$PC' = PC + 2\,PC^2\,C_w/K \quad (1)$$

where PC' is the observed partition coefficient, C_w is the aqueous concentration, and

$$K = [C_m]^2/[C_d] \quad (2)$$

where C_m and C_d signify the concentration of monomer and dimer in the oil phase (24).

Most partition coefficients (*PC*) were taken from the literature (see Table I). The partition coefficients did not include those estimated from HPLC methods or those thought questionable by the authors of the reference, and alkane/water partition coefficients were limited to linear alkanes (*n*-pentane through *n*-decane) and cyclohexane. In cases where multiple values were available, the mean was utilized. There is generally good agreement for the partition coefficients obtained using various types of alkanes. This is to be expected since solute–alkane interactions are limited to London and induced dipole–dipole interactions (the latter only if the solute of interest has a permanent dipole).

RESULTS AND DISCUSSION

Choice of Reference State

In estimating partition coefficients by HPLC, a choice must be made between using a capacity factor determined at a particular mobile phase organic volume fraction or by linearly extrapolating the capacity factor to 0% organic. The latter method has several drawbacks. First, it is well known, both theoretically and experimentally, that the logarithm of capacity factor is related quadratically to the organic volume fraction (27–30). Thus, a linear extrapolation can be performed only over a limited (and impractical) range. Second, the quadratic relationship is dependent on the organic solvent used (27,29,30). Finally, the extrapolation method requires much more time, which defeats a primary advantage of the HPLC method over the shake flask method. Therefore, a 60% methanolic mobile phase was chosen for the present study. This choice was not entirely arbitrary. Methanol is known to produce a less dramatic quadratic curvature in plots of log k' versus volume fraction compared to other commonly used HPLC solvents (27,29,30). This is most likely due to the fact that the solubility parameter of methanol is closer to that of water than the other solvents. Furthermore, the physical properties of methanol/water mixtures have been well studied (31,32), which accounts for why methanol/water mixtures are often used for pK_a determination of compounds with low aqueous solubility (33). A volume fraction of 60% was chosen since it was found to give reasonable retention times for the solutes used in the present study (which is reasonable for many pharmaceutically relevant compounds as well).

Octanol Versus Alkane

Octanol/water partition coefficients have been utilized as a reference system in many fields, due primarily to the vast literature base developed by Hansch and co-workers (8,25,34,35). Unfortunately, the hydrogen bonding capability of octanol reduces the intrinsic usefulness of the octanol/water system. Rytting *et al.* (36) and Anderson (37) have suggested that alkanes provide more information on intermolecular forces than octanol due to the lack of hydrogen bonding and dipole–dipole interactions. Furthermore, alkane/water partition coefficients may be more relevant for biomembrane transport since the interior region of phospholipid membranes present an alkane barrier to transport (36). Young *et al.* (38) have even suggested that the difference

Table I. Octanol–Water, Alkane–Water, and Calculated Partition Coefficients

	Compound	Source[a]	log *PC*		
			Octanol/water[b]	Alkane/water[b]	Calc (alkane/water)
A.	Acetanilide	a	1.23	−1.70	−0.83
B.	Acetophenone	a	1.66	1.16	0.80
C.	Acetylbenzylamine(*N*)	t			−1.00
D.	Acetylbiphenyl(*p* −)	a			3.39
E.	Aniline	m	0.90	−0.01	−0.39
F.	Anisole	s	2.08	2.19	1.91
G.	Benzaldehyde	a	1.45	1.19	0.71
H.	Benzamide	l	0.65	−2.30	−1.50
I.	Benzene	f	1.90	2.30	1.98
J.	Benzoic acid	e	2.03	−1.06	0.10
K.	Benzonitrile	f	1.56	1.04	0.75
L.	Benzophenone	e	3.38	3.29	3.29
M.	Benzylacetate	f	1.96		1.73
N.	Benzylalcohol	e	1.05	−0.62	−0.68
O.	Benzylamine	s	1.09	−0.21	−0.73
P.	Benzylchloride	s	2.30		2.61
Q.	Biphenyl	k	3.88	4.10[c]	4.49
R.	Butylbenzoate	e			3.83
S.	Chlorobenzene	f	2.48	2.95	2.72
T.	Dimethylaniline(*N*,*N*)	s	2.38	2.32	2.15
U.	Ethylacetophenone(*p* −)	a			2.00
V.	Ethylanisole(*p* −)	l			3.18
W.	Ethylbenzene	s	3.15	3.08[c]	3.50
X.	Ethylbenzoate	a	2.42	1.40	2.38
Y.	Methoxybiphenyl(*p* −)	z			4.57
Z.	Methylacetophenone(*p* −)	a	2.19[c]		1.30
a.	Methylanisole(*p*—)	a	2.74[c]		2.53
b.	Methylbenzoate	e	2.18	2.08	1.81
c.	Methylbenzylether	e	1.35		1.28
d.	Methylphenylacetate	a			1.64
e.	Nitrobenzene	s	1.84	1.52	1.51
f.	Nitrobutane	k	1.47[c]	1.14[d]	0.75
g.	Nitroethane	k	0.18[c]	−0.38[d]	−0.58
h.	Nitrohexane	k			2.19
i.	Nitromethane	k	−0.20[c]	−0.93[c]	−1.31
j.	Phenol	m	1.28	−0.81	−0.365
k.	Phenylacetaldehyde	a	1.78		0.838
l.	Phenylacetamide(2 −)	a	0.45		−1.50
m.	Phenylacetate	p	1.49		1.05
n.	Phenylacetic acid	e	1.46	−1.23	−0.11
o.	Phenylacetone	s	1.44	0.98	0.77
p.	Phenylacetonitrile	e	1.56	1.31	0.82
q.	Phenylethylamine(2 −)	s	1.41		−0.32
r.	Propiophenone	a	2.20	2.02	1.83
s.	Propylbenzene(*n* −)	e	3.63	4.11[c]	4.00
t.	Pyridine	a	0.65	−0.31	−1.06
u.	Toluene	f	2.58	2.86	2.74
v.	Trichlorotoluene (ααα)	a	2.92		1.72
w.	Trifluorotoluene (ααα)	a	2.90		2.44

[a] (a) Aldrich Chemical Company, Milwaukee, WI; (e) Eastman Kodak Company, Rochester, NY; (f) Fisher Scientific, Fair Lawn, NJ; (k) Fluka Chemie AG, Buchs.; (l) Lancaster Synthesis Ltd., Windham, NH; (m) Mallinckrodt, Inc., Paris, KY; (p) Pfaltz and Bauer, Inc., Stamford, CT; (s) Sigma Chemical Company, St. Louis, (MO); (t) American Tokyo Kasei, Inc., Portland, OR; (z) Alfa Products, Danvers, MA.

[b] From Ref. 25 if not specified.

[c] From Ref. 26.

[d] From Ref. 17.

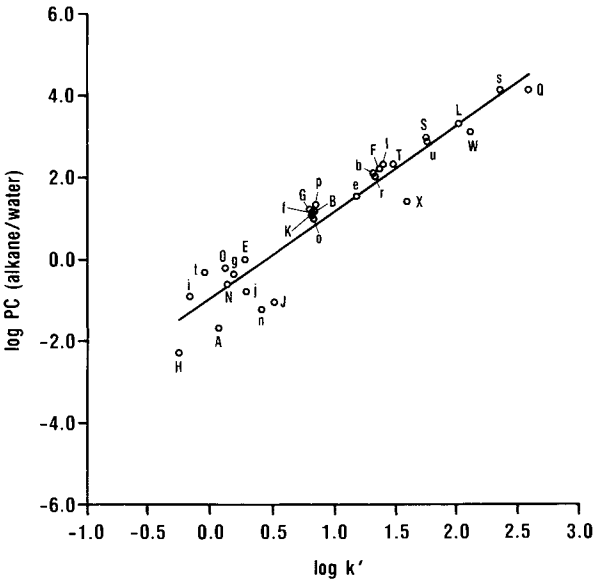

Fig. 1. Relationship between the alkane/water partition coefficient and the capacity factor (60:40 methanol/water mobile phase). The regression line shown is for all compounds. Compounds are represented by letters (see Table I).

between the alkane/water and octanol/water partition coefficients be used to predict transport across biomembranes such as the blood-brain barrier.

Plots of log *PC* versus log *k'* are shown in Figs. 1 and 2 for the alkane/water and octanol/water partition coefficients, respectively. The slopes (*m*), intercepts (*b*), and correlation coefficients (*r*) of the regression lines

$$\log PC = m \log k' + b \qquad (3)$$

are listed in Table II. The observed slope in Fig. 1 is greater

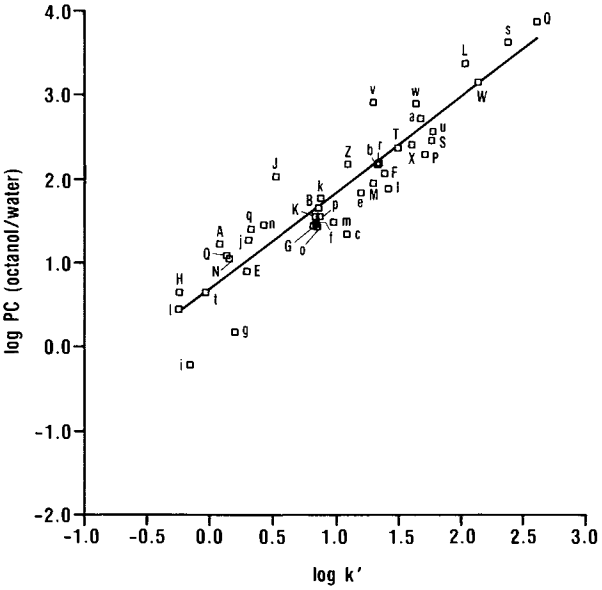

Fig. 2. Relationship between the octanol/water partition coefficient and the capacity factor (60:40 methanol/water mobile phase). The regression line shown is for all compounds. Compounds are represented by letters (see Table I).

Table II. Linear Regression Results from Plots of log *PC* Versus log *k'*

Figure	Slope[a]	*y* intercept[a]	*R*
1 (alkane)	2.11 (11.7%)	−0.998 (30.7%)	0.953
2 (octanol)	1.15 (12.2%)	0.684 (25.3%)	0.932
3 (alkane)[b]	1.61 (55.6%)	−0.0144 (13,000%)	0.957
4 (octanol)[b]	1.69 (12.7%)	−0.444 (101%)	0.998

[a] 95% confidence interval in parentheses.
[b] Non-hydrogen-bonding compounds only.

than unity, as anticipated by the retention theory of Dill (29,39). The correlation between log *k'* and log *PC* appears to be better for the alkane than the octanol system. When silica-based reverse-phase HPLC is utilized, a much better correlation is generally seen for the octanol system (15). This has been attributed to adsorption of methanol to the stationary phase, creating an octanol-like environment (15). While this may be occurring to some extent with the Act-I column, it appears that hydrogen bonding between the stationary phase and the solute is much less important with the Act-I column than with traditional reverse-phase columns. This can also be demonstrated by analyzing the correlation of log *k'* and log *PC* for non-hydrogen-bonding compounds (Table II). For the alkane system, the regression line for the non-hydrogen-bonding compounds is not significantly different from the line for all compounds, and all but three compounds fall within the 95% confidence intervals for the non-hydrogen-bonding regression line (Fig. 3). This is not the case for the octanol system. Hydrogen bonding in octanol causes the majority of the points to be above the 95% confidence interval for non-hydrogen-bonding compounds, including those compounds with partition coefficients similar to the non-hydrogen-bonding compounds (Fig. 4).

The correlation for the alkane/water partition coefficient is welcome considering the wide range of lipophilicities of the solutes (partition coefficients ranging over six orders of magnitude). The log PC (alkane) values calculated from the regression line are listed in Table I. The deviation from the

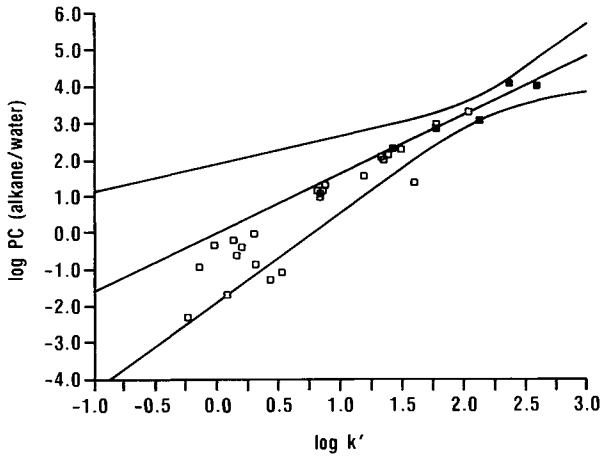

Fig. 3. Relationship between the alkane/water partition coefficient and the capacity factor (60:40 methanol/water mobile phase). The regression line shown (with 95% confidence interval) is for non-hydrogen-bonding compounds (filled symbols).

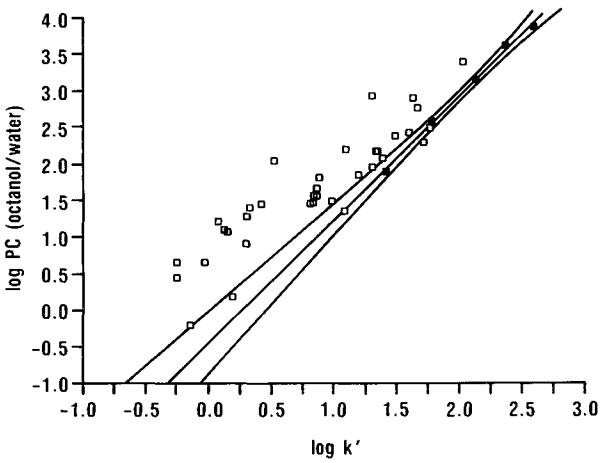

Fig. 4. Relationship between the octanol/water partition coefficient and the capacity factor (60:40 methanol/water mobile phase). The regression line shown (with 95% confidence interval) is for non-hydrogen-bonding compounds (filled symbols).

regression line appears to be most dramatic for compounds with a log *PC* less than 0 (see Fig. 1). This is most likely due to the error in quantitating k' for solutes with retention times approaching t_o. Therefore, investigators may wish to alter the methanol percentage to match the lipophilicities of the compounds under study.

All calculated values in Table I are well within 1 log PC unit of the observed value with the exception of ethyl benzoate, benzoic acid, and phenylacetic acid, which had deviations ranging from 0.98 to 1.16 log PC units. For ethyl benzoate, a low literature value for the alkane/water PC is suspected (see methyl benzoate value). Dimerization of carboxylic acids in the stationary phase would increase retention time and, therefore, lead to a positive deviation of the calculated log PC. To determine if a significant amount of aggregation was taking place, k' for benzoic acid was determined for a series of sample concentrations (0.1 to 10 mg/ml) at 25, 37, 45, and 60°C. There was little or no change (less than 12%) in the capacity factor for a given temperature up to 1 mg/ml. Above 1 mg/ml, k' decreased with increasing concentration (with the exception of 45°C, where no change was observed). The data suggest that for sample concentrations of 1 mg/ml or less, aggregation does not occur. The cause of the deviation in log PC for benzoic acid and phenylacetic acid is therefore unknown.

Test for Specific Interactions

The compounds in Fig. 1 include non-hydrogen-bonding solutes, acids–alcohols, bases, and hydrogen bond acceptors. The inclusion of various classes of solutes suggests there is a lack of any significant specific interaction between the stationary phase and the solutes. However, because the Act-I column is intended to be utilized as a lipophilicity screen, specific interactions were analyzed in a more quantitative manner. Dipole, induced-dipole, and hydrogen bonding interactions between the solutes and the stationary phase were investigated.

Polystyrene-divinylbenzene columns contain dipoles due to electron donation to the aromatic portion of the co-polymer backbone. While these dipoles are probably of a much smaller magnitude than those present in reverse-phase silica-based columns, they may account for anomalous retention behavior that has been observed (19,20). In addition, the polymeric column may be more susceptible to the permanent dipoles of solutes due to a higher polarizability (relative to alkanes). It shall be assumed that a local dipole of the stationary phase may be treated as a dipole of a polar molecule. Then, the interaction energy (E_{int}) for a solute molecule (1) and the stationary phase (2) may be approximated by

$$E_{int} \approx -\frac{1}{(4\Pi\epsilon_0)^2 r^6}\left[\frac{\mu_1^2\mu_2^2}{3kT} + \mu_1^2\alpha_2 + \mu_2^2\alpha_1 + \frac{3}{2}\frac{\alpha_1\alpha_2 I_1 I_2}{(I_1 + I_2)}\right] \quad (4)$$

where μ, α, I, k, T, ϵ_0, and r are the dipole moment, polarizability, ionization potential, Boltzman constant, temperature, permitivity, and separation distance, respectively (40). The first, second and third, and fourth terms in Eq. (4) represent the Keesom, Debye, and London forces, respectively. Interactions between a solute and an alkane molecule would be limited to London and Debye (if the solute has a permanent dipole) forces. A specific interaction between the stationary phase and the solute would be suggested if the polarizability or dipole moment of the solute could be related to the difference between the literature log *PC* (or Π) value and the value calculated from k',

$$\Delta \log PC = \log PC_{lit} - \log PC_{calc} \quad (5)$$

and

$$\Delta\Pi = \Pi_{lit} - \Pi_{calc} \quad (6)$$

The Hansch substituent coefficient Π is defined as the log increase in PC for substitution of a hydrogen of a base compound with the substituent X (35). This subject is discussed in more detail in a later section.

Molar refractivity (MR) is a readily obtainable parameter which is linearly related to polarizability (25). MR values for various substituents have been plotted versus ΔΠ in Fig. 5. The low correlation coefficient and a slope not significantly different from zero (see Table III) suggest that the sum of the London forces and the Debye forces (due to the dipoles of the stationary phase) does not introduce significant deviations relative to bulk alkane. Furthermore, a plot of Δlog *PC* as a function of the square of solute dipole moment suggests that the sum of the Keesom forces and the Debye forces (due to the dipoles of the solute) does not introduce significant deviations relative to bulk alkane (Table III).

The dipole moment does not differentiate between electron withdrawal and donation by benzene substituents. Therefore, a geometrically favorable interaction between the electron-rich pi orbitals of the stationary phase and an electron-deficient pi orbital in a substituted benzene would not be observed in the above analysis. The substituent constant σ_{para} was used to gauge electron withdrawal from the aromatic ring, since this constant is affected by both inductive and resonance effects (25). No correlation was observed between ΔΠ and σ_{para} (Table III), suggesting that no significant pi orbital interactions occur on the column.

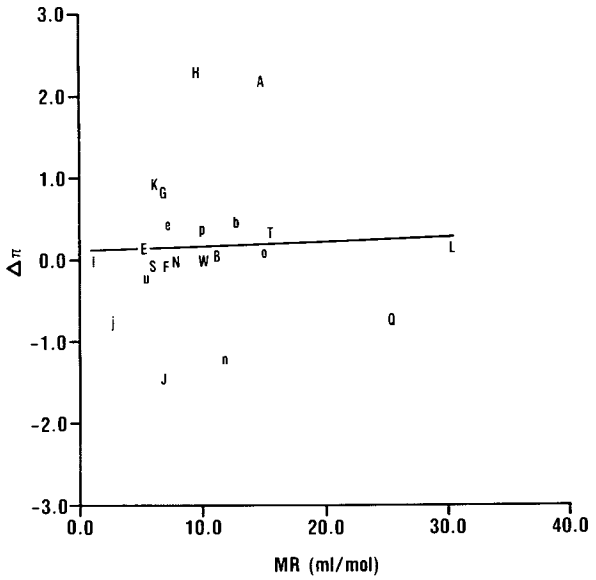

Fig. 5. Relationship between ΔΠ and substituent molar refractivity. Compounds are represented by letters (see Table I). The MR substituent constants were obtained from Ref. 25.

Kiselev (22) and Paleos (23) have suggested that polystyrene-divinylbenzene may act as a hydrogen bond acceptor. If methanol is adsorbed to the stationary phase, the column could also function as a hydrogen bond donor. Therefore, it would be interesting to test if deviations of the HPLC method from the alkane/water system could be accounted for by the acceptance or donating capability of the solute. The hydrogen-bonding acceptance capability of the solute can be measured by pK_{HB} (42,43). No significant deviation is observed as a function of pK_{HB} (Table III). Unfortunately, quantitative studies of the relative strength of hydrogen bonding donors have dealt with a limited number of compounds (42,44), prohibiting their use in the present study.

Effect of Molecular Weight

A molecular size dependence for retention (which is independent of lipophilicity) might be anticipated due to a size exclusion effect or entropic differences between the polymeric stationary phase and bulk alkane. A size exclusion phenomenon would decrease solute retention time with increasing molecular volume (45). The attachment of the C-18 chains to the polymer backbone decreases the degrees of freedom relative to a bulk alkane. Thus, insertion of a solute molecule into the stationary phase will be accompanied by a decrease in the configurational entropy of the grafted chains (29,46). This implies that increasing molecular volume would disfavor solute retention.

In order to test if Δ log PC can be related to molecular size, the cubed root of molecular weight (MW) was utilized as a first approximation of molecular radius (Fig. 6). The slope of this plot is negative, suggesting a larger k' than expected from the PC with increasing molecular weight. A positive slope would be expected if significant entropic expulsion or size exclusion were occurring. The effect of molecular radius on Δ log *PC* is not statistically significant (see Table III) for the molecular weight range of the solutes in Table I (61–196). In order to verify the usefulness of the Act-I column for use as a drug candidate lipophilicity screen, the correlation of k' with PC was investigated for larger molecular weight compounds (MW of approximately 200 to 400). Alkane-water log *PC*'s for alprazolam (Xanax), ibuprofen (Motrin), and prednisolone were found to be −1.53, 1.54, and −1.42, respectively. Acetophenone, benzene, phenol, and toluene were utilized to create a standard curve of log *PC* versus log k'. Calculated log *PC*'s for the three compounds using this standard curve were higher than the experimental values by 1 log *PC* unit or more (0.649, 2.92, and −0.390, respectively). The results for these larger compounds would appear to support the negative slope in Fig. 6. In an attempt to understand the retention mechanisms for the Act-I column and to elucidate the cause of the apparent molecular weight dependence, thermodynamic and extrathermodynamic parameters of retention were investigated.

Table III. Linear Regression Statistics for the "Specific Interaction" Plots

Figure	Slope	95% CI	R	n	Ref. No.
ΔΠ vs *MR*	0.00589	1020%	0.0455	21	25
Δ log *PC* vs μ^2	0.0253	179%	0.306	16	41
Δ Π vs. σ_p	0.432	236%	0.200	21	25
Δ log PC vs pK_{HB}	−0.274	1300%	−0.050	12	42, 43
Δ log PC vs $MW^{1/3}$	−0.498	122%	−0.319	25	

Thermodynamics

HPLC capacity factor is defined as the product of the stationary phase–mobile phase partition coefficient (PC_{sm}) and the phase volume ratio of the column (47),

$$k' = PC_{sm}\, V_s/V_m \qquad (7)$$

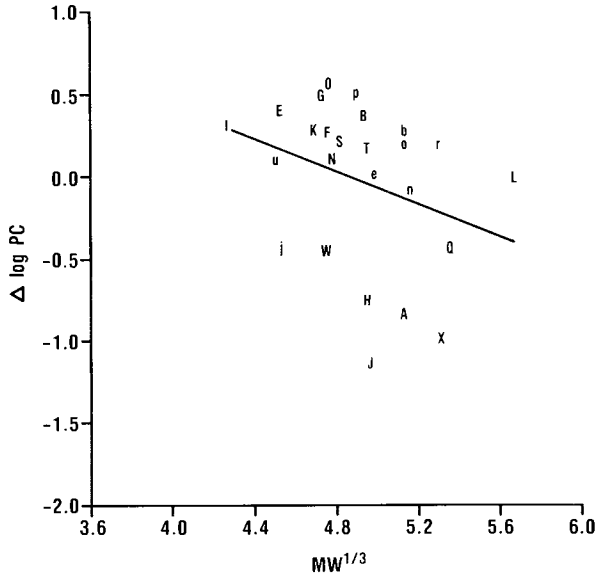

Fig. 6. Relationship between Δ log *PC* and the cubed root of molecular weight. Compounds are represented by letters (see Table I).

where V_s and V_m are the volumes of the stationary phase and mobile phase, respectively. Combining Eq. (7) with the Van't Hoff Equation yields Eq. (8), allowing determination of the enthalpy and entropy of retention (48).

$$\log k' = 0.4343\,(-\Delta H/RT + \Delta S/R) + \log (V_s/V_m) \quad (8)$$

Because the phase volume ratio of the column is typically unknown, only a relative entropy ($\Delta S'$) may be determined from plots of log k' versus $1/T$. Van't Hoff plots are shown for the compounds utilized in the present study in Figs. 7 and 8. All plots appear to be linear over the 25 to 60°C temperature range. Retention times decrease with increasing temperature for all solutes studied. This is consistent with the reports of several investigators who have studied other reverse phase columns (48–52), including a polystyrene-divinylbenzene column (53). Yang and Gilpin (54) recently studied the "onset" temperature at which silica immobilized alkyl chains reorder. Onset temperature with an aqueous mobile phase was found to increase with increasing chain length, to be independent of binding chemistry, and to occur at approximately 60°C for decyl chains. No nonlinearity was observed for the Van't Hoff plots of the present study. This suggests that thermal reordering of the alkyl chains for the Act-I column does not occur in the 25 to 60°C temperature range.

Linear regression statistics and derived enthalpies and relative entropies for the Van't Hoff plots are listed in Table IV. Correlation coefficients were greater or equal to 0.997, with the exception of the correlation coefficient for phenol which was 0.9883 (possibly due to a short retention time). Enthalpies ranged from −8.6 to −3.8 kcal/mol. Relative entropies ranged from −19.7 to −10.4 cal/kmol. As a group, the entropies for alprazolam, ibuprofen, and prednisolone were not larger in magnitude than the smaller solutes, as might have been predicted based on the entropic effect of forming a void in the grafted chains.

Group contribution values ($\Delta\Delta H$) and $\Delta\Delta S$) are listed in Table V. These values are useful for making a priori predictions of retention behavior. As expected, nonpolar substituents have a negative contribution to enthalpy, while polar groups have a positive contribution. It is interesting to note

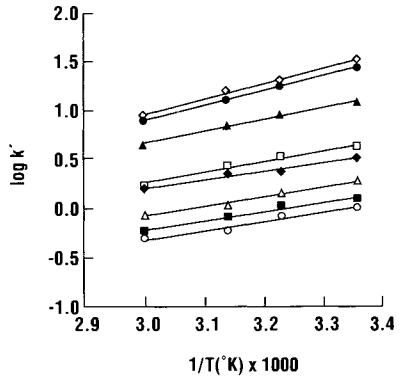

Fig. 7. Van't Hoff plot for retention on the Act-I column. (□) Acetophenone; (♦) alprazolam; (■) aniline; (▲) benzene; (△) benzoic acid; (●) ibuprofen; (○) phenol; (■) prednisolone; (◇) toluene.

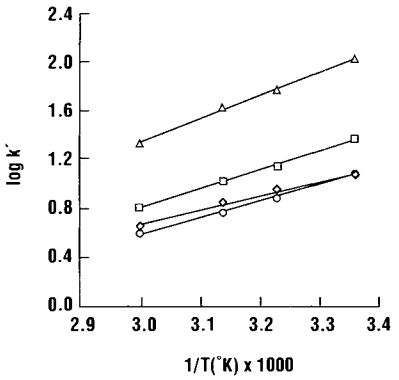

Fig. 8. Van't Hoff plot for retention of benzene and benzoic acid esters on the Act-I column. (○) Methylbenzoate; (□) ethylbenzoate; (△) butylbenzoate; (◇) benzene.

the difference in the group contribution values for the methyl and the methylene groups, the former derived from aromatic substitution and the latter from the benzoate esters. Methyl and methylene group contribution values are often assumed to be identical, which may be unjustified (55). The $\Delta\Delta H$ and $\Delta\Delta S$ values for CH_2 appear to be constant for the limited benzoate series used in the present study. This observation should be treated with caution since a dependence on chain length for $\Delta\Delta H$ and $\Delta\Delta S$ has previously been observed for the transfer of alkanols between water and alkane (55).

Enthalpy–Entropy Compensation

Enthalpy has been empirically observed to be linearly related to entropy for a number of physicochemical processes such as solubility and chemical reaction kinetics (56). An enthalpy–entropy compensation plot (also known as a Barclay–Butler plot) for the retention data in the present report is shown in Fig. 9. The regression line has a slope of 1.52×10^{-3} (26.0%), a y intercept of −6.24 (35.8%) and a correlation coefficient of 0.934 (95% confidence intervals in parentheses). The compensation curve does not seem to differentiate between small and moderate weight compounds, as all points fall near the regression line. In order to avoid statistical errors in examining the compensation effect, it has been suggested (48) that compensation for chromatographic retention be examined by Eq. (9):

$$\log k'_T = -0.4343[\Delta H(1/T - 1/\beta)/R - \Delta G_\beta/R\beta] + \log V_s/V_m \quad (9)$$

where k'_T is the capacity factor at the harmonic mean of the experimental temperatures, and β is the compensation temperature. The so-called Melander–Horvath plot is shown in Fig. 10. The regression line has a slope of -3.70×10^{-4} (22.8%), a y intercept of −1.33 (35.8%), and a correlation coefficient of −0.951. This slope yields a compensation temperature of 652°K, in good agreement with the value from the Barclay–Butler curve (658°K). Melander *et al.* (48) have reported compensation temperatures ranging from 596 to 647°K for octadecyl silica (ODS) stationary phases. The similarity of the compensation temperatures for the ODS columns and the Act-I column may suggest a resemblance in the retention mechanism for the two systems.

Table IV. Linear Regression Statistics, Enthalpy, and Relative Entropy of Retention from Plots of log k' vs $1/T$

Compound	Slope[a]	ΔH (cal/mol)	y intercept[a]	$\Delta S'$ (cal/°K mol)	R
Acetophenone	1027 (16.9%)	−4700	−2.82 (19.6%)	−12.9	0.9985
Alprazolam	823 (15.6%)	−3766	−2.27 (18.0%)	−10.4	0.9987
Aniline	870 (23.8%)	−3982	−2.84 (23.2%)	−13.0	0.9970
Benzene	1139 (19.7%)	−5210	−2.75 (26.1%)	−12.6	0.9979
Benzoic acid	928 (15.0%)	−4246	−2.87 (15.5%)	−13.1	0.9988
Butyl benzoate	1882 (4.2%)	−8612	−4.31 (5.9%)	−19.7	0.9999
Ethyl benzoate	1522 (7.0%)	−6965	−3.76 (9.0%)	−17.2	0.9997
Ibuprofen	1472 (10.0%)	−6732	−3.52 (13.4%)	−16.1	0.9995
Methyl benzoate	1359 (10.2%)	−6216	−3.49 (12.6%)	−16.0	0.9994
Phenol	895 (47.0%)	−4095	−3.01 (44.5%)	−13.8	0.9883
Prednisolone	870 (23.8%)	−3982	−2.84 (19.6%)	−13.0	0.9970
Toluene	1517 (10.8%)	−6940	−3.60 (14.5%)	−16.4	0.9978

[a] 95% confidence interval in parentheses.

Hansch Π Coefficients

While the above analysis failed to provide a mechanistic reason for the observed molecular weight dependence, it suggests that the basic retention mechanisms are similar up to a molecular weight of at least 400. Thus, the lipophilicity for a series of compounds of similar molecular weight can be investigated using the Act-I column. This concept can be tested by comparing Hansch Π coefficients for compounds of markedly different molecular weight (e.g., steroid and benzene derivatives). The substituent constant Π_X is defined as

$$\Pi_X = \log PC_X - \log PC_H \quad (10)$$

where PC_X and PC_H refer to the partition coefficient of a compound with and without a substituent X, respectively (35). Combining Eqs. (3) and (10) gives

$$\Pi_{X,calc} = m(\log k'_X - \log k'_H) \quad (11)$$

where m is the slope of the regression line in Fig. 1. Methylene-group contribution to the partition coefficient can be calculated as

$$\log PC_n = \log PC_0 + n\,\Pi_{CH_2} \quad (12)$$

where n refers to a member of a homologous series with a chain length of n carbon atoms (57). Likewise,

$$\log k'_n = \log k'_0 + n\,\Pi_{CH_2,calc}/m \quad (13)$$

A plot of log k' versus n was used to calculate a mean $\Pi_{CH_2,calc}$ value of 0.66 for five different series of compounds (see Table VI). The correlation coefficients for all five series were greater than 0.996.

Table V. Functional-Group Contribution

Functional group	$\Delta\Delta H$ (cal/mol)	$\Delta\Delta S$ (cal/°K mol)
OH	1115	−1.23
CH_3	−1730	−3.89
$COCH_3$	510	−0.36
COOH	964	−0.55
NH_2	1228	−0.41
$COOCH_3$	−1006	−3.41
$COOCH_2CH_3$	−1755	−4.64
$COO(CH_2)_3CH_3$	−3402	−7.14
CH_2	−802	−1.24

The Π coefficients were calculated from retention times for methyl-, hydroxyl-, and carbonyl-substituted steroids (Table VI and Fig. 11). The substituted steroids have molecular weights of 314 or greater. These Π coefficients may be compared to values obtained for substituted benzene derivatives, all with molecular weights of 178 or less. The hydroxyl and carbonyl substitutions for the benzene derivatives were on alkyl portions, so as to avoid resonance with the aromatic ring. There appears to be good agreement between the Π coefficient for the 6 α methyl group for steroids and the methylene group for homologous series of benzene derivatives. It has been assumed that the methyl and methylene groups have similar Π coefficients, which is consistent with previous observations (55). A large negative value was obtained for the carbonyl group for both benzene and progesterone derivatives. The progesterone value is lower than the benzene value, possibly due to the steric hinderance of the steroid nucleus.

The data in Table VI for the hydroxyl substituted steroids appears to be more complex. The Π coefficients range from 0 to −2.42, depending on the site of substitution and the base steroid. The most dramatic example is seen at position 11. No effect is seen for 11 α substitution of the hydroxyl group for deoxycortisol, while a value of −0.883 is

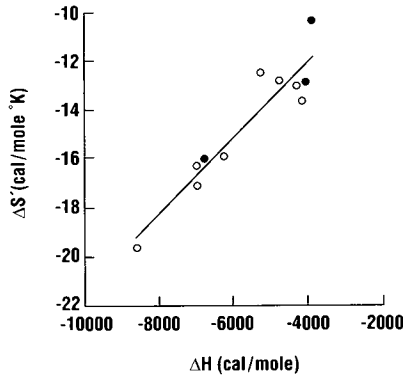

Fig. 9. Barclay–Butler plot for retention on the Act-I column. Compounds with molecular weights greater than 200 are indicated by filled circles.

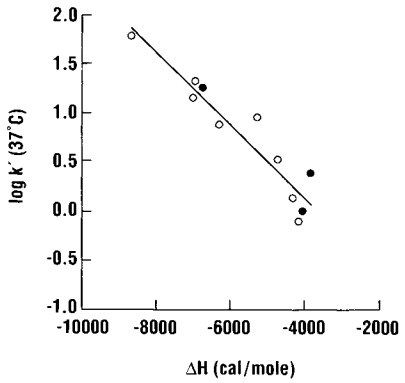

Fig. 10. Melander–Horvath plot for retention on the Act-I column. Compounds with molecular weights greater than 200 are indicated by filled circles.

Table VI. Calculated Π_X Coeffients (Alkane/Water) for Steroid and Benzene Derivatives[a]

X	Prednisolone (I)	Deoxycortisol (II)	Progesterone (III)	Benzene
6 α CH3	0.697	0.685		0.66 (0.05, $n = 5$)[b]
6 β OH		−2.23	−2.00 (0.03, $n = 2$)	−3.48[c]
11 α OH		0.00	−2.42	−3.48[c]
11 β OH		−0.883		−3.48[c]
16 α OH	−0.770	−0.685		−3.48[c]
11 C=O			−2.13 (0.04, $n = 2$)	−3.13[d]

[a] Standard deviation in parentheses; 25°C.
[b] Determined using nitroalkanes, *p*-alkyl anisoles, *p*-alkyl acetophenones, alkylbenzoates, and alkylbenzenes ($X = CH_2$).
[c] Determined using benzyl alcohol and toluene.
[d] Determined using phenyl acetone and propylbenzene.

Base Steroid	Name	6	11	16
I	U-5,962	---	β OH	---
I	U-7,532	α CH3	β OH	---
I	U-19,356	α CH3	β OH	α OH
II	U-1,237	---	---	---
II	U-0,405	β OH	---	---
II	U-1,851	---	β OH	---
II	U-0,461	β OH	α OH	---
II	U-7,240	α CH3	β OH	---
II	U-34,908	---	β OH	α OH
III	U-3,672	---	---	---
III	U-1,258	---	C = O	---
III	U-2,651	β OH	C = O	---
III	U-0,384	---	α OH	---
III	U-27,605	β OH	---	---

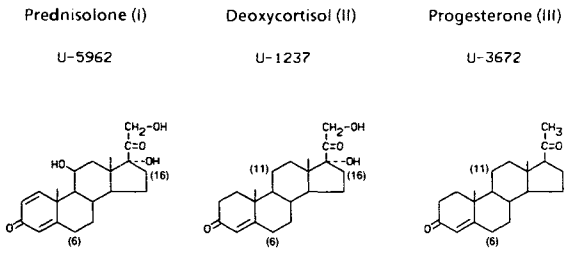

Fig. 11. Steroid derivatives.

Table VII. Π Coefficients at 25°C

X	Octanol/water	Alkane/water	Calc (alkane/water)
C_6H_5X			
H	0	0	0
OH	−0.62	−3.11	−2.34
OCH_3	0.18	−0.11	−0.06
$OC(=O)CH_3$	−0.41		−0.93
NO_2	−0.06	−0.78	−0.46
$NHC(=O)CH_3$	−0.67	−4.00	−2.81
NH_2	−1.00	−2.31	−2.36
$N(CH_3)_2$	0.48	0.02	0.17
Cl	0.58	0.65	0.74
CHO	−0.45	−1.11	−1.27
CH_3	0.68	0.56	0.76
C_6H_5	1.98	1.80	2.51
$C-F_3$	1.00		0.46
$C-Cl_3$	1.02		−0.25
$C(=O)OH$	0.13	−3.36	−1.88
$C(=O)OCH_3$	0.28	−0.22	−0.17
$C(=O)OC_2H_5$	0.52	−0.90	0.40
$C(=O)OC_4H_9$			1.86
$C(=O)NH_2$	−1.25	−4.60	−3.48
$C(=O)CH_3$	−0.24	−1.14	−1.18
$C(=O)C_2H_5$	−0.28	0.12	−0.15
$C(=O)C_6H_5$	1.48	0.99	1.31
C≡N	−0.34	−1.26	−1.22
$C_6H_5CH_2X$			
H	0	0	0
OH	−1.53	−3.48	−3.42
OCH_3	−1.23		−1.46
$OC(=O)CH_3$	−0.62		−1.01
$NHC(=O)CH_3$			−3.73
NH_2	−1.49	−3.07	−3.46
Cl	−0.28		−0.13
CHO	−0.80		−1.90
CH_3	0.57	0.22	0.76
$C(=O)OH$	−1.21	−4.09	−2.85
$C(=O)OCH_3$			−1.10
$C(=O)NH_2$	−2.13		−4.24
$C(=O)CH_3$	−1.14	−1.88	−1.96
C≡N	−1.02	−1.55	−1.92

obtained for 11 β substitution. For progesterone, a value of −2.42 is observed for 11 α substitution. It appears that these effects are due to either intramolecular hydrogen bonding or steric effects of the steroid nucleus, which reduce the apparent polarity of the hydroxyl group.

The data seem consistent with the concept that the retention mechanisms for the steroid and benzene derivatives are similar. Furthermore, it is encouraging to find that the retention is influenced by subtle structural changes. Effects such as intramolecular hydrogen bonding are sure to influence physical properties such as biomembrane transport and biological activity. Thus, it appears that the Act-I column may be utilized as a rapid lipophilicity screen for drug candidates of similar molecular weight.

Since most tabulations of Π coefficients are for the octanol/water system, there is a need in the literature for more extensive listings of Π coefficients for the alkane/water system. The HPLC method described in this report provides a rapid method of calculating these substituent constants.

Alkane, octanol, and calculated (alkane) Π coefficients are listed in Table VII. Alkane and octanol Π values were calculated for common substituents from the log PC values in Table I. In general, there is good agreement between the alkane and the calculated Π value. As expected, the octanol value is greater than the alkane value or the calculated value for most polar substituents.

In conclusion, a significant correlation was found between log *PC* (alkane/water) and log *k'*. There appears to be no significant specific interaction between the solutes and the polymeric stationary phase. This lack of specific interaction provides an advantage over systems utilizing traditional reverse phase columns. Finally, the system can be utilized for the rapid screening of lipophilicity of potential drug candidates of similar molecular weight.

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