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INVESTIGATION OF N-ALKYLBENZAMIDES BY REVERSED-PHASE LIQUID CHROMATOGRAPHY

II. APPLICATION OF THE SOLVOPHOBIC THEORY TO THE PREDICTION OF RETENTION DATA FOR THE C₁-C₅ N-ALKYLBENZAMIDES

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SUMMARY

The relationship between capacity factor (k') and eluent composition for the C₁-C₅ N-alkylbenzamides was not described adequately by either linear or quadratic equations. Using the chromatographic data determined in acetonitrile-water, the relationship between k' and eluent composition was described by the solvophobic theory. Regression coefficients obtained from the solvophobic equation were used to calculate the contact surface area of the solute-bonded ligand complex. The theory was used to calculate k' for each compound in a totally aqueous mobile phase. The results indicate that branched alkyl chains elute before their straight-chain analogues and among isomers the elution volume increases with the distance between the branching point and the amide nitrogen.

INTRODUCTION

The solvophobic theory, developed by Sinanoglu and Abdulnur¹, has been adapted for use in reversed-phase liquid chromatography (RPLC) by Horváth *et al.*². According to this theory, the retention of a solute in RPLC is described by eqn. 1 when the mobile phase composition is varied while the temperature and flow-rate remain constant.

$$\ln k' = A + B\mathcal{D} + C\gamma + D(\kappa^e - 1)V^{2/3}\gamma + E + \ln(RT/P_0V) \quad (1)$$

where

$$A = \phi - \frac{\Delta F_{\text{dw,assoc}}}{RT} \quad (2)$$

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$$B = \frac{1}{RT} \frac{1 - \lambda \mu_s^2}{2\lambda} \frac{1}{v_s} NP \approx \frac{1}{4\pi\epsilon_0 RT} \frac{1 - \lambda \mu_s^2}{2\lambda} \frac{N}{1 - (\alpha_s/v_s)} \quad (3)$$

with the approximation that $\mathcal{G} \approx 1$

$$C = N\Delta A/RT \quad (4)$$

$$D = 4.836 N^{1.3}/RT \quad (5)$$

$$E = \Delta F_{\text{d.w.}}/RT \quad (6)$$

See Appendix for a description of the variables and for a discussion of the derivation of this equation see ref. 2.

Eqn. 1 results from the hypothesis that the formation of a reversible solute-bonded ligand complex is governed by solvophobic effects, *i.e.* a hydrophobic effect when aqueous solvents are used. Studies concerning the nature of the hydrophobic effect have appeared in the literature since the early work of Traube³ in 1891. According to Tanford⁴, a hydrophobic effect (hydrophobic effects, hydrophobic bonds, hydrophobic interactions are used synonymously in the literature) arises when any solute is dissolved in water. A hydrophobic bond has been defined⁵ as one which forms when non-polar groups in an aqueous solvent associate, thereby decreasing the extent of interaction with surrounding water molecules, and liberating water originally bound by the solutes. In the past, the hydrophobic effect was believed to arise from the attraction of non-polar groups for each other⁶. While a like-attracts-like interaction certainly plays a role in this phenomenon, current opinion⁴ views the strong forces between water molecules as primarily responsible for the hydrophobic effect. The detailed molecular structure of liquid water is complex and not well understood. Many of the unusual properties of water are the result of the isotropic network of hydrogen bonds linking individual molecules.

According to the solvophobic theory, interactions in RPLC between the hydrocarbonaceous ligand (bonded stationary phase) and non-polar solutes are not the result of attraction for each other. The formation of a solute-ligand complex reduces the total non-polar surface area and interaction with surrounding water molecules is thereby decreased. The hydrocarbonaceous stationary phase is therefore assumed to play a passive role toward solute retention. This is in contrast to the accepted view of bonded phases in gas chromatography in which the gaseous mobile phase is inactive, selectivity depending primarily upon the stationary phase^{7,8}.

If the hydrophobic effect does indeed govern solute retention in RPLC, then the capacity factor may be viewed as a measure of relative hydrophobicity. The partition coefficient (usually expressed as $\log P$) obtained from immiscible organic/water systems is probably the most commonly used index of hydrophobicity⁹. Various types of thin-layer chromatographic measurements have been made which use R_M as a measure of hydrophobicity⁵. The term R_M is the thermodynamic equivalent of the capacity factor (k'). Both of these parameters have been correlated with biological activities and used to explain structure-activity relationships for improved drug design. The capacity factor determined in pure water (k'_w) is the most desirable RPLC parameter for attempting to identify quantitative retention activity relationships (QRAR). The k'_w can only be obtained directly for a relatively small number of

solutes. Thus, some means of extrapolation of measurable k' data for a solute to the theoretical value in a mobile phase of water is necessary.

Recently, we described¹⁰ the RPLC properties of the C₁–C₅ N-alkylbenz-amides. The k' values for these solutes were observed to vary in a curvilinear fashion with mobile phase composition and increase rapidly at low organic modifier concentration. Treatment of the water–organic k' data as a linear or quadratic relationship did not adequately predict k'_w values. This report describes the use of the solvophobic theory in an attempt to predict k'_w values.

EXPERIMENTAL

The chromatographic data used in this study has been previously described¹⁰. Nuclear magnetic resonance (NMR) studies were performed on a Varian T-60A spectrometer with sample solutions prepared in carbon tetrachloride and tetramethylsilane as the internal standard. Multiple linear regression analyses and calculation of total molecular surface area were performed on an IBM 370/158, Computer Services, Auburn University. The programs used to calculate molecular surface area were obtained from G. L. Amidon, University of Wisconsin, Madison, WI, U.S.A.

RESULTS AND DISCUSSION

Prediction of log k'_w using the solvophobic theory

Rearranging the terms in eqn. 1 produces the relationship in eqn. 7:

$$\ln k' - D(\kappa^e - 1)V^{2.3\gamma} - \ln(RT/P_0V) = (A + E) + B\mathcal{L} + C; \quad (7)$$

The regression coefficients A and E were determined as the sum $(A + E)$. Coefficients A , B and E are unitless and the units of C are $\text{cm}^2 \text{l}^{-1}$. The value of the regression coefficient D was calculated to be $1.6693 \times 10^7 \text{ mole}^{2/3} \text{ l}^{-1} \text{ atm}^{-1}$. Data on density^{11,12}, dielectric constant^{13,14}, surface tension² and κ^e (ref. 2) for solvent mixtures were compiled from the literature and converted to % (v/v) of the organic modifier. Using these data, all terms except $(A + E)$, B and C were calculated for each given solvent concentration at which a capacity factor was measured. This treatment leads to a series of n equations (n equals the number of experimentally determined data points) of the form in eqn. 8:

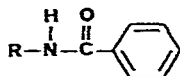
$$X = (A + E) + B\mathcal{L} + C; \quad (8)$$

The regression coefficients of eqn. 8 were evaluated by means of the Statistical Analysis System procedure SYSREG (SAS Institute, Raleigh, NC, U.S.A.).

The calculated regression coefficients for the acetonitrile–water solvent system on the Partisil ODS-2 column are given in Table I while those for the Ultrasphere ODS column are presented in Table II. Thus far a description of the results obtained in methanol–water mixtures on either column by the application of the solvophobic theory has not been possible. The failure to predict reasonable solvophobic parameters in the aqueous methanol system may or may not be a failure of the model (eqn. 1) to account satisfactorily for all factors involved. Results of Melander *et al.*¹⁵ demon-

TABLE I

SOLVOPHOBIC PARAMETERS OF SOME N-ALKYLBENZAMIDES ON PARTISIL ODS-2 IN ACETONITRILE-WATER



<i>R</i>	<i>B</i>	<i>C</i>	(<i>A</i> + <i>E</i>)	<i>r</i>
CH ₃	-327.77	206612152	303.394	0.9995
CH ₃ CH ₂	-298.51	216131391	274.876	0.9994
CH ₃ CH ₂ CH ₂	-275.15	227673700	252.214	0.9999
(CH ₃) ₂ CH	-268.04	219825857	245.461	0.9999
CH ₃ CH ₂ CH(CH ₃)	-168.94	217293234	149.192	0.9990
(CH ₃) ₂ CHCH ₂	-154.07	222333721	134.589	0.9989
CH ₃ (CH ₂) ₃	-148.98	228127478	129.546	0.9992
(CH ₃) ₃ C	-146.49	214732624	127.651	0.9987
(CH ₃ CH ₂) ₂ CH	-122.81	224850132	104.320	0.9961
(CH ₃) ₂ CHCH(CH ₃)	-111.86	225837894	93.644	0.9951
CH ₃ CH ₂ CH ₂ CH(CH ₃)	-106.92	236073206	88.619	0.9968
CH ₃ CH ₂ CH(CH ₃)CH ₂	-105.66	243229778	87.232	0.9966
CH ₃ CH ₂ C(CH ₃) ₂	-103.71	232045062	85.796	0.9964
(CH ₃) ₃ CCH ₂	-99.66	230498308	81.693	0.9956
(CH ₃) ₂ CHCH ₂ CH ₂	-95.04	246301501	76.824	0.9971
CH ₃ (CH ₂) ₄	-85.07	250238960	67.069	0.9970
CH ₂ CH ₂ CH ₂ CH ₂ CH	-138.38	223124385	119.459	0.9953

TABLE II

SOLVOPHOBIC PARAMETERS OF SOME N-ALKYLBENZAMIDES ON ULTRASPHERE ODS IN ACETONITRILE-WATER

For general structure, see Table I.

<i>R</i>	<i>B</i>	<i>C</i>	(<i>A</i> + <i>E</i>)	<i>r</i>
CH ₃	-120.79	175914241	101.202	0.9992
CH ₃ CH ₂	-135.46	186749400	115.728	0.9992
CH ₃ CH ₂ CH ₂	-194.62	206243456	173.633	0.9990
(CH ₃) ₂ CH	-222.02	201887060	200.541	0.9989
CH ₃ CH ₂ CH(CH ₃)	-247.25	220431346	225.178	0.9988
(CH ₃) ₂ CHCH ₂	-236.26	225520204	214.379	0.9974
CH ₃ (CH ₂) ₃	-203.53	227101260	182.433	0.9986
(CH ₃) ₃ C	-204.46	211786479	184.006	0.9983
(CH ₃ CH ₂) ₂ CH	-162.51	218348621	142.932	0.9986
(CH ₃) ₂ CHCH(CH ₃)	-183.22	228066562	162.902	0.9983
CH ₃ CH ₂ CH ₂ CH(CH ₃)	-142.03	226022173	122.859	0.9972
CH ₃ CH ₂ CH(CH ₃)CH ₂	-134.39	231268022	115.285	0.9972
CH ₃ CH ₂ C(CH ₃) ₂	-130.83	218075199	112.398	0.9973
(CH ₃) ₃ CCH ₂	-159.92	231615578	140.145	0.9968
(CH ₃) ₂ CHCH ₂ CH ₂	-120.68	234950318	101.832	0.9979
CH ₃ (CH ₂) ₄	-117.44	238603744	98.653	0.9976
CH ₂ CH ₂ CH ₂ CH ₂ CH	-202.09	224457477	181.194	0.9984

strated no difference in the mechanism of solute–ligand interaction in RPLC whether acetonitrile or methanol was used as the organic modifier. The failure thus far to mathematically describe the aqueous methanol data is being actively pursued.

The regression coefficients obtained from the acetonitrile–water data in Tables I and II were combined with the known solvent properties of an organic–water solvent system to estimate the value of X (from eqn. 8), and from X the values of the capacity factors of these solutes were determined at the desired solvent composition, including a totally aqueous mobile phase. Of the compounds investigated, the only capacity factors actually determined experimentally in pure water were 2.608 ($\log k'_w$) for N-methylbenzamide on Partisil ODS-2 and 1.957 for N-methylbenzamide, 2.362

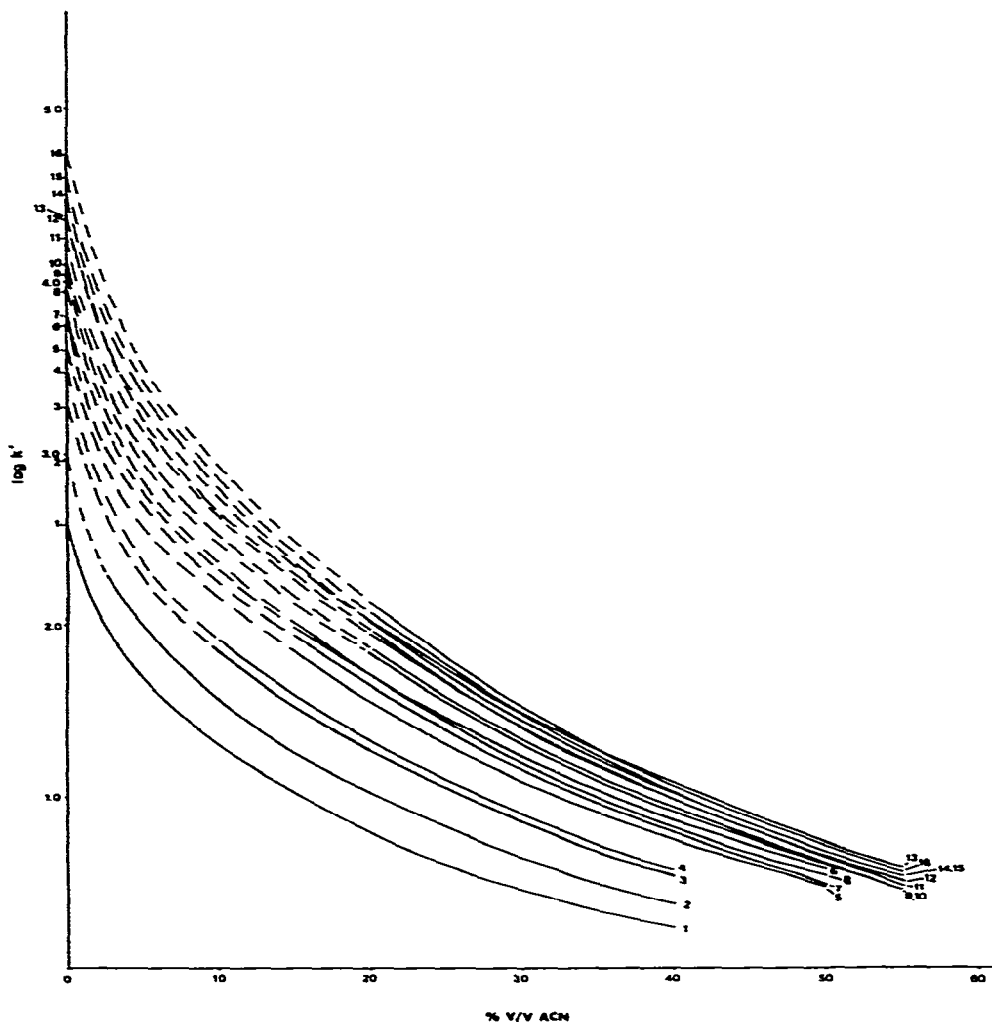


Fig. 1. Eluent composition (ACN = acetonitrile) versus $\log k'$ plots for N-alkylbenzamides on Partisil ODS-2. Curves: 1, R = methyl; 2, R = ethyl; 3, R = isopropyl; 4, R = n-propyl; 5, R = sec.-butyl; 6, R = tert.-butyl; 7, R = isobutyl; 8, R = n-butyl; 9, R = 1-ethylpropyl; 10, R = 1,2-dimethylpropyl; 11, R = neopentyl; 12, R = sec.-pentyl; 13, R = tert.-pentyl; 14, R = 2-methylbutyl; 15, R = isopentyl; 16, R = n-pentyl. See general structure in Table I.

for N-ethylbenzamide, and 2.601 for N-isopropylbenzamide, on Ultrasphere ODS. The experimentally measured k'_w values were omitted from the regression analyses in order to test the ability to predict these values. The measured and calculated $\log k'$ data are plotted as a function of mobile phase composition in Figs. 1 and 2. The $\log k'$ data was calculated for each solute in 5% mobile phase composition increments from the last measured data point to 0% organic. At very low organic compositions a $\log k'$ was calculated at each 2.5% change in solvent composition. The plots resulting from the calculated data show the same dramatic increase in slope at low organic mobile phase composition that was observed in the measured data¹⁰. Due to the complexity of the graphs, the data points have been omitted. In these figures the curves seem to vary in a regular fashion, except for the *tert.*-butyl and *tert.*-pentyl isomers.

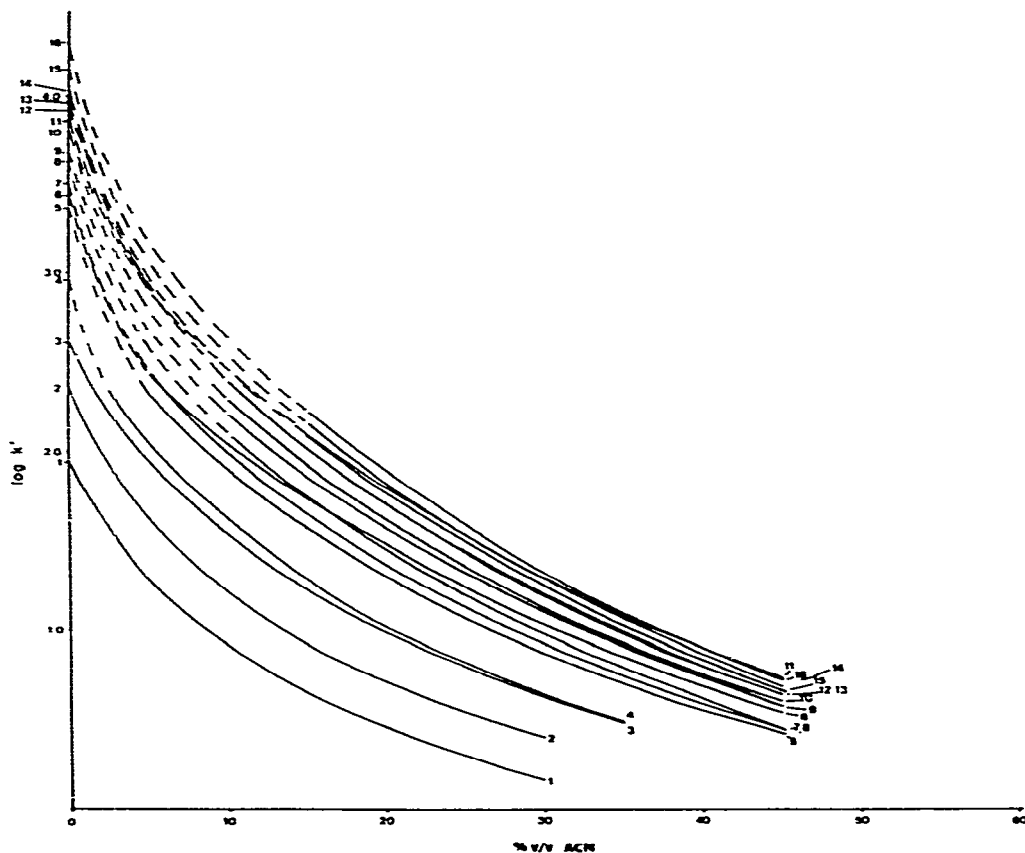


Fig. 2. Eluent composition versus $\log k'$ plots for N-alkylbenzamides on Ultrasphere ODS. Curves: 1. R = methyl; 2, R = ethyl; 3, R = isopropyl; 4, R = propyl; 5, R = *sec.*-butyl; 6, R = *tert.*-butyl; 7, R = isobutyl; 8, R = *n.*-butyl; 9, R = 1-ethylpropyl; 10, R = 1,2-dimethylpropyl; 11, R = *tert.*-pentyl; 12, R = *sec.*-pentyl; 13, R = neopentyl; 14, R = 2-methylbutyl; 15, R = isopentyl; 16, R = *n.*-pentyl. See general structure in Table I.

The $\log k'$ values predicted for 0% acetonitrile on both the Partisil ODS-2 and Ultrasphere ODS columns are listed in Table III. The order predicted for each column is nearly the same, differing only in exceptions denoted by an asterisk. In Fig.

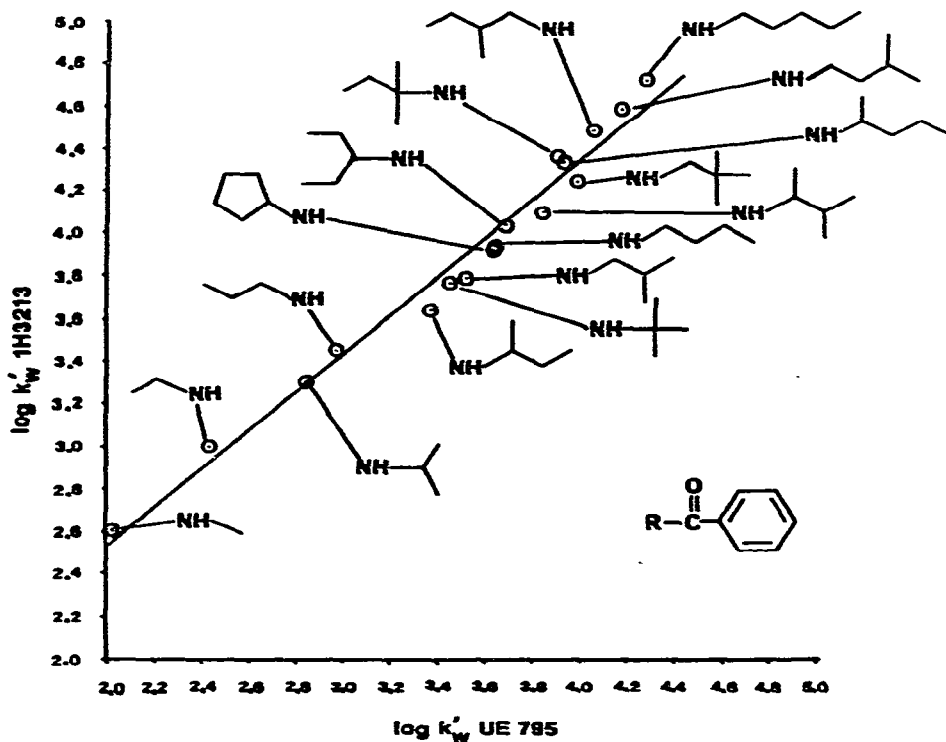
TABLE III

CALCULATED CAPACITY FACTORS AT 0% ACETONITRILE

For general structure, see Table I.

R	$\log k'_w$ (0% acetonitrile)	
	Partisil ODS-2	Ultrasphere ODS (UE795)
CH ₃	2.612	2.034
CH ₃ CH ₂	2.987	2.427
(CH ₃) ₂ CH	3.305	2.850
CH ₃ CH ₂ CH ₂	3.452	2.972
CH ₃ CH ₂ CH(CH ₃)	3.638	3.373
(CH ₃) ₃ C	3.768	3.456
(CH ₃) ₂ CHCH ₂	3.786	3.522
CH ₃ (CH ₂) ₃	3.946	3.642
(CH ₃ CH ₂) ₂ CH	4.039	3.692
(CH ₃) ₂ CHCH(CH ₃)	4.098	3.842
(CH ₃) ₃ CCH ₂ *	4.248	3.995
CH ₃ CH ₂ CH ₂ CH(CH ₃)*	4.337	3.937
CH ₃ CH ₂ C(CH ₃) ₂ *	4.353	3.920
CH ₃ CH ₂ CH(CH ₃)CH ₂	4.490	4.065
(CH ₃) ₂ CHCH ₂ CH ₂	4.589	4.177
CH ₃ (CH ₂) ₄	4.722	4.289
CH ₂ CH ₂ CH ₂ CH ₂ CH	3.926	3.635

* The predicted elution orders are the same on each column except for the compounds noted.

Fig. 3. $\log k'_w$ versus $\log k'_w$ plot for the C₁-C₅ N-alkylbenzamides as measured on Partisil ODS-2 (IH3213) and Ultrasphere ODS (UE795) at 25°C.

3. the $\log k'_w$ values from the Partisil ODS-2 column are plotted against those obtained from the Ultrasphere ODS column, and the observed relationship is described by eqn. 9:

$$\begin{aligned} (\log k'_w \text{ Partisil ODS-2}) &= 0.906 (\log k'_w \text{ Ultrasphere ODS}) + 0.711 \\ r &= 0.988 \end{aligned} \quad (9)$$

There are some interesting patterns evident in the elution orders predicted for 0% acetonitrile. Branched compounds elute before their straight chain analogs, even though this is not always the case at higher concentrations of acetonitrile in the mobile phase. For those benzamides containing one single carbon branch, the compounds elute in the order of *sec.*-butyl < *isobutyl* and *sec.*-pentyl < 2-methylbutyl < *isopentyl*. In each instance, the compound having the branch on the carbon adjacent to the amide group is predicted to elute first, followed successively by compounds having the branch on the next carbon atom down the chain.

Analysis of solvophobic regression coefficients

The data reported in Tables I and II compare the values of B and $(A + E)$ derived from the Partisil ODS-2 and Ultrasphere ODS columns, respectively. The benzamides in Table I are arranged in order of increasing values for the coefficient B , which correspond to decreasing values of $(A + E)$. The benzamides in Table II are arranged in the same order as those in Table I but the ordering of the values of B and

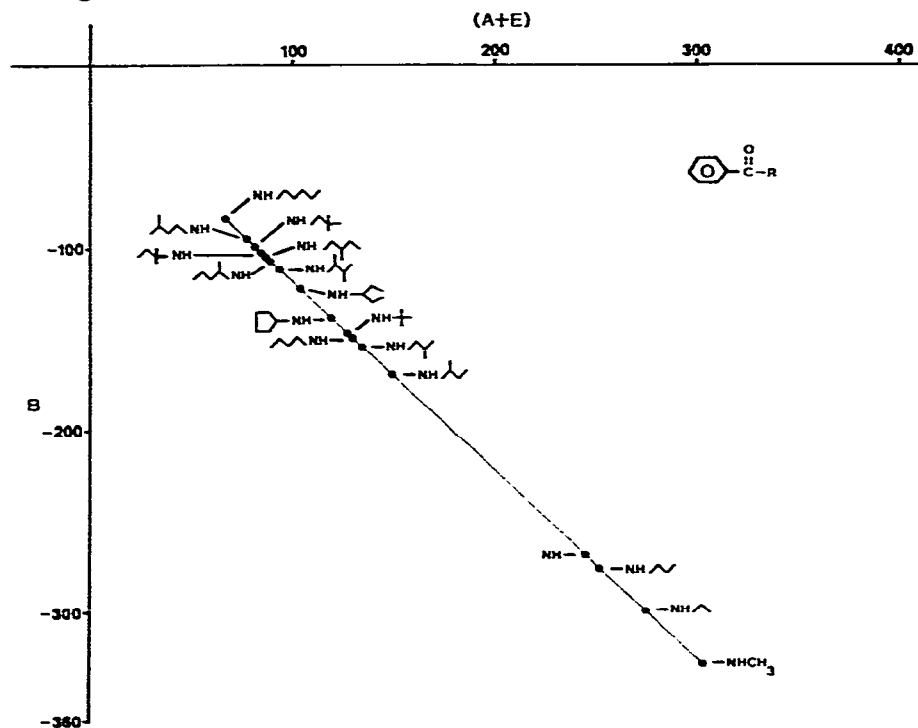


Fig. 4. Plot of the solvophobic parameters B versus $(A + E)$ as derived from data obtained using Partisil ODS-2.

($A + E$) are different. The regression coefficients A , B and E were defined in eqns. 2, 3 and 6, respectively. The correlation between coefficients B and ($A + E$) derived from the Partisil ODS-2 data is described by eqn. 10 and plotted in Fig. 4.

$$B = -1.028 (A + E) - 15.714 \quad r = -0.999 \quad (10)$$

For these compounds, B becomes less negative, and ($A + E$) less positive from N-methylbenzamide to N-*n*-pentylbenzamide. Fig. 5 is a plot of B versus ($A + E$) calculated from the data obtained on the Ultrasphere ODS column, and is described by eqn. 11:

$$B = -1.025 (A + E) - 16.270 \quad r = -0.999 \quad (11)$$

The similarity between eqns. 10 and 11 is remarkable, yet within each plot (Figs. 4 and 5) the individual compounds are not arranged in the same order. In Fig. 5, the methyl, ethyl, propyl and isopropyl derivatives appear in reverse order to that in Fig. 4. Other order changes among individual isomers can be noted. The coefficient B involves several solute properties which are all independent of the chromatographic system with the exception of λ . The λ term is the molecular volume of the solute-bonded ligand complex and is assumed to be a multiple of the molecular volume of the solute. This parameter varies from column to column with configurational differences in the bonded stationary phase. The coefficient A involves φ and $\Delta F_{\text{vdw, assoc}}$

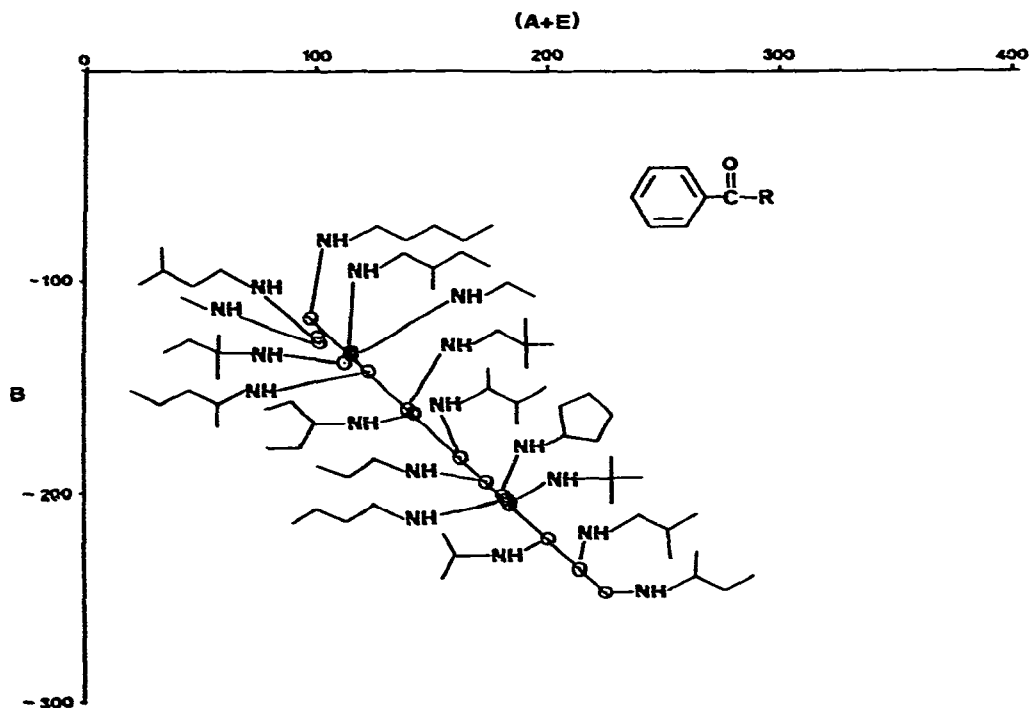


Fig. 5. Plot of the solvophobic parameters B versus ($A + E$) as derived from data obtained using Ultrasphere ODS.

which are both expected to be dependent upon the individual column. Coefficient E is related to the interaction of solute and solvent, and, therefore, is independent of column properties. In spite of these variables, the fact that eqns. 10 and 11 are virtually identical suggests that some underlying factors in the chromatographic retention process are the same in these two columns. If the identical nature of these plots were simply a result of the solute properties being the same, one might expect the ordering of the compounds to be the same. The calculated regression parameters for the compound N-cyclopentylbenzamide have been included in Table I and II. Although it is not an isomer of the other C_5 derivatives, the point $((A + E), B)$ for the cyclopentyl derivative lies on the same line as the other compounds in both Figs. 4 and 5.

From eqn. 4, and the calculated values of regression coefficient C given in Tables I and II, ΔA , the contact surface area of the associated solute-bonded ligand complex, can be evaluated. The values obtained for ΔA are given in Table IV. The total surface area (TSA) for each solute was calculated in a manner totally independent of chromatographic measurements using standard bondlengths and bond angles¹⁶ and compiled in Table IV. The purpose of comparing the solute-bonded ligand contact surface area to the total surface area is to attempt to lend credibility to the values predicted for ΔA . The values for ΔA appear reasonable as they are approximately equal to or less than one-half of the total molecular surface area. While for some solutes, the contact surface area calculated from the Ultrasphere ODS column is greater and in other cases ΔA from the Partisil ODS-2 column is greater.

TABLE IV

CONTACT SURFACE AREA AND TOTAL SURFACE AREA

For general structure, see Table I.

R	Partisil ODS-2 ΔA (\AA^2)*	Ultrasphere ODS (UE795) ΔA (\AA^2)	TSA (\AA^2)**
CH ₃	83.9	71.4	165.0
CH ₃ CH ₂	87.8	75.8	187.8
CH ₃ CH ₂ CH ₂	92.5	83.8	210.5
(CH ₃) ₂ CH	89.3	82.0	210.2
CH ₃ CH ₂ CH(CH ₃)	88.3	89.5	231.7
(CH ₃) ₂ CHCH ₂	90.3	91.6	227.7
CH ₃ (CH ₂) ₃	92.7	92.2	233.2
(CH ₃) ₃ C	87.2	86.0	226.4
(CH ₃ CH ₂) ₂ CH	91.4	88.7	248.7
(CH ₃) ₂ CHCH(CH ₃)	91.8	92.6	246.2
CH ₃ CH ₂ CH ₂ CH(CH ₃)	95.9	91.8	251.7
CH ₃ CH ₂ CH(CH ₃)CH ₂	98.8	93.9	250.3
CH ₃ CH ₂ C(CH ₃) ₂	94.3	88.6	241.3
(CH ₃) ₃ CCH ₂	93.6	94.1	245.0
(CH ₃) ₂ CHCH ₂ CH ₂	100.1	95.4	247.6
CH ₃ (CH ₂) ₄	101.7	96.9	255.9
CH ₂ CH ₂ CH ₂ CH ₂ CH	90.6	91.2	—

* Contact surface area in square Angstroms.

** Total surface area in square Angstroms.

overall, the values for ΔA calculated from each of the two columns investigated are similar.

In Figs. 6 and 7, $\log k'_w$ is plotted against the contact surface area in square angstroms (\AA^2) as determined in acetonitrile–water. The plots demonstrate that compounds that have similar contact surface areas with the bonded ligand can also exhibit vastly differing capacity factors in water. Therefore, while the contact surface area between the solute and the hydrocarbonaceous stationary phase may contribute in part to determining retention in water, it is not the controlling factor. If one looks at only the four isomeric butylbenzamides in Fig. 6, a line of correlation $r = 0.9996$ can be passed through the *sec.*-butyl, isobutyl, and *n*-butyl derivatives, omitting the

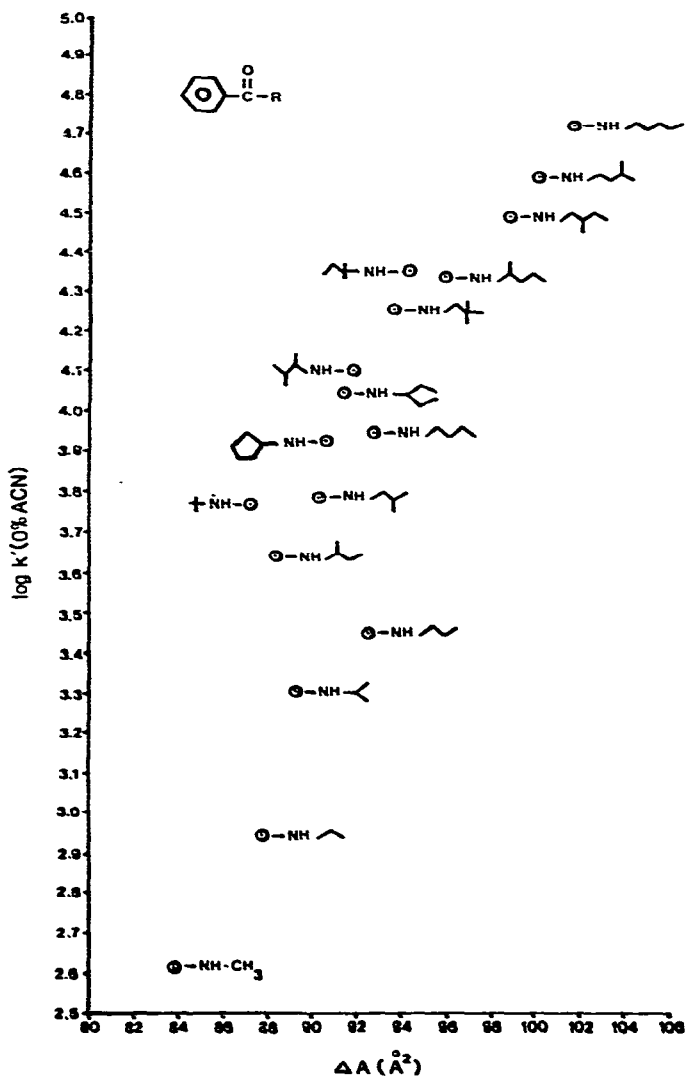


Fig. 6. $\log k'_w$ versus solute-ligand contact surface area, ΔA , determined from data obtained on Partisil ODS-2.

tert.-butyl derivative. Similarly, a line of correlation $r = 0.9941$ can be drawn through the isomeric pentylbenzamides if the *tert.*-pentylbenzamide is excluded. If in each case, the excluded points are projected onto the line defined by the other isomeric benzamides, it can be seen that for these two compounds, the capacity factor in water is much greater than would be expected according to the contact surface area. This behavior of the *tert.*-butyl and *tert.*-pentyl derivatives is also observed to occur on the Ultrasphere ODS column (see Fig. 7).

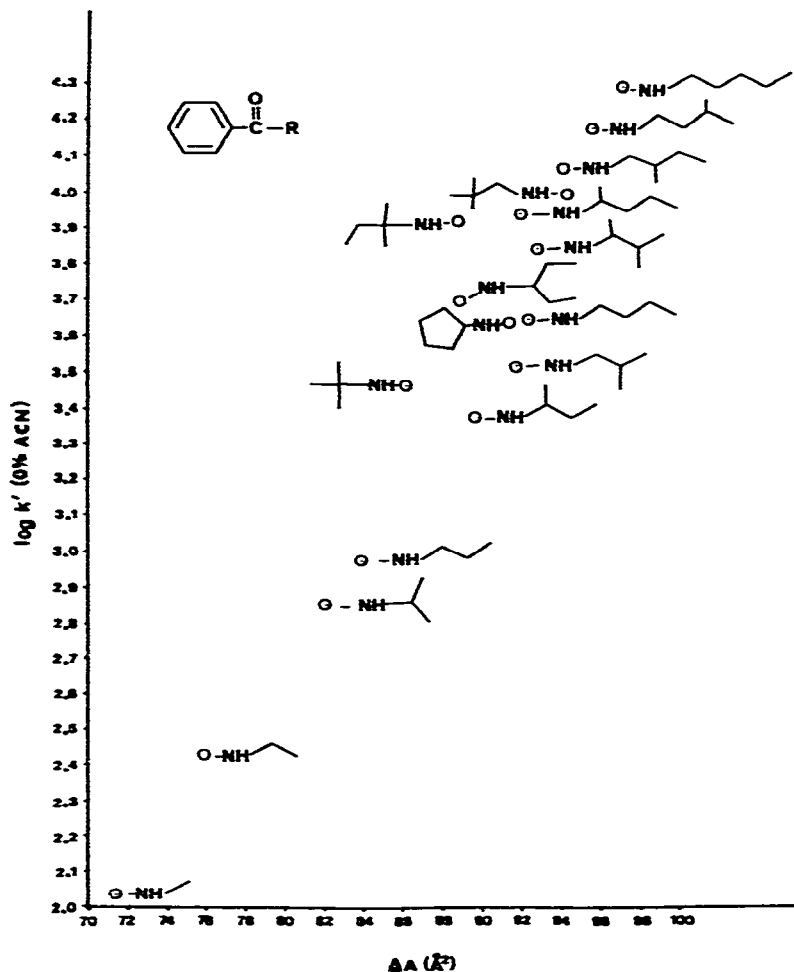


Fig. 7. $\log k'_w$ versus solute-ligand contact surface area. ΔA , determined from data obtained on Ultrasphere ODS.

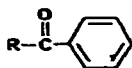
Anomalous retention of the tertiary alkylbenzamides

The unusual behavior of the two tertiary alkylbenzamides is also demonstrated in the overall profile of this series of compounds when the values for $\log k'$ for these solutes are plotted versus the percent of the organic modifier in the mobile phase (Figs. 1 and 2). In these figures, the curves seem to vary in a regular fashion, except for the curves of the *tert.*-butyl and *tert.*-pentyl isomers which cross the curves of other

compounds. This is further pointed out by a closer look at the retention order of the compounds in various concentrations of acetonitrile-water listed in Table V. In each of the three cases presented in this table, where the order has been determined at 20% and at 40% acetonitrile and predicted at 0% acetonitrile, the elution order is the same if the *tert.*-butyl- and *tert.*-pentylbenzamides are again omitted. The *tert.*-pentylbenzamide changes from 13th to 14th to 15th in order of elution, while the *tert.*-butylbenzamide changes from 6th to tie for 7th and finally 8th in elution order as the percentage of the organic modifier increases. The elution orders at 0, 20 and 40% acetonitrile using the Ultrasphere ODS column are the same as those given in Table V for the Partisil ODS-2 column, except for the differences already noted (see Table III)

TABLE V

RETENTION ORDER OF THE C₁-C₅ N-ALKYLBENZAMIDES AT 0, 20 AND 40% (v/v) ACETONITRILE-WATER ON PARTISIL ODS-2



Elution order	Acetonitrile (% v/v)		
	0	20	40
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
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and for the elution of the *tert.*-pentylbenzamide after the *n*-pentyl derivative at 40% acetonitrile on the Ultrasphere ODS column. Therefore, both the *tert.*-butyl and *tert.*-pentyl solutes tend to elute after their straight-chain analogues as the percentage of acetonitrile in the mobile phase is increased.

When the structures of these two compounds are scrutinized, it appears that the factor they have in common is a quaternary carbon atom bonded to the amide nitrogen. Perhaps an explanation for this behavior concerns the energy required to place solutes back into the solvent after contact with the bonded ligand: (1) the structural bulkiness about this carbon atom should increase the energy required to prepare a solvent cavity of the proper shape and size, and (2) the arrangement about this carbon atom could obstruct the hydrogen bonding between the amide group and the solvent, thereby affecting the interaction energy between the solute and the solvent. The net result can be viewed as an increase in the contact time between the two solutes and the bonded phase and a concomitant increase in time of elution. A proton NMR study of hydrogen bonding effects in the butylbenzamides was conducted in an attempt to provide a means, independent of chromatography, to establish the validity of the above points.

Amide N-H groups produce somewhat broad NMR signals in the 5.0–8.5 δ region¹⁷. The position of resonance for amide protons is dependent upon concentration because these protons are involved in the formation of intermolecular hydrogen bonds (solute-solute and/or solute-solvent hydrogen bonds). The amide proton experiences a difference in magnetic shielding in the associated (hydrogen-bonded) and unassociated states. Dilution causes dissociation of solute-solute hydrogen bonds altering the populations of associated and unassociated states in non-hydrogen bonding solvents. Thus, the resonance frequency for amide protons is concentration dependent¹⁸.

The concentration dependence of the chemical shift of the amide proton in *N-n*-butyl-, *N-isobutyl*-, *N-sec.*-butyl- and *N-tert.*-butylbenzamide was investigated by proton NMR in carbon tetrachloride and is demonstrated in Fig. 8. The ordinate represents the δ value of the amide proton in ppm relative to TMS, and the abscissa, the mole fraction of each benzamide in carbon tetrachloride. In such a non-hydrogen bonding solvent, the intermolecular interaction can be described by eqn. 12 and the equilibrium constant by eqn. 13:



$$K_{\text{eq}} = \frac{[\text{R-H} \cdots \text{R-H}]}{[\text{R-H}]^2} \quad (13)$$

Curve 1 in Fig. 8 represents the concentration dependencies of both the *N-n*-butyl- and the *N-isobutyl*benzamides; curve 2 is for *N-sec.*-butylbenzamide and curve 3 for *N-tert.*-butylbenzamide. Fewer data points are recorded for *N-tert.*-butylbenzamide due to its limited solubility in carbon tetrachloride compared to the other isomeric butylbenzamides. The relative displacement of the three curves at any given concentration can be attributed to the electron donating effects of the groups bonded to the carbon attached to the amide nitrogen atom. As the mole fraction of

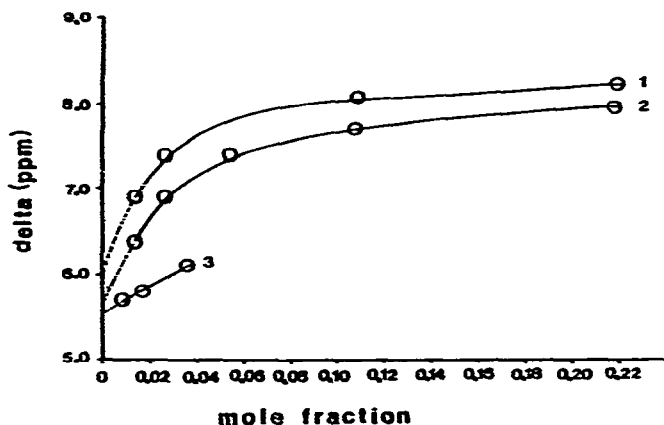


Fig. 8. Concentration versus NMR chemical shift for the N-H proton of the butylbenzamides. Curves: 1, *N-n*-butyl and *N-isobutyl*benzamide; 2, *N-sec.*-butylbenzamide; 3, *N-tert.*-butylbenzamide.

each benzamide in carbon tetrachloride is increased, the NMR signal of the amide proton is displaced to lower field due to an increase in the population of the associated state.

From these data, K_{eq} values of 4.4 for the *N-n*-butyl and *N-isobutyl* isomers, and 2.3 for the *N-sec.*-butyl derivative were calculated. The calculation of K_{eq} was based on the assumption that the δ value obtained by extrapolation to zero concentration represents the completely non-hydrogen-bonded form, and that the δ value obtained at high solute concentration represents the totally associated form. Due to the lack of data points at high concentration for *N-tert.*-butylbenzamide, calculation of the equilibrium constant for this compound was impossible. By qualitatively comparing the slopes of the plots in Fig. 8 at increasing dilutions, the tendency for the reaction in eqn. 12 to be displaced to the right (*i.e.*, toward dimerization) occurs in the following order: *N-n*-butyl-, *N-isobutyl*- > *N-sec.*-butyl- > *N-tert.*-butylbenzamide. It is reasonable to assume that this same trend occurs between a molecule of an individual isomer and a molecule of a hydrogen-bonding solvent used in chromatography. Steric crowding about the carbon adjacent to the amide nitrogen (due to the amount of substitution on this carbon) influences the ability of the forward reaction in eqn. 12 to occur. Therefore, the NMR data support the conclusions drawn chromatographically. Furthermore, the anomalous behavior of the tertiary alkylbenzamides was also observed chromatographically in methanol-water eluents. The behavior of *tert.*-butyl- and *tert.*-pentylbenzamide does not appear to result from solvent selectivity since it occurs in both aqueous methanol and acetonitrile solvents.

CONCLUSIONS

The 16 isomeric C_1 - C_5 *N*-alkylbenzamides exhibit significant non-linearity of k' as a function of solvent composition in aqueous acetonitrile and methanol. The experimentally measured k' values were observed to increase dramatically at low concentrations of organic modifier. The relationship between k' and eluent composition for these compounds is not described by linear or quadratic equations. Using

the chromatographic data determined in acetonitrile–water, the relationship between k' and eluent composition was described by the solvophobic theory. Regression coefficients obtained from eqn. 1 were used to determine various fundamental chromatographic parameters. The calculated contact surface area of the solute-bonded ligand complex was found to be one-half or less of the total molecular surface area of the solute. Calculated k'_w data predicts that branched alkyl chains elute before their straight-chain analogues. In a compound with a single carbon branch, the elution volume increases with the distance between the methyl-branch and the amide nitrogen.

APPENDIX

Definition of Mathematical Variables

<i>Variables</i>	<i>Definition</i>
A, B, C, D and E	Regression coefficients
$\mathcal{L} = 2(\epsilon - 1)/(2\epsilon + 1)$	\mathcal{L} is a function of ϵ , the static dielectric constant of the solvent
γ	Surface tension of the solvent
κ^c	Microscopic cavity factor; curvature correction to convert the macroscopic surface tension to microscopic dimensions
V	Average mole volume of solvent
R	Gas law constant
T	Temperature in degrees Kelvin
P_0	Atmospheric pressure
$k' = (V_R - V_0)/V_0$	Chromatographic capacity factor
V_R	Retention volume
V_0	Column void volume
φ	Characteristic constant for a given column (logarithm of the phase ratio)
N	Avogadro's number
$\Delta F_{\text{dw,assoc}}$	Free energy change for the interaction of solute and ligand in a hypothetical gas phase; assumed to occur by Van der Waals forces only
$\Delta F_{\text{dw,s}}$	The Van der Waals component of the free energy change for the interaction of the solute with the solvent
$\lambda = v_{\text{SL}}/v_s$	The molecular volume of the complex, v_{SL} , is assumed to be a multiple of the molecular volume of the solute, v_s
μ_s	Static dipole moment of the solute
$P = \left[4\pi\epsilon_0 \left(1 - \mathcal{L} \frac{\alpha_s}{v_s} \right) \right]^{-1}$	where ϵ_0 is the permittivity constant, and α_s is the polarizability of the solute
A_A	Contact surface area of the solute ligand complex

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