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INVESTIGATION OF N-ALKYLBENZAMIDES BY REVERSED-PHASE LIQUID CHROMATOGRAPHY

V. CHARACTERISTICS OF SOME TERTIARY ALKYLBENZAMIDES

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SUMMARY

The capacity factors (k') for sixteen tertiary N-alkylbenzamides in a reversed-phase chromatographic system are reported. Applying the solvophobic theory, the measured log k' data from an acetonitrile–water eluent were used to calculate log k'_w (the log k' in 100% water). The values for the contact surface area, ΔA, between the benzamide-bonded ligand (stationary phase) complex were also calculated. The measured and calculated capacity factor data and other chromatographic parameters calculated from the solvophobic equations were found to be highly correlated with those molecular connectivity indices which quantitatively describe molecular bulk.

INTRODUCTION

In reversed-phase liquid chromatography (RPLC), control of the characteristics of the mobile phase is essential for the optimization of separation. The mobile phase composition significantly affects retention due to its hydrophobic/hydrophilic functional group selectivity[1,2]. Understanding the rôle of the eluent in solute retention in RPLC is facilitated by considering the solvophobic theory as originally described by Sinanoglu and Abdulnur[3,4] and adapted to RPLC by Horváth *et al.*[5] (see Appendix). According to this theoretical treatment, creation of a suitable cavity in the mobile phase is just as important to the retention of a solute as the formation of a reversible solute-bonded ligand complex. The size and shape of the cavity formed in the solvent depends not only upon the molecular volume and surface area of the solute, but also on the properties of the solvent, especially surface tension and dielectric constant[6]. RPLC is widely recognized as a valuable analytical technique; however, its use for the study of molecular phenomena in solution is just beginning.

Previous reports in this series[7–10] have described the RPLC elution properties

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of several secondary N-alkylbenzamides, the prediction of capacity factor data for a totally aqueous mobile phase using the solvophobic theory and the determination of quantitative structure–retention relationships (QSRRs) by molecular connectivity indices[11]. The results of these studies showed, in general, that the length of the alkyl chain (carbon number), chain branching and the position of the branch relative to the amide nitrogen were the major factors controlling retention in the secondary N-alkylbenzamides. In this report, a similar theoretical treatment is used to describe the RPLC retention of some tertiary N-alkylbenzamides (benzamides of N,N-dialkylamines and cyclic secondary amines).

EXPERIMENTAL

Tertiary benzamides were prepared from the appropriate secondary amines and benzoyl chloride as previously described[7] except that the reaction mixture was heated to reflux for 12 h. A Waters (Milford, MA, U.S.A.) Model 6000A solvent delivery system, Model U6K injector, Model 440 UV absorbance detector, an Alltech (Deerfield, IL, U.S.A.) HPLC column water jacket and a Haake constant-temperature circulator were used to determine chromatographic data. An Ultrasphere ODS (5-μm spherical porous particle) column (15 cm × 4.6 mm I.D.) from Altex Scientific (Berkeley, CA, U.S.A.) was preceded by a 7 cm × 2.1 mm I.D. guard column dry packed with Whatman (Clifton, NJ, U.S.A.) CO:PELL ODS (30–38 μm). The columns were maintained at 25 ± 0.2°C. The mobile phase consisted of acetonitrile–water mixtures pumped at a rate of 1.5 ml/min. The ultraviolet detector was operated at 254 nm and 0.005 a.u.f.s. Other equipment, reagents, chemicals and procedures have been described previously[7].

Regression analyses and molecular connectivity calculations were performed using an IBM 370/158 (Computer Services, Auburn University). The program (CFUNC) used to calculate molecular connectivity indices was obtained from Dr. L. H. Hall, Eastern Nazarene College, Quincy, MA. More detailed discussion of these calculations is found in ref. 9. Calculations involving the solvophobic theory are described in the Appendix.

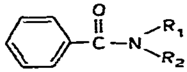
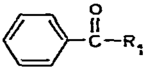
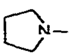
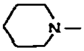
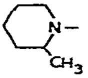
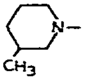
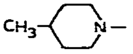
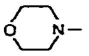
RESULTS AND DISCUSSION

Ten N,N-disubstituted benzamides were prepared from the appropriate secondary amines and benzoyl chloride; in addition, six tertiary benzamides were prepared from cyclic amines. The physical properties of the compounds are presented in Table I. Most of these amides are oils at ambient temperature and were purified by column chromatography on silica gel[7]. The available infrared and ultraviolet absorption data suggest that the amides are electronically similar.

The experimentally measured values of the capacity factors, k', in acetonitrile–water are reported in Table II. As with various other benzamides prepared in this laboratory[8–10], data gathered in aqueous methanol eluents were not adequately described by the solvophobic theory and are not included in this report. Application of the solvophobic theory (see Appendix) to the measured k' data in acetonitrile–water did however produce satisfactory results. The values of the solvophobic regression coefficients B, C and $(A + E)$ for the N,N-dialkyl derivatives and the benzamides

TABLE I

PHYSICAL PROPERTIES OF SOME TERTIARY BENZAMIDES

Structure	*Compound No.*	R_1	R_2	*M.p. (°C)*	λ_{max} *(nm)*	*Carbonyl stretch (cm^{-1})*
	1	CH_3-	CH_3-	Oil	–	1620
	2	CH_3CH_2-	CH_3CH_2-	Oil	–	1618
	3	$CH_3CH_2CH_2-$	$CH_3CH_2CH_2-$	Oil	–	1615
	4	$(CH_3)_2CH-$	$(CH_3)_2CH-$	65–68	–	1615
	5	$CH_3(CH_2)_3-$	CH_3-	Oil	–	1620
	6	$CH_3(CH_2)_3-$	CH_3CH_2-	Oil	–	1615
	7	$CH_3(CH_2)_3-$	$CH_3(CH_2)_3-$	Oil	–	1615
	8	$CH_3(CH_2)_3-$	$(CH_3)_3C-$	Oil	–	1622
	9	$(CH_3)_2CHCH_2-$	$(CH_3)_2CHCH_2-$	60–65	–	1618
	10	$CH_3(CH_2)_5-$	$CH_3(CH_2)_5-$	Oil	–	1615
	11			Oil	220.5	1610
	12			Oil	–	1615
	13			Oil	–	1612
	14			Oil	–	1615
	15			Oil	–	1611
	16			70–74	–	1625

of cyclic amines are reported in Table III. The correlation between *B* and (*A* + *E*) for the N,N-dialkylbenzamides is described by eqn. 1

$$B = -1.032\ (A + E) - 15.947 \qquad r = -0.9999 \tag{1}$$

and plotted in Fig. 1, while that for the benzamides of cyclic amines is given in eqn. 2

$$B = -1.030\ (A + E) - 16.614 \qquad r = -0.9999 \tag{2}$$

and plotted in Fig. 2. Eqns. 1 and 2 are remarkably similar to each other and to the regression of *B versus* (*A* + *E*) for the secondary benzamides analyzed on this column (see eqn. 11 in ref. 8 and eqn. 9 in ref. 10). In the determination of eqn. 1, the point for N,N-di-*n*-hexylbenzamide was omitted. This point lies well off the line defined by the remaining N,N-dialkyl derivatives and may be another example of the anomalous

TABLE II

EXPERIMENTALLY DETERMINED LOG k' VALUES FOR TERTIARY BENZAMIDES

Compound No.	*Acetonitrile (%, v/v)*							
	2.5	*5*	*10*	*15*	*20*	*25*	*30*	*35*
1	2.090	1.782	1.324	0.982	0.713	0.522	0.340	0.209
2			2.109	1.698	1.368	1.087	0.846	0.669
3					2.120	1.783	1.461	1.218
4					2.054	1.730	1.429	1.197
5				2.220	1.819	1.498	1.196	0.993
6					2.184	1.808	1.488	1.240
7							2.142	1.819
8							2.196	1.874
9							2.018	1.692
10								
11		2.385	1.845	1.435	1.117	0.833	0.606	0.468
12			2.278	1.852	1.494	1.209	0.941	0.754
13				2.186	1.800	1.470	1.191	0.975
14				2.274	1.884	1.548	1.263	1.026
15				2.336	1.944	1.594	1.298	1.068
16	2.184	1.840	1.318	0.946	0.672	0.444	0.311	0.140

behavior similarly exhibited by large alkyl chain solutes described in a previous report[10]. This anomalous behavior for long alkyl chain benzamides can be attributed to solute conformational changes or a critical chain length effect. A folding back of the hydrocarbon chain upon itself or association of the two alkyl chains in the case of the long chain N,N-dialkylbenzamides can produce an intramolecular hydrophobic effect. The result of this association is a drastic alteration in the size and shape of the solvent cavity displaced by this compound as compared to the unassociated state. Alternatively, these observations can also be explained by the critical chain length effect described by Berendsen and De Galan[12]. This theory suggests that the degree of

TABLE III

SOLVOPHOBIC PARAMETERS OF SOME TERTIARY BENZAMIDES

Compound No.	*B*	*C*	*(A + E)*	*r*
1	−344.12	224.851,898	318.551	0.9982
2	−213.39	243,967,276	191.228	0.9984
3	−110.34	271,707,584	90.946	0.9936
4	−114.00	259,295,238	94.863	0.9925
5	−150.29	263,559,239	129.699	0.9969
6	−114.82	284,526,230	94.955	0.9953
7	5.64	264.101,335	−20.774	0.9917
8	7.51	254,864,581	−22.167	0.9805
9	−9 34	247,930,912	−5.878	0.9859
10	−231.14	1,524,156,405	173.900	0.9897
11	−261.14	251.441,922	237.126	0.9987
12	−197.82	252,187,065	175.962	0.9984
13	−147.38	259,404,897	126.953	0.9976
14	−121.89	259,647,066	102.171	0.9981
15	−135.54	267,178,469	115.366	0.9977
16	−334.12	236,083,040	308.215	0.9988

40	45	50	55	60	65	70	75	80
0.158								
0.528		0.285						
1.023		0.669	0.425					
1.011		0.652	0.425					
0.811		0.514						
1.041		0.691	0.434					
1.561	1.308	1.115	0.766					
1.622	1.376	1.170	0.833					
1.454	1.224	1.032	0.702					
		2.007	1.735	1.539	1.347	1.168	1.024	0.836
0.340	0.099							
0.602	0.349							
0.800	0.498							
0.845	0.522							
0.893	0.575							
0.031								

association between the solute and bonded ligand (alkyl stationary phase) varies with the chain length of each species and reaches a maximum at some critical chain length.

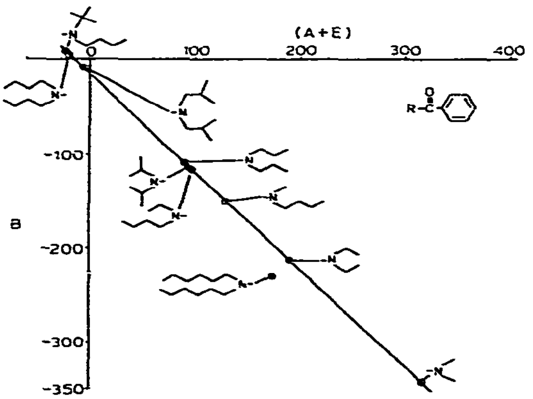
The values of B and $(A + E)$ for all sixteen tertiary benzamides were regressed on the molecular connectivity indices, and the best two-variable combinations are given in eqns. 3 and 4:

$$B = 458.371(48.776)\,{}^0\chi^v - 18.232(2.090)\,({}^0\chi)^2 - 2232.750(206.254)$$

$$r = 0.9501 \quad (3)$$

$$(A + E) = -419.597(42.819)\,{}^0\chi^v + 16.469(1.834)\,({}^0\chi)^2 + 2061.877(181.064)$$

$$r = 0.9581 \quad (4)$$



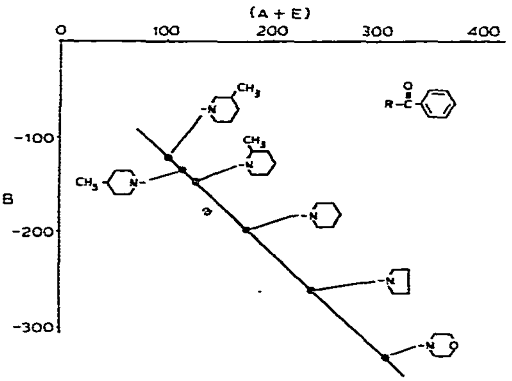


Fig. 1. Plot of the solvophobic parameters B *versus* $(A + E)$ as derived from data obtained on the Ultrasphere ODS column (UE795) for some N,N-dialkylbenzamides.

Fig. 2. Plot of the solvophobic parameters B *versus* $(A + E)$ as derived from data obtained on the Ultrasphere ODS column (UE795) for some cyclic benzamides of secondary amines.

The indices chosen represent steric bulk and do not encode relative degree of branching. The numbers in parentheses in these equations represent the standard error.

The values for the solvophobic parameter C (Table III) were used to calculate ΔA, the contact surface area of the solute-bonded ligand complex (see Appendix, eqn. A1). The calculated contact surface areas of the N,N-dialkylbenzamides and the benzamides of cyclic amines are reported in Table IV. In general, the ΔA values increase with an increase in the total number of alkyl carbons and decrease with chain branching. These trends can be seen in compounds 1–3 and 7–9 in Table IV. Interestingly, the N,N-di-*n*-butyl derivative (compound 7) was predicted to exhibit about the same contact surface area as the N-methyl-N-butyl amide (compound 5) and less than that of the N-ethyl-N-butyl derivative (compound 6). However, the calculated log k'_w values for these compounds increase with the total number of alkyl carbons. Thus, the contact surface area for the solute–ligand complex is not the only factor involved in the reversed-phase retention of these solutes. The 2- and 3-methylpiperidine derivatives (compounds 13 and 14) were predicted to have equivalent ΔA values while the 4-methylpiperidine derivative exhibited a slightly larger value for ΔA. The contact surface area predicted for the morpholine derivative was the smallest of the cyclic benzamides studied.

The morpholine and piperidine derivatives differ only in the replacement of a methylene group with a polar oxygen atom. A comparison of the properties of the morpholine (compound 16) and piperidine (compound 12) amides should reflect the RPLC differences between an ether-type oxygen and the methylene group. Table IV shows a small decrease in ΔA from piperidine to morpholine; however, there is a large decrease in log k'_w. These observations again demonstrate that a major influence upon the retention of these compounds is the interaction of the solute with the solvent and the lowered k'_w for the amide of morpholine is the result of the solvophilic nature of the oxygen and not due to any large decrease in ΔA.

Regression of the molecular connectivity indices on ΔA, for the sixteen tertiary benzamides, produced the quadratic combination described by eqn. 5:

TABLE IV

CALCULATED CAPACITY FACTORS AT 0% ACETONITRILE (ACN) AND CALCULATED CONTACT SURFACE AREAS FOR SOME TERTIARY BENZAMIDES

Compound No.	*log k'_w (0% ACN)*	*ΔA (Å^2)*
1	2.790	91.3
2	3.781	99.1
3	4.989	110.4
4	4.748	105.3
5	4.547	107.0
6	5.217	115.6
7	5.647	107.3
8	5.554	103.5
9	5.235	100.7
10	28.208	619.1
11	3.601	102.1
12	4.038	102.4
13	4.466	105.4
14	4.571	105.5
15	4.718	108.5
16	2.908	95.9

TABLE V

SELECTED MOLECULAR CONNECTIVITY INDICES FOR SOME TERTIARY BENZAMIDES

Compound	$^0\chi$	$^0\chi^v$	$^1\chi$
1	8.26758	6.74221	5.21521
2	9.68180	8.15642	6.29122
3	11.09601	9.57064	7.29122
4	11.42228	9.89691	7.03658
5	10.38890	8.86353	6.75322
6	11.09601	9.57064	7.29122
7	12.51022	10.98485	8.29122
8	12.88890	11.36353	7.96454
9	12.83649	11.31112	8.00290
10	15.33865	13.81328	10.29122
11	9.09601	7.57064	6.37701
12	9.80312	8.27774	6.87701
13	10.67336	9.14798	7.28769
14	10.67336	9.14798	7.27085
15	10.67336	9.14798	7.27085
16	9.80312	7.97888	6.87701

$$\Delta A = 36.941(4.699)\,(^1\chi)^2 - 486.608(73.810)\,^1\chi + 1680.118(286.742)$$
$$r = 0.9619 \qquad (5)$$

The molecular connectivity values chosen for the tertiary benzamides are listed in Table V.

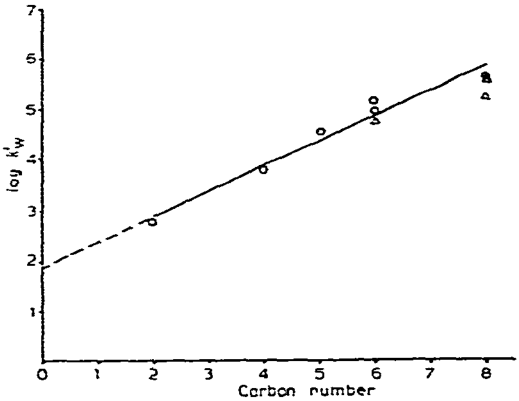
The values predicted for log k'_w (the capacity factor in a totally aqueous eluent) by the solvophobic theory are given in Table IV. A linear relationship exists (Fig. 3) between the predicted log k'_w and the total number of alkyl carbon atoms (sum of the number of carbons in each chain) in those unbranched N,N-dialkylbenzamides (Table IV). Eqn. 6 was derived from the linear regression of both the symmetrically and unsymmetrically disubstituted, unbranched benzamides:

$$\log k'_w = 0.504\ (\text{no. of C atoms}) + 1.891 \qquad r = 0.980 \qquad (6)$$

When only the symmetrically disubstituted, unbranched compounds were considered, the correlation coefficient was 0.994. Those disubstituted benzamides containing branched portions were scattered about the line described by eqn. 6.

Eqn. 6 is plotted in Fig. 4 (curve B) along with a curve which represents the log k'_w *versus* carbon number plot obtained using the same chromatographic column for the unbranched C_1–C_8 mono-N-alkylbenzamides (see Fig. 2 and Table V, ref. 10). According to eqn. 6 (curve B, Fig. 4), the log k'_w value predicted for N-methylbenzamide (C_1) is 2.395, and 1.891 for benzamide (C_0). However, the log k'_w value predicted by the solvophobic theory from data on mono-N-alkylbenzamides (curve A, Fig. 4) is 2.034 for N-methylbenzamide, and that actually measured is 1.957. If curve A is extended to the y axis, a value of 1.540 is predicted for benzamide (C_0).

The offset of the two lines at the left-hand side of Fig. 4 (lower carbon numbers) is probably due to the lack of including (in the extrapolation of eqn. 6 to the y axis) the interaction of the amide proton (C_1) or protons (C_0) with the surrounding solvent. This interaction was studied previously by NMR spectrometry[8]. Differences in the electronic environment in the amide portion of secondary and tertiary benzamides are also reflected in the UV and IR spectra. Conformational changes in the



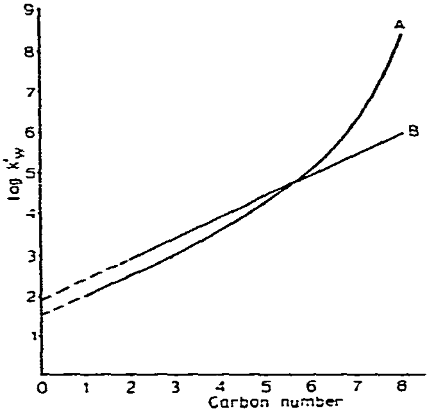


Fig. 3. The relationship between predicted log k'_w values derived from data collected on the Ultrasphere ODS column (UE795) and the total number of alkyl carbon atoms for some N,N-dialkylbenzamides. ---, Extrapolated, △ = branched, ○ = unbranched.

Fig. 4. The relationship between predicted log k'_w values derived from data collected on the Ultrasphere ODS column (UE795) and the total number of alkyl carbon atoms for unbranched N-alkylbenzamides (A) and unbranched N,N-dialkylbenzamides (B). ---, Extrapolated.

monosubstituted benzamides (as the number of carbon atoms increases) could reduce the interaction between water and the amide proton, and explain the crossover of curves A and B shown in Fig. 4 at higher carbon number.

There appear to be at least two factors operating in the substitution of an amide hydrogen with an alkyl group: the increased solvophobic effect resulting from adding the alkyl group; and the decreased solvophilic effect caused by loss of the amide N–H. Thus, the addition of a methyl group to N-methylbenzamide to yield N,N-dimethylbenzamide produces a greater net increase in reversed-phase retention than the addition of a methyl group to yield N-ethylbenzamide. The use of the curves in Fig. 4 to predict the log k'_w value for benzamide (unsubstituted) probably yields an invalid estimate. In benzamide, there are two amide protons capable of interacting with the environment. A better estimate of the log k'_w of benzamide may be to use the difference in k'_w values ($\Delta k'_w$) of N,N-dimethyl- and N-methylbenzamide to measure the total effect (increased solvophobic and decreased solvophilic) produced by adding the methyl group to the amide nitrogen ($\Delta k'_w = 1.891 - 1.540 = 0.351$). Then assuming, of course, that the first substitution will have the same effect on log k'_w as the second substitution (and it may well not) the log k'_w for benzamide is predicted to be 1.189. Unfortunately however, the log k'_w of benzamide was not measured during this study. But this situation dramatically demonstrates the caution with which "parent" molecules for substituent contribution analysis should be chosen.

A quadratic relationship in the first order connectivity level index was chosen as the best description of the predicted capacity factors in pure water for the sixteen tertiary benzamides:

$$\log k'_w = 3.551(0.419)\,({}^1\chi)^2 - 45.049(6.587)\,{}^1\chi + 149.506(25.590)$$
$$r = 0.9737 \qquad (7)$$

Eqn. 8 is the regression equation

$$\log k'_{30\%\,\mathrm{ACN}} = 2.037(0.267)\,{}^0\chi^v - 1.637(0.270)\,{}^0\chi + 0.109(0.474)$$
$$r = 0.9928 \qquad (8)$$

for the molecular connectivity indices chosen to describe the retention of fifteen of the tertiary benzamides at 30% (v/v) acetonitrile (ACN) in water. (Data at this solvent composition were not collected for N,N-di-*n*-hexylbenzamide.) Eqns. 7 and 8 contain no branching indices, although six of the sixteen tertiary benzamides studied were branched. The chosen indices are primarily descriptors of bulk with only one valence level term appearing in eqn. 8. The selection of valence level terms, χ^v, indicates that the nature of the atom is important to the correlation, however the choice of connectivity level terms, χ, means that the identity of the atom is not a significant factor in the correlation.

CONCLUSIONS

The log k'_w values for these tertiary benzamides as well as other fundamental chromatographic parameters can be predicted from measured k' data in acetonitrile–water using the equations of the solvophobic theory. The calculated contact surface area for the solute–ligand complexes increased with the number of alkyl carbons in the solute and decreased with chain branching. Molecular connectivity regressions on the measured and calculated chromatographic parameters showed excellent correlations with those indices quantitatively describing molecular size.

APPENDIX

Eqn. A1 describes the reversed-phase liquid chromatographic retention of a solute when the mobile phase composition varies while temperature and flow-rate remain constant

$$\ln k' = A + B\mathscr{D} + C\gamma + D(\kappa^e - 1)\, V^{2/3}\gamma + E + \ln (RT/P_0V) \tag{A1}$$

where

$$A = \varphi - \frac{\Delta F_{\text{vdw,assoc}}}{RT} \tag{A2}$$

$$B = \frac{1}{RT}\cdot\frac{1-\lambda}{2\lambda}\cdot\frac{\mu_s^2}{v_s}\cdot N\mathscr{P} \approx \frac{1}{4\pi\varepsilon_0 RT}\cdot\frac{1-\lambda}{2\lambda}\cdot\frac{\mu_s^2}{v_s}\cdot\frac{N}{1-(\alpha_s/v_s)} \tag{A3}$$

with the approximation that $\mathscr{D} \approx 1$

$$C = N\Delta A/RT \tag{A4}$$

$$D = 4.836\ N^{1/3}/RT \tag{A5}$$

$$E = \Delta F_{\text{vdw,s}}/RT \tag{A6}$$

These expressions were derived from the application of the solvophobic theory[3,4] to chromatographic measurements[5]. Table AI enumerates the variables involved in eqns. A1–A6.

TABLE AI

DEFINITION OF MATHEMATICAL VARIABLES

Variables	*Definition*
A, B, C, D and E	Regression coefficients
$\mathcal{D} = 2(\varepsilon - 1)/(2\varepsilon + 1)$	$\mathcal{D}$ is a function of ε, the static dielectric constant of the solvent
γ	Surface tension of the solvent
κ^e	Microscopic cavity factor; curvature correction to convert the macroscopic surface tension to microscopic dimensions
V	Average molar volume of solvent
R	Gas law constant
T	Temperature in °K
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
$\Delta F_{\mathrm{vdw,assoc}}$	Free energy change for the interaction of solute and ligand in a hypothetical gas phase; assumed to occur by van der Waals forces only
$\Delta F_{\mathrm{vdw,s}}$	Van der Waals component of the free energy change for the interaction of the solute with the solvent
$\lambda = v_{\mathrm{SL}}/v_{\mathrm{S}}$	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
$\mathrm{P} = \left[4\pi\varepsilon_0\left(1 - \mathcal{D}\frac{\alpha_{\mathrm{S}}}{v_{\mathrm{S}}}\right)\right]^{-1}$	ε_0 is the permittivity constant, and α_{S} is the polarizability of the solute
ΔA	Contact surface area of the solute–ligand complex

Rearranging eqn. A1 yields

$$\ln k' - D(\kappa^e - 1)\,V^{2/3}\gamma - \ln(RT/P_0V) = (A + E) + B\mathcal{D} + C\gamma \qquad \text{(A7)}$$

which can be reduced to:

$$X = (A + E) + B\mathcal{D} + C\gamma \qquad \text{(A8)}$$

The statistical analysis system procedure SYSREG (SAS Institute, Inc., P.O. Box 10066, Raleigh, NC 27605) was used on an IBM 370/158 computer (Computer Services, Auburn University, AL) to determine the regression coefficients B and C as well as the intercept $(A + E)$. This process has been discussed in detail elsewhere[8].

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