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INVESTIGATION OF N-ALKYLBENZAMIDES BY REVERSED-PHASE LIQUID CHROMATOGRAPHY

IV. THE STUDY OF A HOMOLOGOUS SERIES OF N-ALKYLBENZAMIDES USING THE SOLVOPHOBIC THEORY AND MOLECULAR CONNECTIVITY

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SUMMARY

The capacity factors for eighteen normal-chain N-alkylbenzamides in a reversed-phase chromatographic system are reported. Measured and calculated chromatographic parameters indicate that an adequate description of the structure-retention process can be achieved only if the amides are considered in groups of short, intermediate and long chain length. The inconsistent chromatographic behavior observed when all eighteen amides are considered may be due to conformational or critical chain-length effects.

INTRODUCTION

There are basically two types of solute retention mechanism which have been proposed for reversed-phase liquid chromatography (RPLC): partition and adsorption, along with variations of each type^{1–3}. Indeed, retention on a bonded phase may not result from a pure adsorption or a pure partition process⁴. With low-coverage bonded-phase materials where an increased number of silanol groups are accessible to the mobile phase, a mixed retention mechanism may be in operation⁵. The most widely accepted explanations of retention in RPLC are based upon processes involving adsorption of the solute on the non-polar stationary phase. In this respect, RPLC would be similar to liquid-solid chromatography (LSC). But in classical LSC, the interactions between solute and adsorbent are controlled by strong polar forces, whereas the stationary phase-solute attractions in RPLC are probably due to weak, non-specific, Van der Waals forces¹. This prompted Colin *et al.*⁶ to term the interaction a “most unusual type of adsorption”, since it does not result from a particular affinity for the adsorbent⁴ as in classical LSC.

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The solvophobic theory⁷⁻⁸ has been proposed by Horváth *et al.* as a theoretical explanation of retention in RPLC. The formation of a reversible solute-bonded ligand complex is hypothetically governed by solvophobic effects, *i.e.* a hydrophobic effect when aqueous solvents are used. Since the non-attractive interactions between the solute and stationary phase are apparently solvent-mediated, the solute-solvent interactions assume a primary role. An understanding of solution phenomena is therefore essential¹¹.

It has been demonstrated previously¹² that the solvophobic theory can be used to predict RPLC parameters for some *N*-alkylbenzamides. Furthermore, predicted and measured chromatographic data are correlated with a topological index, *i.e.* the molecular connectivity index, $\chi^{13,14}$. This report describes the application of these methods to the analysis of the retention behavior of other secondary benzamides.

EXPERIMENTAL

Equipment

Melting points were determined on a Thomas-Hoover melting point apparatus in open capillary tubes and are uncorrected. Ultraviolet spectra were determined in absolute ethanol using a Hitachi (Tokyo, Japan) Model 60 or a Perkin-Elmer (Norwalk, CT, U.S.A.) Model 200 UV spectrophotometer. Infrared spectra were determined with a Beckman (Irvine, CA, U.S.A.) 4230 or an Acculab 6 spectrophotometer. Solution infrared (IR) spectra were taken in sodium chloride cells using spectrophotometric grade chloroform. Refractive index measurements were determined on an Erma (Tokyo, Japan) refractometer. The liquid chromatograph consisted of a Waters (Milford, MA, U.S.A.) Model 6000A solvent delivery system, Model U6K injector, Model 440 UV absorbance detector and an Alltech (Deerfield, IL, U.S.A.) HPLC column water jacket. Constant temperature in both the high-performance liquid chromatographic (HPLC) column water jacket and the refractometer was maintained by a Haake (Saddle Brook, NJ, U.S.A.) Model FE constant-temperature circulator.

Reagents and chemicals

All chemicals were of reagent grade quality or better and were used as purchased without further purification. Methylamine was obtained from Matheson, Coleman and Bell (Norwood, OH, U.S.A.), and *n*-heptadecylamine and *n*-nonadecylamine from Pfaltz and Bauer (Stamford, CT, U.S.A.). Spectrophotometric grade acetonitrile (ACN) was obtained from Burdick & Jackson (Muskegon, MI, U.S.A.), or from Fisher Scientific (Fair Lawn, NJ, U.S.A.). Sodium nitrate was obtained from Fisher Scientific and *n*-hexylamine was purchased from Eastman Kodak (Rochester, NY, U.S.A.). All other chemicals were purchased from Aldrich (Milwaukee, WI, U.S.A.). Doubly distilled water was further purified by pumping (Waters Model 6000 solvent pump) through a 7 cm \times 2.1 mm I.D. column dry-packed with Whatman CO:PELL ODS (30–38 μ m) prior to preparation of solvent mixtures.

Synthesis of amides

A solution of the appropriate primary amine (0.03–0.07 mole) in 35 ml of tetrahydrofuran was mixed with 200 ml of a 20% (w/v) solution of potassium carbonate in a three-necked flask equipped with a magnetic stirrer, reflux condenser, addition funnel, and a heating mantle. A solution of benzoyl chloride (two-fold molar

excess) in 35 ml of tetrahydrofuran was added dropwise. The resulting mixture was heated at 50°C for 3 h. The mixture was maintained at or above pH 8 during the reaction. The solution was cooled to room temperature and extracted with chloroform (3 × 100 ml). The extract was dried with magnesium sulfate and evaporated. The resulting residue was purified by recrystallization from light petroleum (b.p. 30–60°C)–benzene or by column chromatography on silica gel (40 mesh) using a light petroleum–diethyl ether step gradient.

Mobile phase characteristics

The mobile phase solvent mixtures were prepared and allowed to equilibrate for at least 1 h before use. The specific gravity and refractive index were measured for each batch of HPLC solvent prepared (acetonitrile–water) as a means of quality control. The specific gravity of the mobile phase was determined by adding solvent to fill completely a 25-ml Kimax specific gravity bottle equipped with a thermometer, a side-arm and a side-tube cap with vent. The bottle and contents were weighed (± 0.1 mg) at 25°C. The refractometer was calibrated to a value of 1.3325 at 25°C using the purified water. Refractive index values of the mobile phase solutions were determined at 25°C using the Erma refractometer.

Chromatographic procedures

An Ultrasphere ODS (5 μ m, spherical porous particle) column (15 cm × 4.6 mm I.D.) was obtained from Altex Scientific (Berkeley, CA, U.S.A.) and used in this study. The analytical column was preceded by a guard column (7 cm × 2.1 mm I.D.) dry-packed with Whatman CO:PELL ODS (30–38 μ m). The guard column and analytical column were contained inside a column jacket and maintained at $25.0 \pm 0.2^\circ\text{C}$ by circulating water from a constant-temperature bath through the column jacket. The mobile phase flow-rate was 1.5 ml/min and the ultraviolet detector was operated at 254 nm and 0.005 a.u.f.s. Sufficiently dilute solutions of the benzamides were prepared in acetonitrile to give least detectable peaks (less than 20% of scale).

Capacity factors were calculated in the usual manner and are based upon the average of at least two injections. The column void volume was taken as the elution volume for a 0.5- μ l injection of an aqueous solution containing 0.3 μ g of sodium nitrate. The void volume was measured during each chromatographic session.

CALCULATIONS

Solvophobic parameters

According to the solvophobic theory the RPLC retention of a solute on a given column is described by eqn. 1 when the mobile-phase composition is varied while the temperature and flow-rate remain constant. A description of the variables in eqn. 1 is found in Table I. A discussion of the derivation of this equation can be found elsewhere^{7,8,10}.

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

where

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

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

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

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

$$D = 4.836 N^{1/3}/RT \quad (5)$$

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

Rearranging terms, eqn. 1 becomes:

$$\ln k' - D(\kappa^c - 1)V^{2/3} - \ln(RT/P_0V) = (A + E) + B\mathcal{Q} + C\gamma \quad (7)$$

TABLE I

DEFINITION OF MATHEMATICAL VARIABLES IN EQNS. 1-6

Variable	Definition
A, B, C, D and E	Regression coefficients
$\mathcal{Q} = 2(\epsilon - 1)/(2\epsilon + 1)$	\mathcal{Q} 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
σ	Characteristic constant for a given column (logarithm of the phase ratio)
N	Avogadro's number
$\Delta F_{\text{d.w.assoc}}$	Free energy change for the interaction of solute and ligand in a hypothetical gas phase; assumed to occur by Van der Waals forces only
$\Delta F_{\text{d.w.s.}}$	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
$P = \left[4\pi\epsilon_0 \left(1 - \frac{\mathcal{Q}\alpha_s}{v_s} \right) \right]^{-1}$	Where ϵ_0 is the permittivity constant, and α_s is the polarizability of the solute
ΔA	Contact surface area of the solute-ligand complex

where $\ln k'$ is the natural logarithm of experimentally determined k' values. The regression coefficients A and E were not determined separately, but as the sum ($A + E$). Coefficients A , B , and E are unitless. The units of C are $\text{cm}^2 \text{l}^{-1} \text{atm}^{-1}$. The value of the regression coefficient D was calculated to be $1.6693 \cdot 10^7 \text{ mole}^{2/3} \text{l}^{-1} \text{atm}^{-1}$. Data on density^{15,16}, dielectric constant^{17,18}, surface tension¹⁰, and κ^c (ref. 10) for mobile phase 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 determined. This treatment leads to a series of n equations (n equals the number of experimentally determined data points) of the form:

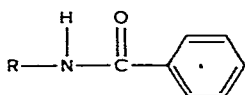
$$X = (A + E) + B\varnothing + C\gamma \quad (8)$$

The regression coefficients of eqn. 8 were evaluated by means of the Statistical Analysis System (SAS Institute, Inc., P.O. Box 10066, Raleigh, NC 27605, U.S.A.) procedure SYSREG on an IBM 370/158 (Computer Services, Auburn University).

Molecular connectivity indices

Calculations of molecular connectivity were performed using the IBM 370/158. The program (CFUNC) used to calculate molecular connectivity indices was obtained from Dr. L. H. Hall, Eastern Nazarene College, Quincy, MA, U.S.A.

TABLE II
PHYSICAL PROPERTIES OF BENZAMIDES



	<i>R</i>	<i>M.p.</i> (°C)	λ (nm) max	Carbonyl stretch (cm^{-1})
Methyl	CH ₃	79–82	225.5	1655
Ethyl	CH ₃ CH ₂	68–70	225.5	1652
<i>n</i> -Propyl	CH ₃ CH ₂ CH ₂	82–84	225.5	1650
<i>n</i> -Butyl	CH ₃ (CH ₂) ₃	oil	225.5	1652
<i>n</i> -Pentyl	CH ₃ (CH ₂) ₄	31–33	225.2	1650
<i>n</i> -Hexyl	CH ₃ (CH ₂) ₅	41–45	225.5	1650
<i>n</i> -Heptyl	CH ₃ (CH ₂) ₆	35–39	225.5	1652
<i>n</i> -Octyl	CH ₃ (CH ₂) ₇	41–45	225.5	1655
<i>n</i> -Nonyl	CH ₃ (CH ₂) ₈	46–48	225.5	1655
<i>n</i> -Decyl	CH ₃ (CH ₂) ₉	55–58	225.5	1652
<i>n</i> -Undecyl	CH ₃ (CH ₂) ₁₀	56–61	225.5	1655
<i>n</i> -Dodecyl	CH ₃ (CH ₂) ₁₁	67–71	225.5	1655
<i>n</i> -Tridecyl	CH ₃ (CH ₂) ₁₂	70–73	225.5	1650
<i>n</i> -Tetradecyl	CH ₃ (CH ₂) ₁₃	73–77	225.5	1652
<i>n</i> -Hexadecyl	CH ₃ (CH ₂) ₁₅	75–78	225.5	1655
<i>n</i> -Heptadecyl	CH ₃ (CH ₂) ₁₆	82–85	225.5	1658
<i>n</i> -Octadecyl	CH ₃ (CH ₂) ₁₇	84–87	225.5	1650
<i>n</i> -Nonadecyl	CH ₃ (CH ₂) ₁₈	85–90	225.5	1655

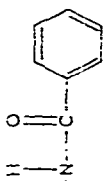


TABLE III

EXPERIMENTALLY DETERMINED LOG K' VALUES ON ULTRASPHERE ODS (UJ795)

(a)

R	% (v/v) Acetonitrile												
	0	0.5	1.0	2.5	5.0	10	15	20	25	30	35	40	45
CH ₃	1.957	1.847	1.741	1.549	1.292	0.930	0.646	0.458	0.311	0.158			
CH ₃ CH ₂	2.362		2.118	1.902	1.625	1.235	0.929	0.713	0.530	0.404			
CH ₃ CH ₂ CH ₂			2.352	2.051	1.628	1.297	1.050	0.833	0.640	0.498			
CH ₃ (CH ₂) ₃				2.120	1.749	1.456	1.204	0.977	0.792	0.621	0.449		
CH ₃ (CH ₂) ₄				2.224	1.749	1.456	1.204	0.977	0.792	0.621	0.449		

(b)

R	% (v/v) Acetonitrile												
	15	20	25	30	35	40	45	50	55	60	65	70	80
CH ₃ (CH ₂) ₅			1.964	1.647	1.365	1.127		0.747		0.434			
CH ₃ (CH ₂) ₆			2.016	1.680	1.410	1.168	0.951	0.747		0.640			
CH ₃ (CH ₂) ₇				1.988	1.687	1.420	1.191	0.948	0.948	0.800			
CH ₃ (CH ₂) ₈				2.310	1.966	1.686	1.417	1.191	0.999	0.999	0.844	0.680	
CH ₃ (CH ₂) ₉					2.248	1.941	1.648	1.417	1.390	1.203	1.018	0.840	
CH ₃ (CH ₂) ₁₀						2.197	1.876	1.603	1.603	1.401	1.206	1.025	0.705
CH ₃ (CH ₂) ₁₁							2.113	1.820	1.820	1.605	1.397	1.206	0.862

(c)

R	% (v/v) Acetonitrile										
	55	60	65	70	75	80	85	90	95	99	100
CH ₃ (CH ₂) ₁₂	2.040	1.811	1.592	1.389		1.018	0.847	0.727	0.588	0.520	0.581
CH ₃ (CH ₂) ₁₃		2.016	1.785	1.573	1.394	1.188	0.998	0.858	0.682	0.615	0.677
CH ₃ (CH ₂) ₁₄			2.175	1.943	1.743	1.514	1.304	1.134	0.929	0.804	0.879
CH ₃ (CH ₂) ₁₅				2.128	1.918	1.680	1.452	1.275	1.046	0.910	0.980
CH ₃ (CH ₂) ₁₆					2.092	1.850	1.605	1.413	1.169	1.011	1.079
CH ₃ (CH ₂) ₁₇					2.270	2.016	1.761	1.554	1.290	1.118	1.185

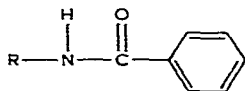
RESULTS AND DISCUSSION

The C_1 – C_{14} and C_{16} – C_{19} straight-chain primary amines were used to prepare the secondary benzamides. The physical characteristics of these compounds are given in Table II. Experimentally measured values of the capacity factors for the compounds at several mobile phase compositions in acetonitrile–water are given in Table III. Retention data were measured up to a $\log k'$ of 2 in most cases. This corresponds to a retention time of 1.46 h for this column. Higher concentrations of acetonitrile in the mobile phase were needed to reach a $\log k'$ of 2 as the carbon content of the solute increased. Indeed, significant retention was noted for the amides of carbon content greater than C_{12} in a mobile phase of 100% acetonitrile.

The capacity factors for the chain lengths of C_{13} and longer exhibit a minimum in the 95–100% acetonitrile solvent composition range (see Table III). Concave minima in $\log k'$ vs. solvent composition plots at high organic modifier content have been observed recently in the reversed-phase chromatography of such large molecules as crown ethers^{19,20} and peptides²⁰. This irregular retention behavior was attributed to silanophilic interactions.

Column void volume in this study was measured for each chromatographic run using sodium nitrate without background electrolyte. This procedure actually determines the interstitial column volume²¹, which is related to the true column void volume through a constant. Periodic measurements using sodium nitrate with

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



<i>R</i>	<i>B</i>	<i>C</i>	(<i>A</i> + <i>E</i>)	<i>r</i>
CH ₃	– 120.79	175,914,241	101.202	0.9992
CH ₃ CH ₂	– 135.46	186,749,400	115.728	0.9992
CH ₃ CH ₂ CH ₂	– 194.62	206,243,456	173.633	0.9990
CH ₃ (CH ₂) ₃	– 203.53	227,101,260	182.433	0.9986
CH ₃ (CH ₂) ₄	– 117.44	238,603,744	98.653	0.9976
CH ₃ (CH ₂) ₅	– 65.26	291,535,851	46.584	0.9938
CH ₃ (CH ₂) ₆	– 8.77	292,315,990	– 7.919	0.9881
CH ₃ (CH ₂) ₇	18.69	411,946,853	– 37.790	0.9899
CH ₃ (CH ₂) ₈	– 23.86	570,930,895	– 0.540	0.9898
CH ₃ (CH ₂) ₉	– 61.67	895,988,947	27.018	0.9852
CH ₃ (CH ₂) ₁₀	– 284.54	1,985,483,246	211.871	0.9852
CH ₃ (CH ₂) ₁₁	– 247.76	1,769,074,522	182.955	0.9955
CH ₃ (CH ₂) ₁₂	– 207.97	1,727,588,446	145.987	0.9838
CH ₃ (CH ₂) ₁₃	– 185.06	1,712,031,155	124.669	0.9704
CH ₃ (CH ₂) ₁₅	– 142.49	1,622,258,008	86.861	0.9318
CH ₃ (CH ₂) ₁₆	– 121.64	1,559,324,047	68.893	0.8937
CH ₃ (CH ₂) ₁₇	– 119.92	2,019,778,645	54.386	0.8754
CH ₃ (CH ₂) ₁₈	– 100.94	1,999,904,386	36.994	0.8384

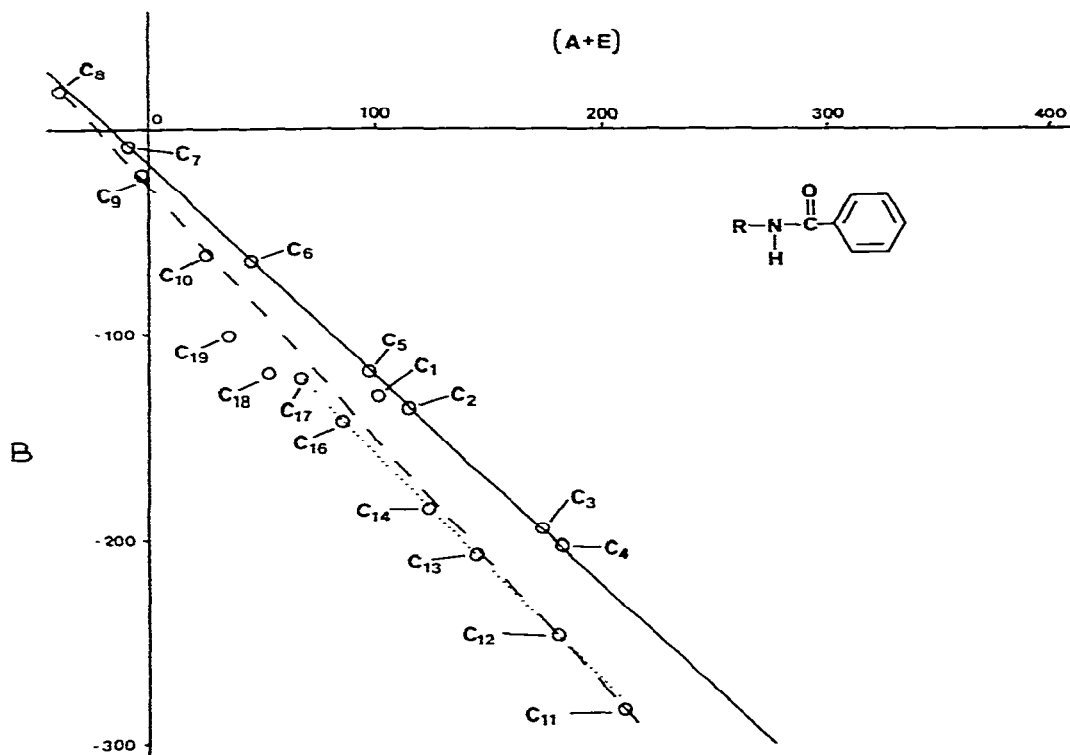


Fig. 1. Plot of the solvophobic parameters B vs. $(A + E)$ as derived from data obtained from an Ultrasphere ODS (UE795) column. —, Eqn. 9; ---, eqn. 10; ···, eqn. 11.

background electrolyte (0.1 M phosphate buffer, pH 6.9) produced the true column void volume. The resulting void volume is higher than that measured without background electrolyte due to the electrochemical exclusion of sodium nitrate from the pore structure of the silica particle in the absence of background electrolyte²². Using either method for measuring the column void volume produces no more than a 3% variation in calculated k' values.

Application of eqn. 1 to the data obtained on Ultrasphere ODS in acetonitrile-water predicted the values for the solvophobic parameters $(A + E)$, B , and C , reported in Table IV. The correlation coefficient for the regression analysis decreased as the length of the hydrocarbon chain increased in these amides. Both positive and negative values of B and of $(A + E)$ were obtained. The calculated values of B were all negative and those of $(A + E)$ were all positive for the sixteen C_1 - C_5 N-alkylbenzamides¹².

In Fig. 1, the values of B and $(A + E)$ from Table IV are plotted with $(A + E)$ on the abscissa and B as the ordinate. Three linear relationships are defined by this plot and described by eqns. 9-11. Eqn. 9 is plotted as a solid line in Fig. 1 and is derived for the C_1 - C_8 compounds. This line does not differ significantly from the plot of $(A + E)$ vs. B obtained in the regression of the sixteen C_1 - C_5 analogues¹².

$$B = -1.014(A + E) - 18.132 \quad r = -0.9999 \quad (9)$$

The C_8 , C_9 , C_{10} , and C_{11} homologues define another linear relationship, however, which is given by eqn. 10 and is drawn as short dashes in Fig. 1.

$$B = -1.217(A + E) - 26.814 \quad r = -0.9999 \quad (10)$$

The dotted line in Fig. 1 represents the regression for the six benzamides: C_{11} , C_{12} , C_{13} , C_{14} , C_{16} , and C_{17} which is given in eqn. 11.

$$B = -1.126(A + E) - 44.18 \quad r = -0.9997 \quad (11)$$

The reason for this type of behavior is not clear, but it could be the result of conformational changes in the alkyl groups. The hydrocarbon portion of a molecule in the solid state has been observed to exist in a fully extended form²³. The extended form is also a stable conformation in the liquid state, but it may not be predominant because its statistical weight is small compared with the sum of all other conformers²⁴. Large organic molecules are capable of an increasing number of differences in shape, *i.e.* conformations²⁵. In aqueous solutions, the C_5 and larger aliphatic hydrocarbons²⁶ are proposed to exist in coiled or folded conformations^{27,28}. This shape presents the least amount of surface area for contact with water molecules. The space inside the coil may consist of a non-solvated, empty interior volume²⁷ stabilized by intramolecular interactions²⁶, or the hydrocarbon arms may be separated by one or more layers of solvent molecules²⁸.

The question is, however, whether or not this coiled behavior can be extended to polar derivatives of hydrocarbons, *i.e.* amphiphiles, particularly the N-*n*-alkylbenzamides. The term "amphiphile" is used in the sense given by Tanford²⁹ as designating a species having a polar or ionic head group and a hydrocarbon tail.

In a study involving α,ω -aminocarboxylic acids and corresponding betaines, Kirchnerova *et al.*³⁰ calculated intercharge distances for these zwitterionic molecules in water and demonstrated that the overall molecular conformation represented some coiling of the chain for those compounds having flexible aliphatic chains. Their results showed that when three or less methylene groups separated the charges, these molecules existed in a fully extended conformation. However, as the chain became longer the results were compatible with restricted carbon-carbon bond rotation causing a coiled conformation. Walder³¹ has shown that, for long-chain α,ω -amino acids (greater than C_6), internal rotational motion is allowed in aqueous solution, whereas lower members of the series appear to exist in the extended arrangement. On the basis of dielectric behavior, these workers found that for 7-aminoheptanoic acid through 10-aminodecanoic acid the rotational motion was not entirely unrestricted. There is also evidence to suggest that the side-chains of proteins reduce exposure to water by folding of the side-chains against the backbone³². However, Tanford²⁹ contends that the linear relationship between the free energy of transfer from an aqueous buffer to pure hydrocarbon solvent for undissociated fatty acids up to behenic acid (C_{22}) precludes the possibility that the chains of these molecules fold upon themselves in aqueous solution.

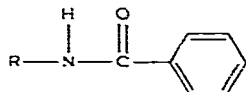
Fig. 1 appears to be consistent with conformational changes in the alkyl portions of these N-*n*-alkylbenzamides. The C_1 - C_8 compounds (eqn. 9) may exist predominantly in an extended conformation whereas the C_8 - C_{11} compounds (eqn. 10)

could represent stages of a conformational transition, *i.e.* an increasing tendency to exist in partially folded conformations. The C_{11} - C_{14} , C_{16} and C_{17} amides redefine a new line (eqn. 11) nearly parallel to the solid line. This may indicate a stable, highly populated, coiled conformer common to these long-chain derivatives. A C_{12} chain folded back upon itself might resemble, longitudinally at least, a C_6 derivative. Why then does it not lie on the same line as the C_6 derivative rather than on a parallel line, especially since the terms B and $(A + E)$ do not involve ΔA ? Even though the total surface areas of an extended C_6 and a folded C_{12} derivative may be similar in magnitude, the molecular volumes which are a component of the term B should differ. The folded conformation should have a relatively small cavity surface area compared with an extended conformation²⁸. These effects could result in an offset of the intercept while the lines remain approximately parallel.

However, the observations in Fig. 1 can be explained by an argument that is not based upon conformational changes in the solute. The findings of Berendsen and De Galan³ supplied evidence to support the concept of a critical chain length, which indicates that a solute interacts only with a certain part of the bonded chains. They measured the retention properties of selected compounds while varying the bonded chain length from RP-1 to RP-22. The complementary situation is presented here. The bonded chain length was held constant (RP-18), while the chain length of the non-polar portion of *N*-substituted benzamides was varied from C_1 to C_{14} , and from C_{16} to C_{19} . The observed effects then may be viewed as a change in the interactions of the solute and ligand as the solute size changes. These possibilities present some interesting avenues for future research.

TABLE V

CALCULATED CAPACITY FACTORS AT 0% ACETONITRILE AND CALCULATED SURFACE AREA DATA ON ULTRASPHERE ODS FOR SOME *N*-ALKYLBENZAMIDES



<i>R</i>	$\log k'_w$ (0% ACN)	$\langle \Delta A \rangle (\text{\AA}^2)$
CH ₃	2.034	71.4
CH ₃ CH ₂	2.427	75.8
CH ₃ CH ₂ CH ₂	2.972	83.8
CH ₃ (CH ₂) ₃	3.642	92.2
CH ₃ (CH ₂) ₄	4.289	96.9
CH ₃ (CH ₂) ₅	5.541	118.4
CH ₃ (CH ₂) ₆	5.962	118.8
CH ₃ (CH ₂) ₇	8.380	167.4
CH ₃ (CH ₂) ₈	11.337	231.9
CH ₃ (CH ₂) ₉	17.230	364.0
CH ₃ (CH ₂) ₁₀	36.187	806.5
CH ₃ (CH ₂) ₁₁	32.619	718.6
CH ₃ (CH ₂) ₁₂	32.236	701.9
CH ₃ (CH ₂) ₁₃	32.258	695.4
CH ₃ (CH ₂) ₁₅	31.204	659.0
CH ₃ (CH ₂) ₁₆	30.341	633.4
CH ₃ (CH ₂) ₁₇	38.986	820.6
CH ₃ (CH ₂) ₁₈	38.905	812.4

When the values of B and $(A + E)$ for the straight-chain homologues (C_1 – C_{14} , C_{16} – C_{19}) were regressed on the molecular connectivity indices (an index which encodes structural information about the solute), the best two-variable combination had a correlation coefficient of 0.4619. In other words, there was no correlation with the topological index when all straight chain homologues were considered together, which is not surprising in view of Fig. 1.

The contact surface area, ΔA , of the associated solute–bonded ligand complex was evaluated (eqn. 4) using the values for C given in Table IV. The results are reported in Table V. The values obtained for ΔA appear to substantiate the results of Fig. 1. The contact surface area increases through the C_{10} homologue with an abrupt increase at C_{11} followed by a leveling off of the ΔA value. These observations may be explained on the basis of a solute conformational change, or in terms of the compulsory absorption mechanism proposed by Berendsen and De Galan³, in that large solutes need longer RP chains to become fully enclosed. A leveling-off effect is often seen when comparing the data of a homologous hydrocarbon family with various parameters, because the addition of a methylene group has a larger effect in the shorter chains than in the longer ones⁴.

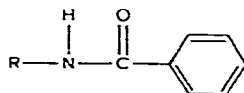
The relationship between ΔA and molecular connectivity for all eighteen of the straight-chain homologues studied is described by eqn. 12.

$$\Delta A = 2522.209 \pm 874.892 \text{ }^6\chi_p^{\wedge} - 236.106 \pm 107.689 \text{ }^5\chi_p^2 + 200.009 \pm 130.532$$

$$r = 0.9340 \quad (12)$$

TABLE VI

SELECTED MOLECULAR CONNECTIVITY INDICES FOR THE C_1 – C_{14} AND C_{16} – C_{19} N-ALKYLBENZAMIDES



R	$^{\circ}\chi$	$^{\circ}\chi^*$	$^5\chi_p$	$^5\chi_p^{\wedge}$	$^6\chi_p^{\wedge}$
CH ₃	7.397 34	5.79500	1.417 11	0.41874	0.13065
CH ₃ CH ₂	8.104 44	6.502 10	1.583 77	0.49639	0.17548
CH ₃ CH ₂ CH ₂	8.811 55	7.209 21	1.836 71	0.58003	0.23039
CH ₃ (CH ₂) ₃	9.518 66	7.916 32	1.886 74	0.63516	0.28953
CH ₃ (CH ₂) ₄	10.225 76	8.623 42	2.021 24	0.73427	0.32852
CH ₃ (CH ₂) ₅	10.932 87	9.330 53	2.146 24	0.87444	0.39860
CH ₃ (CH ₂) ₆	11.639 98	10.037 64	2.271 24	0.99944	0.49771
CH ₃ (CH ₂) ₇	12.347 08	10.744 74	2.396 24	1.12444	0.58610
CH ₃ (CH ₂) ₈	13.054 19	11.451 85	2.521 24	1.24944	0.67449
CH ₃ (CH ₂) ₉	13.761 30	12.158 96	2.646 24	1.37444	0.76288
CH ₃ (CH ₂) ₁₀	14.468 40	12.866 06	2.771 24	1.49944	0.85126
CH ₃ (CH ₂) ₁₁	15.175 51	13.573 17	2.896 24	1.62444	0.93965
CH ₃ (CH ₂) ₁₂	15.882 62	14.280 28	3.021 24	1.74944	1.02804
CH ₃ (CH ₂) ₁₃	16.589 72	14.987 38	3.146 24	1.87444	1.11643
CH ₃ (CH ₂) ₁₅	18.003 92	16.401 58	3.396 24	2.12444	1.29320
CH ₃ (CH ₂) ₁₆	18.711 01	17.108 69	3.521 24	2.24944	1.38159
CH ₃ (CH ₂) ₁₇	19.418 11	17.815 78	3.646 24	2.37444	1.46998
CH ₃ (CH ₂) ₁₈	20.125 20	18.522 87	3.771 24	2.49944	1.55837

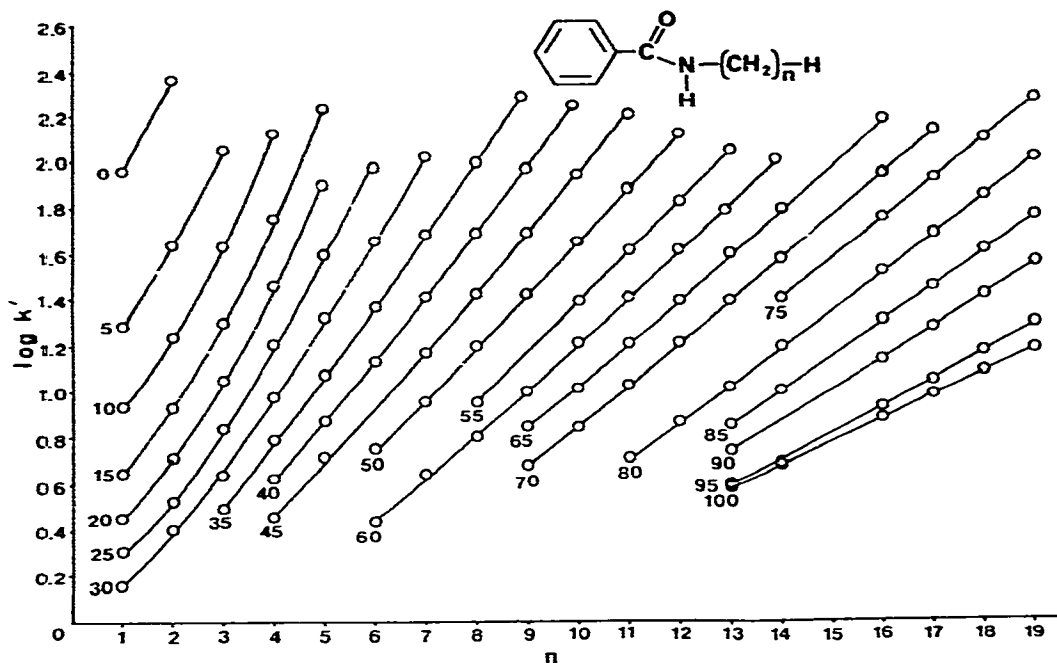


Fig. 2. $\log k'$ values plotted against the alkyl carbon number (n) for *N*-*n*-alkylbenzamides. Each plot is labeled with a number representing the % (v/v) acetonitrile in the mobile phase.

Both of the molecular connectivity indices chosen in eqn. 12 are path indices; no branching indices were selected to describe this homologous series of straight-chain compounds. The pertinent indices chosen are described in Table VI.

In Fig. 2 the measured $\log k'$ values from Table III for every 5% solvent composition change are plotted against the number of carbon atoms in the unbranched alkyl portion of the solute molecule. A trend toward lower slope values as the quantity of organic in the mobile phase increases is immediately obvious. The slope for the plot at 5% acetonitrile is 0.338 ($r = 0.998$) while that at 95% acetonitrile is 0.118 ($r = 0.999$); a 3.2-fold slope decrease. The decrease in slope demonstrates the inconsistent ability of the chromatographic system toward discrimination between sequential members of the homologous series. This effect appears to be related to both the hydrophobicity of the solute and the composition of the mobile phase. The above slopes are based on linear regression; however, upon close examination of Fig. 2, a trend toward increasing non-linearity with increased water content in the mobile phase is noted. $\log k'$ was demonstrated to be linearly related to the number of alkyl carbon atoms in the unbranched chains of alcohols, carboxylic acids, and alkanes by Tanaka and Thornton³³, and of methyl esters, alkanes, and alkylbenzenes by Colin and Guiochon³⁴. In these cases however, the capacity factors measured were much smaller than reported here.

As an extension of Fig. 2, the predicted values for $\log k'_w$ (at 0% ACN) given in Table V are graphed vs. carbon number in Fig. 3. The capacity factors in a totally aqueous eluent (k'_w) were actually determined for the methyl ($\log k'_w = 1.957$) and ethyl ($\log k'_w = 2.362$) homologues (see Table IIIa). The $\log k'_w$ values predicted by

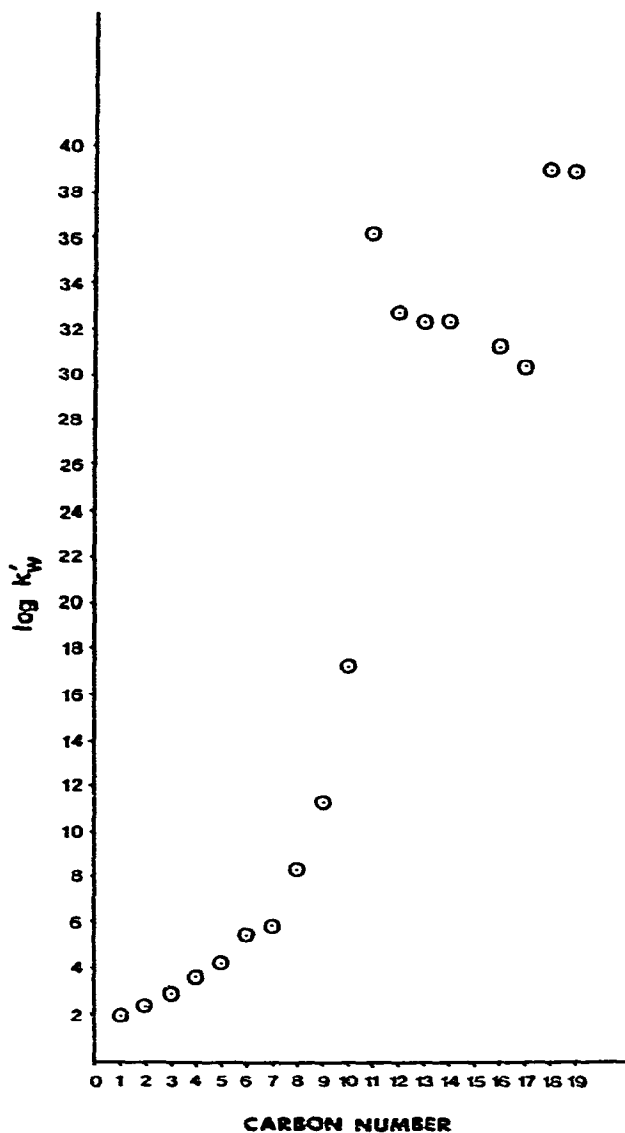


Fig. 3. Plot of the predicted $\log k'_w$ values derived from data collected on the Ultrasphere ODS (UE795) column versus carbon number.

the solvophobic theory for each of these two derivatives are the same as those actually measured (see Table V). The graph in Fig. 3 of $\log k'_w$ vs. carbon number is approximately linear through C_7 . At C_7 either the molecular conformation of the amide changes, or the ligand is no longer able to interact with the entire solute. Compounds C_8 , C_9 , C_{10} , and C_{11} indicate some type of transitional phase. The remaining solutes show little or no chromatographic discrimination in a totally aqueous system. The addition of methylene groups to a folded solute conformation should provide only a small increase in the total surface exposed to the solvent.

The results of the regression of $\log k'_w$ (for the eighteen straight-chain homologues) on the molecular connectivity indices is given in eqn. 13.

$$\log k'_w = 255.268 \pm 84.466 {}^6\chi_p^1 - 23.148 \pm 10.397 {}^5\chi_p^2 + 16.990 \pm 12.602 \quad (13)$$

$$r = 0.9485$$

The same indices were chosen as those in eqn. 12. The results of molecular connectivity regression on the capacity factors obtained at a single solvent composition, acetonitrile (ACN)-water (60:40) (eqns. 14 and 15) show excellent correlations requiring only one connectivity variable.

$$\log k'_{60\% \text{ ACN}} = 1.580 \pm 0.015 {}^5\chi_p^1 - 0.960 \pm 0.021 \quad (14)$$

$$n = 9 \quad r = 0.9997$$

$$\log k'_{60\% \text{ ACN}} = 0.279 \pm 0.003 {}^0\chi^1 - 2.184 \pm 0.033 \quad (15)$$

$$n = 9 \quad r = 0.9997$$

Only a small selection of the longer chain derivatives were included in this regression study (C_6 - C_{14} , $n = 9$) because of the diverse capacity factors represented by the eighteen straight-chain homologues. Two different indices, ${}^5\chi_p^1$ and ${}^0\chi^1$, gave equivalent correlation coefficients for the data at 60% acetonitrile. The indices chosen in eqns. 14 and 15 indicate that both the nature of the atoms as well as the size of the molecule are important factors in describing the structure-retention relationship for these compounds.

CONCLUSIONS

The $\log k'$ values for a series of eighteen N-*n*-alkylbenzamides were determined on a hydrocarbonaceous stationary phase. The capacity factors were determined in varying compositions of water and acetonitrile. Significant retention was observed for the longer chain alkylbenzamides in a mobile phase of pure acetonitrile. Molecular connectivity regressions on the measured capacity factors showed excellent correlations requiring only one connectivity variable. Application of the solvophobic theory to the measured k' data allowed for the calculations of the capacity factors in pure water ($\log k'_w$) and other fundamental chromatographic parameters. The correlation coefficient for the regression analysis decreased as the length of the hydrocarbon chain increased in these amides. The solvophobic parameters appear to divide the compounds into groups of short, intermediate, and long chain alkylbenzamides. The inconsistent chromatographic behavior of this diverse groups of amides can be explained on the basis of conformational changes in the hydrocarbon moiety or a critical chain length effect.

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