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STOICHIOMETRIC DISPLACEMENT OF SOLVENT BY NON-POLAR SOLUTES IN REVERSED-PHASE LIQUID CHROMATOGRAPHY

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SUMMARY

This paper proposes a retention model which predicts that the displacement of non-polar solutes from a reversed-phase chromatographic column is accompanied by the adsorption of a stoichiometric number (Z) of solvent molecules. The number of solvent molecules involved in this process is a function of both solute and solvent contact surface areas. Increasing solute contact surface area would increase Z whereas increasing solvent contact surface area would decrease the Z value for a specific solute. The experimental observations presented are consistent with this model.

Further predictions of the model are that (1) plots of log k' *versus* the inverse log of solvent concentration will be non-linear at solvent concentrations where the surface of a reversed-phase support is not fully solvated, and (2) only a portion of the total non-polar surface area of a molecule actually contacts the surface of a reversed-phase support. Non-linearity in plots of log k' *versus* the inverse log of solvent concentration was in fact observed at solvent concentrations where solvation of the reversed-phase support is incomplete.

INTRODUCTION

The problem of predicting solute retention in reversed-phase chromatography (RPC) has occupied the attention of liquid chromatographers for decades. At present the most popular approach is to relate the logarithm of solute capacity factor (k') to the volume fraction (Φ_b) of organic solvent B in the mobile phase[1], as shown in eqn. 1.

$$\log k' = \log k_w - S\Phi_b \tag{1}$$

The intercept (log k_w) is an extrapolated value of the capacity factor in pure water and S is the slope of the plot of log k' *versus* Φ_b. S is said to be an indicator of the

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strength of pure solvent B as a mobile phase. A larger value of S leads to a faster decrease in k' with increases in Φ_b. The fact that the same volume fraction of two different solvents will produce different k' values with the same solute indicates that S is dependent on the type of solvent.

Although eqn. 1 adequately expresses the relationship of k' to Φ_b for k' values between 0.5 and 10, it has been suggested by Schoenmakers et al.[2] that the equation

$$\ln k' = A_g\Phi_b^2 + B_g\Phi_b + C_g + D_g(\Phi_b) \tag{2}$$

provides a more adequate description of the process. The terms A_g, B_g and C_g are constants and $D_g(\Phi_b)$ is a size correction term. This equation fits experimental data at least as well as eqn. 1 and in some cases it is superior. The principal difference is that eqn. 2 shows $\ln k'$ to be a quadratic rather than a linear function of solvent composition Φ_b. Non-linearity of $\ln k'$ vs. Φ_b plots at extremes of k' confirms the assertion that eqn. 2 provides a more adequate description of the process. At present, no reason has been given for non-linearity of $\ln k'$ vs. Φ_b plots at extremes of k'.

Horváth et al.[3] have provided the most detailed analysis of the interaction of small non-polar solutes with solvent. Through application of the solvophobic theory[3] they have been able to account for many of the factors that control solute retention; even making it possible to characterize eluent strength of mixed solvents with different compositions. The basic premise in this treatment is that mobile phase surface tension provides the driving force for retention in RPC. Attenuation of the solute–sorbent and water–water interactions is achieved by the addition of organic solvents. Unfortunately, the relatively large number of surface tension measurements and physicochemical constants required to use the equations developed in this treatment are not always available.

The influence of stationary phase on retention in RPC is well known, yet most retention models do not include the contribution of stationary phase in solute retention. Jandera et al.[4] have recently proposed a semi-empirical model based on "interaction indices", in which the importance of stationary phase modifiers and silanol groups are considered. However, the main assumption of this model is that retention in RPC is primarily controlled by interactions in the mobile phase.

Several studies[5-7] have also been carried out on macromolecular retention in RPC. Although the same mechanism is probably involved in the retention of both small and macromolecular species, molecular size has been found to contribute to the retention process. Much smaller changes in mobile phase composition are required for the elution of macromolecules than small molecules[8]. Grego et al. proposed[7] that in the case of proteins this is due to the larger contact area of macromolecular solutes than small peptides. In a recent treatment of gradient elution, Larmann et al.[9] and Snyder et al.[10] showed with polystyrene that the slope (S) of plots of $\ln k'$ versus Φ_b increases with solute molecular weight. Since S varies with solute size, it may be questioned whether S is truly a measure of solvent strength.

The recent papers[5,6] by Geng and Regnier have proposed that retention of proteins on an alkylsilane support can be described by the equation

$$k' = I/[\mathrm{D_0}]^Z \tag{3}$$

where $[D_0]$ is the concentration of the organic solvent in the mobile phase, Z is the number of solvent molecules involved in the displacement of a protein from the reversed-phase surface and I is a constant. This approach is based on the assumption that a stoichiometric number of solvent molecules is required for solute displacement from an RPC column. Instead of examining the forces that promote adsorption, this model is directed more toward contact surface area between the stationary phase and solutes and how solvent stoichiometry may be used to predict contact surface area.

The question of whether there is also a solvent–solute displacement stoichiometry (Z) for small molecules has not been examined. The objectives of this paper are (1) to examine the possibility that eqn. 3 also describes the retention of small molecules in RPC, (2) to determine Z values for a series of low-molecular-weight, non-polar species, (3) to examine the relationship between Z number and non-polar surface area (A_u), (4) to examine the relationship between $\log I$ and A_u, (5) to examine the relationship between Z and I and (6) to give a physical meaning to I.

THEORETICAL

The driving force for solvation of an alkylsilane bonded phase is provided by solvophobic effects in the mobile phase. It is probable that the thickness of either the adsorbed solvent or solute layer should in no case be more than a bilayer[11]. Layer thickness probably also depends on the solvent dipole. Solvents such as methanol and ethanol that interact with the alkyl bonded phase in such a way as to provide a gradient between the hydrophobic bonded phase and a hydrophilic mobile phase would be expected to form a monolayer. In contrast, molecules with a smaller dipole could be in the form of a bilayer.

Adsorption of a hydrophobic molecule onto an RPC column can be envisioned as an interaction between non-polar portions of the bonded phase and non-polar areas of the solute[11]. Polar groups of a solute, in contrast, will be directed away from the hydrophobic support toward the more hydrophilic mobile phase. Since this is a surface phenomenon, only a portion (f, to be referred to henceforth as a geometric factor) of either the non-polar surface area (A_m) of a monomeric group within a molecule or of the total non-polar surface area (A_u) of the solute will be involved in interactions with the RPC support surface.

Solvent displacement stoichiometry

The basic premise of this model[5,6] is that there is a stoichiometric relationship between solute adsorption and the number (Z) of solvent molecules displaced from an RPC column. It has been shown[6] that

$$S_m + nL_d \rightleftarrows S_b + (nr+q)D_0 \tag{4}$$

where S_m is solvated solute in the mobile phase, n is the number of alkyl ligands onto which a solute is adsorbed, L_d is organic displacing agent adsorbed on alkyl ligands, S_b is adsorbed solute, r is the amount of solvent displaced from a single ligand when solute is adsorbed and D_0 is the organic displacing agent in the mobile phase. Letting $(nr+q) = Z$, this displacement process may be expressed in terms of the formation constant (K_1) shown in eqn. 5.

$$K_1 = \frac{[S_b][D_0]^{(nr+q)}}{[S_m][L_d]^n} = \frac{[S_b][D_0]^Z}{[S_m][L_d]^n} \tag{5}$$

Since solute displacement occurs with changes of only a few percent in $[D_0]$, it is assumed that the activity coefficient of the solvent is constant over this range and it is valid to use formation constants to describe these equilibria. This model is simpler than that for macromolecules in that structural transitions and changes in the degree of solvation during elution do not have to be considered with small molecules. The ratio of solute concentration in the stationary phase to that in the mobile phase, $[S_b]/[S_m]$, may be related to chromatographic retention and the phase ratio (φ) of the column through the distribution coefficient (K_D) by the equation

$$[S_b]/[S_m] = K_D = k'/\varphi \tag{6}$$

Substituting k'/φ for $[S_b]/[S_m]$ in eqn. 5 shows that

$$k' = K_1 \varphi [L_d]^n \cdot \frac{1}{[D_0]^Z} \tag{7}$$

Since K_1, φ and n are constants and $[L_d]$ is a constant over the range of $[D_0]$ in which $0.5 < k' < 30$ (ref. 6), these constants may be clustered into a single constant, I, where

$$I = K_1 \varphi [L_d]^n \tag{8}$$

This allows eqns. 7 and 8 to be combined and written in logarithmic form as

$$\log k' = Z \log 1/[D_0] + \log I \tag{9}$$

Solutes that behave according to this stoichiometric displacement model would be expected to give linear plots of $\log k'$ *versus* $\log 1/[D_0]$, with a slope of Z and intercept of $\log I$.

Relationship between Z and solute surface area

The contact surface area (C_m) of any non-polar monomeric unit within a molecule may be expressed as

$$C_m = f_m A_m \tag{10}$$

where f_m and A_m are the geometric factor and non-polar surface area of the monomer, respectively. The total non-polar contact surface area (C_u) of a molecule with multiple hydrophobic residues will be equal to the sum of the contributions of individual non-polar groups, as seen in eqn. 11:

$$C_u = \sum_{n=1}^{i} f_{mi} A_{mi} \tag{11}$$

The term A_{mi} represents the non-polar surface area of a particular ith group which is experiencing partial contact (f_{mi}) with the support surface, and n is the number of ith non-polar groups in the molecule. Unfortunately it is difficult to determine C_u. Although n can be found by examining the structure of the molecule, and A_{mi} may be obtained by calculating the van der Waals surface area[12], the geometric factor (f_{mi}) is not available. In addition, the geometric factor may vary between species and be dependent on the position of a non-polar group in the molecule.

For convenience, this treatment will be limited to solutes of the same shape in which it is assumed that f_m is the same for all monomeric units of the same type and that

$$C_M = n_m f_m A_m = f_m A_M \tag{12}$$

where n_m is the number of a particular monomeric unit, C_M is the total contact surface area of this type of monomeric unit, and A_M is the total non-polar surface area of these identical monomeric units. It will be shown later using a homologous series as an example, that eqn. 12 makes it possible to develop a relationship between Z and the non-polar van der Waals surface area (A_u).

The total non-polar contact surface area (C_u) of a solute is

$$C_u = C_M + C_E + C_F + C_I \tag{13}$$

where C_M is as defined above, C_E is the contact surface area of all terminal methyl groups, C_F is the contact surface area of all functional groups and C_I is the contact surface area of other moieties in the molecule that serve as points of branching. An expansion of eqn. 11 into its contributing variables is seen in eqn. 14:

$$C_u = n_m f_m A_m + n_e f_e A_e + n_i f_i A_i + n_f f_f A_f \tag{14}$$

where A_m, A_e, A_f and A_i are the non-polar van der Waals surface areas of monomers, end-groups, functional groups and branching groups, respectively. The terms n_m, n_e, n_f and n_i represent the number of each species of functional group, while f_m, f_e, f_f and f_i designate the corresponding geometric factors.

The assumption has been made in eqn. 14 that f_m, f_e and f_f will be independent of the position of a group in a molecule. This may not always be true. Strong electronic, steric or solvent effects of neighboring groups may alter the geometric factor (f) of a group such that its contact surface area will not be equivalent to other identical moieties in the molecule. This point will be further examined later.

In the case of a homologous series with a normal alkyl chain, $n_m > n_e$, $C_M > C_E$, and it will be assumed that $f_m A_m \approx f_e A_e$. This allows the methyl group to be treated as a methylene group and eqns. 13 and 14 to be reduced to

$$C_u = C_{M+E} + C_F + C_I \tag{15}$$

and

$$C_u = n_{(m+e)} f_m A_m + n_f f_f A_f + n_i f_i A_i \tag{16}$$

This means that

$$n_{(m+e)} = f_m A_M / f_m A_m = A_M / A_m \tag{17}$$

where $n_{(m+e)}$ is the combined number of end and monomer groups.

Geometric factors will also limit the contact of solvents with both support and solute surfaces such that

$$C_v = f_v A_v + C_\alpha \tag{18}$$

where C_v is non-polar contact surface area of a solvent, A_v is its total non-polar surface area, f_v is the geometric factor for non-polar groups in the solvent and C_α allows for the contribution made by functional groups when present.

The stoichiometric displacement model holds that when a substance is displaced from a surface, nr molecules of solvent solvate the support surface in an area C_{spt}, and q molecules of solvent solvate the solute in an area C_u. The area of solvation (C_{spt}) on the support would be related to C_v by the equation

$$C_{spt} = \frac{nrC_v}{S_{cs}} + C_\beta \tag{19}$$

where C_β is a constant that accounts for any functional group contributions and S_{cs} represents support surface coverage by solvent molecules, where a value of $S_{cs} = 1$ would indicate a monolayer.

Contact surface area of the solute may also be described in terms of the contact surface area of the solvent:

$$C_u = \frac{qC_v}{S_{cu}} + C_\gamma \tag{20}$$

where S_{cu} represents solvent surface coverage on the solute surface and C_γ is a constant accounting for functional-group contributions. Actual surface area (C_u) should be larger than the average geometric area occupied by solvent unless the solute is adsorbed in multiple layers. For convenience, this treatment uses average geometric area. As long as the surface of the bonded phase and solute are of the same chemical nature, S_{cs} will equal S_{cu}. In the case of more polar solutes, it is possible that $S_{cs} > S_{cu}$. Values of one for S_{cs} and S_{cu} indicate a monolayer. In all cases the two terms will be proportional and it is possible to write

$$S_{cs} = S_{cu} C_\delta \tag{21}$$

where C_δ is a proportionality constant.

The total non-polar contact surface area (C_T) of both the support and solute is

$$C_T = C_{spt} + C_u \tag{22}$$

According to eqns. 19 and 20, it may be shown that

$$C_T = \frac{nrC_v}{S_{cs}} + \frac{qC_v}{S_{cu}} + C_\beta + C_\gamma \tag{23}$$

Recalling from eqn. 5 that $Z = nr + q$, and taking the special case where $S_{cu} = S_{cs}$, eqn. 23 reduces to

$$C_T = \frac{ZC_v}{S_{cu}} + C_\beta + C_\gamma \tag{24}$$

Solution of the general case in which $S_{cs} \neq S_{cu}$ is achieved by assuming that for a homologous series the ratio of displacing agent in the contact surface area on the support (nr) to that in the contact surface area on the solute (q) is a contant (C_ε):

$$C_\varepsilon = \frac{nr}{q} \tag{25}$$

This allows eqn. 23 to be converted into the more general equation

$$C_T = \frac{C_v \cdot q}{S_{cu}} \left[\frac{C_\varepsilon}{C_\delta} + 1 \right] + C_\mu \tag{26}$$

where $C_\mu = C_\beta + C_\gamma$.
    It may be shown, by solving eqns. 19 and 20 for C_v and setting them equal, that there should be a constant relationship between C_{spt} and C_u in a homologous series; i.e. $C_{spt} = C_\eta C_u + C_\rho$ where $C_\eta = C_\varepsilon/C_\delta$ and $C_\rho = (C_\gamma C_\varepsilon/C_\delta) + C_\beta$. In this case (substituting in eqn. 22), $C_T = C_u(1 + C_\eta) + C_\rho$. Using these terms and letting $q = Z/(1 + C_\delta)$, it may be shown through substitution in eqn. 26 that

$$Z = \frac{S_{cu}}{C_v} [C_u C_\sigma + C_\omega] \tag{27}$$

where $C_\sigma = 1 + C_\eta$ and $C_\omega = C_\rho - C_\mu$. Since S_{cu}, C_v, C_σ and C_μ are all constants, this equation may be reduced to

$$Z = sC_u + i \tag{28}$$

where $s = \dfrac{S_{cu}}{C_v} \cdot C_\sigma$ and $i = \dfrac{S_{cu}}{C_v} \cdot C_\omega$. This equation shows that, for a non-polar

homologous series, it is expected that Z will be directly proportional to non-polar contact surface area (C_u).
    Z may also be related to the non-polar surface area (A_u) of a substance, as seen in eqn. 29 by combining eqns. 11 and 28:

$$Z = s \sum_{n=1}^{i} f_{mi}A_i + i \tag{29}$$

In a homologous series such as normal aliphatic hydrocarbons in which there are no functional groups, the geometric factor (f_u) for the molecule is approximately equal to that of the individual monomers (f_{mi}) and $\sum_{n=1}^{i} A_1 = A_u$. Eqn. 29 may be reduced to

$$Z = sf_uA_u + i \tag{30}$$

in this special case. Addition of functional groups in the more general case will provide a constant but different contribution than that of non-polar groups. This functional-group contribution is accommodated in the equation

$$Z = sA_u(f_{mi}a_{mi} + F) + i \tag{31}$$

where a_{mi} is the fraction of the total non-polar surface area contributed by a monomer and F is the functional-group contribution. On the basis of the linear relationship between Z and C_u predicted by eqn. 28, for a homologous series, the same is expected of other solutes. Through the use of eqns. 29 and 31, it should be possible to use Z values and the non-polar surface areas (A_{mi}) to determine values for f_{mi}.

Relationship between I and surface area

    Other than being the product of a cluster of constants (see eqn. 8), there is no known relationship between I and the molecular properties of a solute. Since the objective here is to couple contact surface area (C_u) to retention, this treatment will develop a relationship between C_u and I.

    The Martin equation is often used to describe retention properties

$$\log k' = A + Bn_{(m+e)} \tag{32}$$

of the members of a homologous series where A and B are constants and n is the number of monomeric units in the solute[13]. Total non-polar contact surface area (C_u) of a solute in such a series may be represented as

$$C_u = n_{(m+e)}C_m + C_\psi \tag{33}$$

where C_m is the contact surface area of a single monomeric unit such as a methylene (CH$_2$) group and $C_\psi = C_F + C_I$. Substituting for $n_{(m+e)}$ in eqn. 32, chromatographic retention in a homologous series may be related to non-polar contact surface area:

$$\log k' = A + B\left[\frac{C_u}{C_m} - \frac{C_\psi}{C_m}\right] \tag{34}$$

When a homologous series is separated isocratically on an RPC column, the log $1/[D_0]$ term in eqn. 9 is a constant (d), and eqns. 9, 28 and 34 may be combined to produce the expression

$$\log I = C_u\left[\frac{B}{C_m} - sd\right] + A - id - \frac{BC_\varphi}{C_m} \tag{35}$$

Combining constants in eqn. 35 produces the expression

$$\log I = aC_u + C_\lambda \tag{36}$$

where $a = (B/C_m) - sd$ and $C_\lambda = A - id - (BC_\omega/C_m)$. Through eqn. 11, log I of a solute may be related to non-polar surface area by the equation

$$\log I = a \sum_{n=1}^{i} f_{mi}A_i + C_\lambda \tag{37}$$

when $f_{mi} \approx f_u$ and $\sum_{n=1}^{i} A_i = A_u$ as in equation 30, then

$$\log I = af_uA_u + C_\lambda \tag{38}$$

In those cases where functional groups are present, functional-group contributions are accommodated by the equation

$$\log I = aA_u(f_{mi}a_{mi} + F) + C_\lambda \tag{39}$$

as in eqn. 31. The slope (a) in eqns. 36–39 indicates that there should be a constant incremental increase in log I with the addition of each monomeric unit in a homologous series.

Relationship between I and Z

It has been shown in eqns. 28 and 36 that both I and Z may be related to contact surface area (C_u). Solving these equations for C_u and setting them equal shows, after rearranging terms, that

$$\log I = aZ/s - ai/s - C_\lambda = JZ - C_\theta \tag{40}$$

where $J = a/s$ and $C_\theta = ai/s - C_\lambda$. The derivative of this equation, $d(\log I)/dZ = J$, shows that there should be a constant relationship between I and Z for solutes in a homologous series.

Relationship of I to K_D

It has been shown in RPC of proteins[5,6] that species of nearly identical Z values can be resolved in an isocratically eluted system because their intercept terms

(*I*) differ. It will be assumed here that solutes of the same Z value are of identical contact surface area. In this case, the term $([D_0]^Z/[L_d]^n)$ in eqn. 5 will be identical for both solutes, and it is possible to write

$$(K_1)_A \frac{[S_m]_A}{[S_b]_A} = (K_1)_B \frac{[S_m]_B}{[S_b]_B} \tag{41}$$

The symbols A and B identify the first and second members, respectively, of this solute pair to elute from the column. Since $[S_b]/[S_m] = K_D$ and I is directly proportional to K_1 (eqn. 8), eqn. 41 becomes

$$I_A/(K_D)_A = I_B/(K_D)_B \tag{42}$$

This means that when contact surface area has been normalized, the relative difference between the I terms of solutes will be directly related to the differences between their partition coefficients.

Relationship between I and P

    The octanol–water partition coefficient (P) of a molecule is a property used to predict transport across cell membranes. Yalkowsky and Valvani have reported[14] that hydrocarbon surface area (A_{hsa}) of a molecule is proportional to the logarithm of its partition coefficient, where

$$\log P = 0.0275 \, A_{hsa} - 0.863 \tag{43}$$

Since A_{hsa} is proportional to non-polar van der Waals surface area (A_u), this allows eqn. 43 to be rewritten as

$$\log P = mA_u - v \tag{44}$$

Combining eqns. 43 and 44 it may be shown that

$$\log I = (a/m)(f_{mi}a_{mi} + F) \log P + (av/m)(f_{mi}a_{mi} + F) + C_\lambda \tag{45}$$

When functional-group contributions are small and $f_{mi}a_{mi} \gg F$, then $a_{mi} \simeq 1$ and $f_{mi} \simeq f_u$. In this special case eqn. 41 may be reduced to

$$\log I = g \log P + C_\theta \tag{46}$$

by letting $g = a/m$ and $C_\theta = (avf_u/m) + C_\lambda$. Eqn. 46 will apply in those cases where a molecule has no functional groups or where the non-polar portion of the molecule is sufficiently large that it dominates retention. Eqns. 45 and 46 suggest that chromatographic methods will be useful in predicting P of large hydrophobic molecules but will fail in the case of solutes containing small functional groups because f_{mi} and F are unknown in eqn. 45.

MATERIALS AND METHODS

Equipment

All separations were carried out on a chromatographic system equipped with a Beckman-Altex (Berkeley, CA, U.S.A.) Model 110 gradient pumping system, a Rheodyne (Berkeley, CA, U.S.A.) Model 7120 injection valve fitted with a 100-μl loop, a Varian Model UV-50 detector operated at 254 nm and a Fisher Recordall (Austin, TX, U.S.A.) series 5000 recorder.

Supports

SynChropak C-1, C-8 and C-18 supports of 6-μm particle diameter were obtained from SynChrom (Linden, IN, U.S.A.). Columns of 50 × 4.6 mm I.D. were slurry-packed using 2-propanol as solvent.

Mobile phases

HPLC grade water was prepared in the laboratory. 2-Propanol and methanol were obtained in HPLC grade from Mallinckrodt (Paris, KY, U.S.A.) HPLC grade acetonitrile was purchased from Fisher (Fair Lawn, NJ, U.S.A.). Mobile phases were prepared with the pumping system of the instrument by mixing a weak solvent (A) with a strong solvent (B) to achieve the desired mobile phase composition. Weak solvents used in these studies were water, water–methanol (80:20; v/v), water–acetonitrile (90:10; v/v) and water–tetrahydrofuran (90:10; v/v). Strong solvents used in these studies were water–methanol (20:80; v/v), water–acetonitrile (40:60; v/v) and water–acetonitrile (40:60; v/v). When pure water was used as the weak solvent the strong solvent was water–methanol (80:20; v/v), water–acetonitrile (90:10; v/v) or water–tetrahydrofuran (90:10; v/v). Mobile phase flow-rate was 1 ml/min in all cases. Columns were equilibrated with 40 ml of mobile phase before any retention measurements were made.

Solutes

All solutes (Table I) were obtained from Aldrich (Milwaukee, WI, U.S.A.) and used at a concentration of 5.0 mg/ml in pure solvent B. Sample volumes were 5–10 μl.

Column permeation volume

The total permeation volume ($k' = 0$) of columns was measured by injecting several microliters of sodium dichromate and calculating the dead volume according to published methods[3].

RESULTS

Testing the model

The relationship between the capacity factor (k') of selected solutes and the organic solvent concentration $1/[D_0]$ with a water–methanol mobile phase and octylsilane stationary phase is seen in Fig. 1 and Table I. With the exception of benzene and toluene (Fig. 1b), the curves in Fig. 1a are linear. The data presented in the figure for nine compounds are representative of the family of 24 compounds studied. Slope

TABLE I

RETENTION DATA FOR SOME ALKYLBENZENES

Solute No.	Solute	Correlation coefficient	Slope (Z)	Intercept (log I)	Standard deviation
1	Benzene	0.995	1.71	2.09	0.026
2	Bibenzyl	0.999	6.49	8.35	0.024
3	Biphenyl	0.999	4.92	6.28	0.019
4	m-Diisopropylbenzene	0.999	6.90	9.02	0.025
5	p-Diisopropylbenzene	0.998	7.07	9.26	0.031
6	2,2'-Diphenylpropane	0.999	6.72	8.72	0.020
7	3,3'-Dimethylbiphenyl	0.999	6.79	8.81	0.017
8	4,4'-Dimethylbiphenyl	0.999	6.77	8.79	0.025
9	Mesitylene	0.997	4.38	5.89	0.033
10	m-Xylene	0.991	3.59	4.50	0.042
11	n-Pentylbenzene	0.999	6.74	8.81	0.024
12	Naphthalene	0.997	3.83	4.83	0.029
13	n-Propylbenzene	0.996	4.44	5.73	0.036
14	Pentamethylbenzene	0.996	4.93	6.60	0.033
15	4-Phenyltoluene	0.994	5.37	7.02	0.055
16	p-Xylene	0.996	3.36	4.32	0.035
17	sec-Butylbenzene	0.997	5.08	6.64	0.032
18	1,2,3,4-Tetramethylbenzene	0.997	4.52	5.95	0.036
19	1,2,3,5-Tetramethylbenzene	0.997	4.57	6.04	0.029
20	1,2,4,5-Tetramethylbenzene	0.995	4.84	6.34	0.036
21	Toluene	0.997	2.65	3.31	0.020
22	1,2,3-Trimethylbenzene	0.997	3.92	5.09	0.025
23	1,2,4-Trimethylbenzene	0.999	4.43	5.72	0.018
24	o-Xylene	0.900	3.17	4.09	0.057

(Z) and intercept (I) data for the 24 compounds are presented in Table I. To facilitate discussion of the proposed model, linear and non-linear portions (when applicable) of these curves will be treated separately. The data presented in Table I are derived from the linear portion of these curves. Correlation coefficients for the linear segments of these curves were greater than 0.99 in all cases; indicating that the stoichiometric retention model (eqn. 9) is consistent with the data.

Observations of non-linearity at elution extremes, *i.e.* as k' approaches either zero or large values, have also been made by other workers[1,2] without explanation as to the origin of the phenomenon. A question is whether the stoichiometric retention model is capable of predicting or explaining these deviations. An important condition of the model is that linearity will only be achieved when activity coefficients and the concentration of adsorbed displacing agent $[L_d]$ are constant while $[D_0]$ is varied to effect elution. Variations in activity coefficients or $[L_d]$ would cause both Z and I to very and produce non-linear plots of log k' *vs.* log $1/[D_0]$. McCormick and Karger[15] and Slaats *et al.*[16] have examined the relationship between $[L_d]$ and $[D_0]$ in RPC and found $[L_d]$ to be relatively constant in the 30–80% (v/v) range for methanol–water and in the 50–70% (v/v) range for acetonitrile–water. As the concentration of the displacing agent approached the extremes (0–30 and 80–100%) with methanol–water, $[L_d]$ decreased. This means that the stationary phase in the column
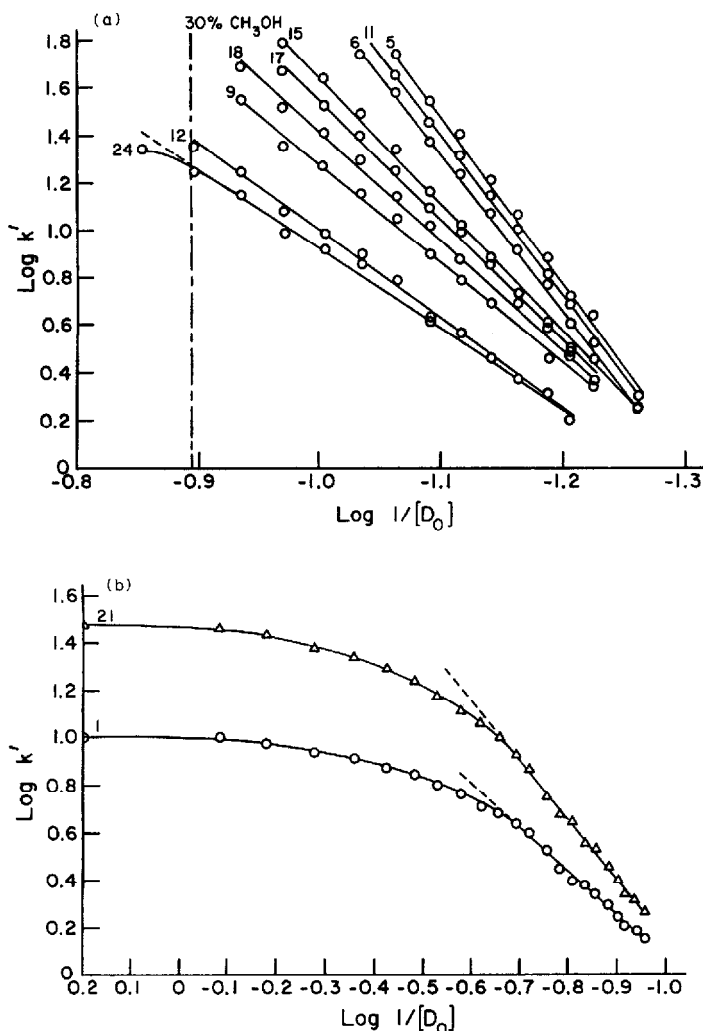
Fig. 1. Plots of log k' vs. log $1/[D_0]$ for a non-homologous series of alkylbenzenes. Separations were achieved on a 5.0 × 0.41 cm I.D. SynChropak C_8 column eluted with a methanol–water mobile phase at ambient temperature. Solutes in the figure are numbered and identified in Table I. (a) Data were obtained at methanol concentrations of greater than 30% (v/v); (b) data were derived at less than 30% methanol concentration (v/v).

is changing at the extremes of solvent concentration. In view of the contribution of the stationary phase to retention through $[L_d]$ in the stoichiometric displacement model, the data of McCormick and Karger[15] and Slaats et al.[16] would lead to the conclusion that non-linearity is to be expected with methanol and acetonitrile at the extremes of organic solvent concentration $[D_0]$, and that the observed deviations are consistent with variations in $[L_d]$. Support for this concept may also be derived from experimental data in the literature[16,17]. Slaats et al.[16] reported non-linearity in plots of ln k' vs. $[D_0]$ that can be explained in terms of variations in $[L_d]$. In addition, when
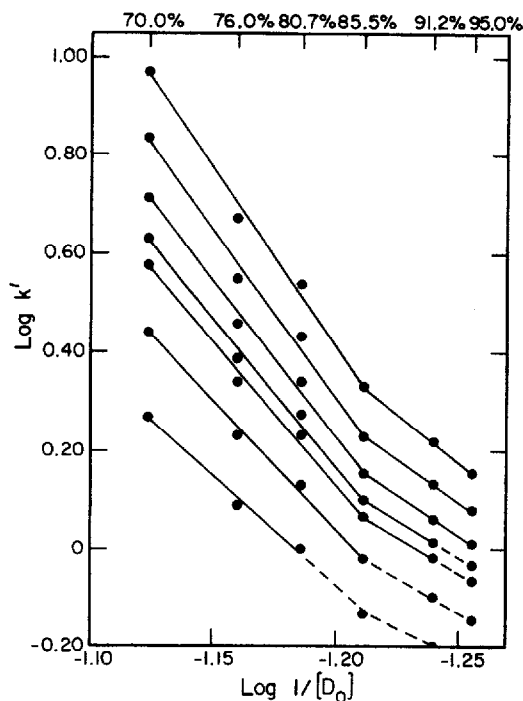
Fig. 2. Plots of log k' $vs.$ log $1/[D_0]$ for some aromatic hydrocarbons. Data for this figure were derived from ref. 17. Separations were achieved on a C_{18} column eluted with acetonitrile–water at ambient temperature. Identification of solutes in the figure is as follows: 1 = 1,4-diisopropylbenzene; 2 = sec-pentylbenzene; 3 = 1,3-diethylbenzene; 4 = 1,2,3,4-tetramethylbenzene; 5 = 1,3,5-trimethylbenzene; 6 = n-propylbenzene; 7 = ethylbenzene.
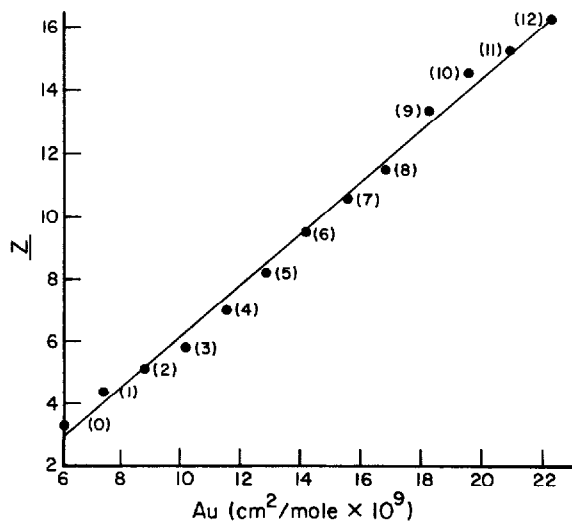

Fig. 3. A plot of Z $vs.$ non-polar van der Waals surface area (A_u) for n-alkylbenzenes. Retention data for this plot were obtained from ref. 18. Separations were achieved on a C_{18} column eluted with a methanol–water mobile phase at ambient temperature. Solutes ranged from benzene to n-dodecylbenzene. The symbols 0 to 12 designate the combined number of methyl and methylene groups in the alkylbenzene.

the data of Jinno and Ishigaki[17] describing retention of non-polar solutes on a re-versed-phase column eluted with acetonitrile–water are recalculated and plotted as log k' vs. log $1/[D_0]$ (Fig. 2), non-linear portions of the curve are seen to be beyond the 80% (v/v) concentration range[16]. The stoichiometric-displacement model and the experimental data of McCormick and Karger[15], Slaats et al.[16] and Jinno and Ishi-gaki[17] strongly suggest that at least part of the non-linearity observed when retention data are plotted according to eqns. 1, 2 and 9 are the result of variations in $[L_d]$ at both extremes of $[D_0]$. Although changes in the activity coefficient of the mobile phase have not been eliminated as a causitive factor of non-linearity, correlation of non-linearity with changes in $[L_d]$ diminish the possible contribution of activity coef-ficient.

Relationship of Z to non-polar surface area

The theoretical basis for a relationship between solvent displacement stoi-chiometry (Z) and non-polar van der Waals surface area (A_u) in a homologous series has been established in eqns. 30 and 31. This relationship was examined with a hom-ologous series of alkylbenzenes by plotting their Z values *versus* the van der Waals surface area (A_u), as shown in Fig. 3*; retention data were taken from the paper by Colin et al.[18] and surface areas calculated according to Bondi[12]. As predicted in eqn. 30, there is a linear relationship between Z and A_u. When retention data from a non-homologous series of hydrocarbons were examined in the same manner (Fig. 4), correlation coefficients and standard deviations indicated a poorer fit of the experi-mental data to eqns. 30 and 31. This is as expected, because the geometric factors f_u and f_{mi} that relate A_{mi} to contact surface area of the various groups in a molecule are not necessarily the same as they are in a homologous series, even though the same monomeric unit is being added to the molecule.
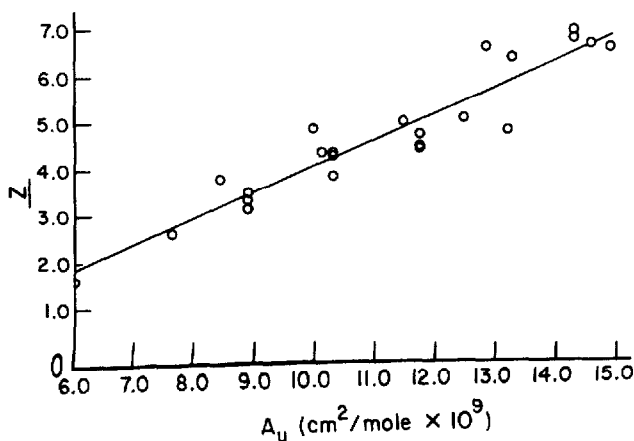

Fig. 4. A plot of Z vs. non-polar van der Waals surface area (A_u) for the non-homologous series of alkylbenzenes in Table I. Experimental conditions are given in Fig. 1.

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* It should be noted that the Z value for benzene in this figure is different than in Table I. It has been noted previously[1] that the S value in eqn. 1 may also vary between different columns. The reason for this is unknown.
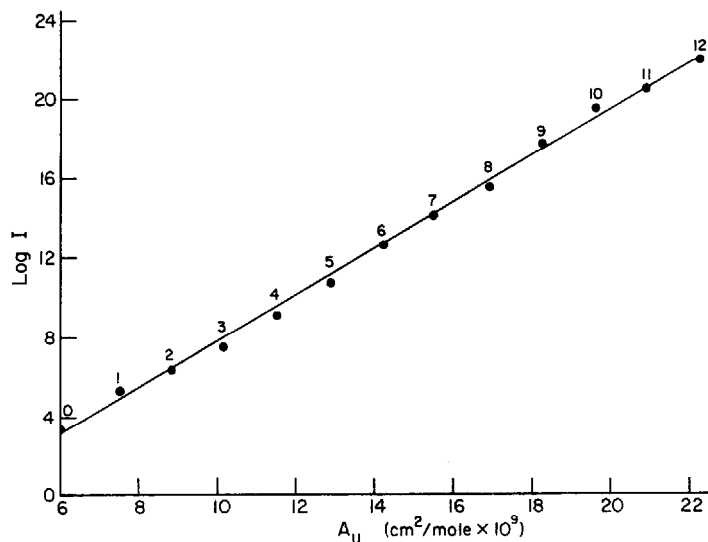
Fig. 5. A plot of log I vs. non-polar van der Waals surface area (A_u) for the n-alkylbenzenes. Retention data were derived from ref. 18. Experimental conditions are as given in Fig. 3.

Relationship between I and non-polar surface area

Fig. 5 is a plot of log I vs. non-polar van der Waals surface area (A_u) of a homologous series of alkylbenzenes. As expected from eqn. 38, this plot is linear and there is general agreement between the model and experimental data. Although not a homologous series, data from the hydrocarbons used in Fig. 4 also exhibit linear behavior as shown in Fig. 6. Again the geometric factors (f_{mi}) of the various groups are sufficiently different that there is some scatter in the plot.
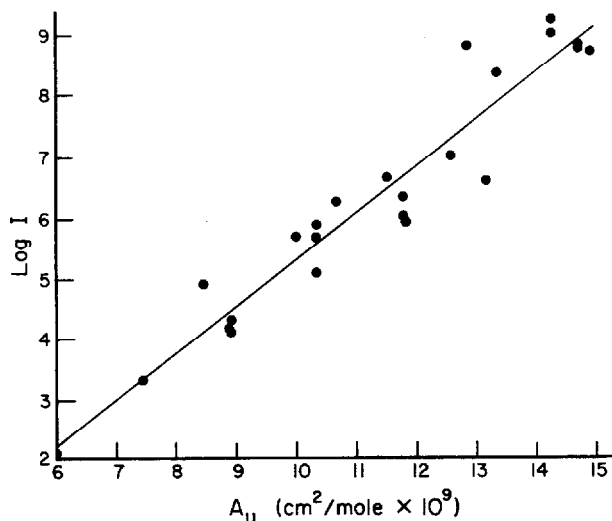

Fig. 6. A plot of log I vs. non-polar van der Waals surface area (A_u) for the non-homologous series of alkylbenzenes in Table I. Experimental conditions are given in Fig. 1.

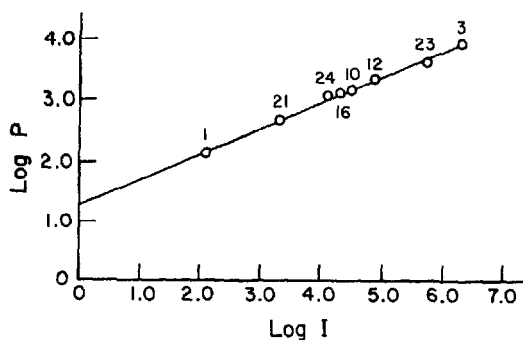Fig. 7. A plot of log P vs. log I for some aromatic hydrocarbons. Octanol–water partition coefficient (P) values were taken from ref. 14. Chromatographic conditions are given in Fig. 1. Solutes are numbered according to Table I.

Relationship between I and P

The Yalkowsky and Valvani treatment[14] of the relationship between hydrocarbon surface area and the partition coefficient (P) of a substance was obtained from the hydrophobic fragment constants of Nauta and Rekker[19]. Because of the ease of calculation, van der Waals surface areas of non-polar portions of solutes were substituted for Rekker constants in these studies. Justification for this was obtained from a plot (data not shown) of octanol–water partition coefficients versus A_u; linearity was equivalent to that obtained by Yalkowsky and Valvani[14]. Plots of log I versus log P shown in Fig. 7 are linear, as suggested by eqn. 46. Numerical values of 0.445 and 1.2223 were calculated for the slope (g) and intercept (C_0) respectively. The correlation between log I of this hydrocarbon series on the octysilane column and log P for an octanol–water system is sufficiently good that treating an octylsilane
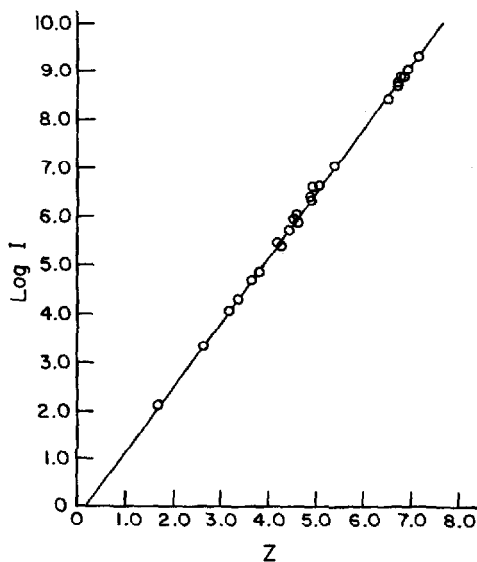

Fig. 8. A plot of log I vs. Z for the non-homologous series of alkylbenzenes given in Table I. Experimental conditions are given in Fig. 1.
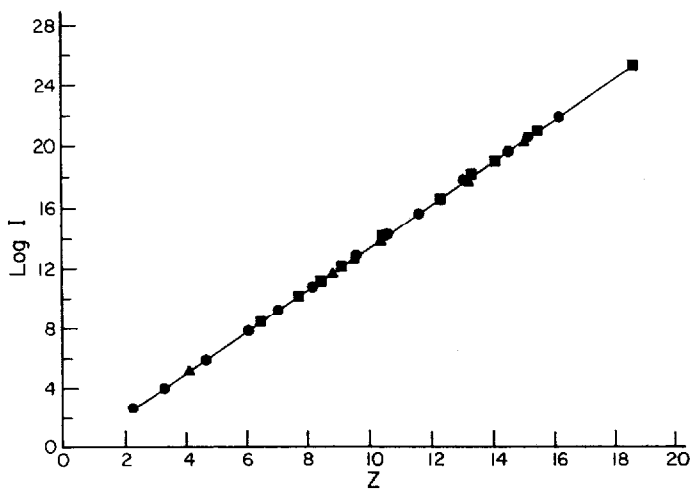
Fig. 9. Plots of log I vs. Z for three homologous series. The retention data for this figure were obtained from ref. 18. The chromatographic conditions are given in Fig. 3. Identification symbols: ● = n-alkylbenzenes; ▲ = n-methyl esters; ■ = n-alkanes.

column as a liquid–liquid partition system with non-polar solutes would seem to be valid.

Relationship between I and Z

Since both Z and I have been related to non-polar contact surface area (C_u) in eqns. 28 and 36, respectively, it is to be expected that plots of log I vs. Z will be linear with a slope of J, as indicated in eqn. 40. This has been confirmed experimentally, with a non-homologous series of hydrocarbons (Fig. 8) and three homologous series (Fig. 9). Since C_u is used here instead of A_u, and geometric factors (f) do not appear in any of these equations, it is to be expected that a non-homologous series will show linearity equivalent to that of a homologous series, and this is indeed observed in the figures. These plots confirm again that as the number (Z) of solvent molecules involved in displacement increases, the affinity (I) of a solute for the bonded phase increases logarithmically.

The slope J of Fig. 8 for the 24 non-polar compounds from Table I was 1.328 in comparison to 1.384 for the n-alkylbenzenes in Fig. 9. These values are relatively close to J values of 1.418 for n-alkanes and 1.400 for n-methyl esters that may be calculated from the data of other workers[18] with different columns (figures not shown). In all these cases, the monomeric unit was a methylene group (CH_2), and it is seen that the contribution of a monomer to retention is only slightly influenced by the rest of the molecule and the column.

CONCLUSIONS

This paper is the first to examine a model predicting that displacement of small non-polar solutes from an RPC support involves the participation of a stoichiometric number (Z) of solvent molecules. This stoichiometric displacement model predicts

that there is a specific contact surface area of both solvents and solutes with the organosilane bonded phase and that this contact surface area is a fraction of the total non-polar surface area of the molecule. An increase in the non-polar surface area of a solute is accompanied by increasing retention and the number (Z) of solvent molecules required for displacement. In contrast, increasing the surface area of the solvent decreases Z for a particular solute.

The model also predicts that the fraction of the non-polar surface area of a solute that contacts the surface of a support is a function of a series of group specific steric factors within a molecule that limit the contact of individual groups. Total contact surface area of a molecule would be the sum of the contributions of individual groups in a molecule. In all cases experimental data were found to be consistent with this model. Non-linearity in plots of log k' *versus* log $1/[D_0]$ at extremes of $[D_0]$ were concluded to be the result of variations in the concentration of solvent adsorbed on either the support or the solute.

GLOSSARY OF SYMBOLS

$a = (B/C_m) - sd = d(\log I)/dC_u$ from eqn. 36.

a_{mi} is the fraction of the total non-polar surface area contributed by a monomer.

A is a constant in eqn. 32 (see ref. 13 for a more detailed discussion).

A_e represents the non-polar surface area of an end group.

A_f represents the non-polar surface area of a functional group.

A_g is a constant in eqn. 2 (see ref. 2 for a more extensive discussion).

A_{hsa} designates hydrocarbon surface area of a solute according to Yalkowsky and Valvani[14].

A_i represents the non-polar surface area of a branching group.

A_m represents the non-polar van der Waals surface area of a monomeric unit in a solute.

A_M represents the non-polar van der Waals surface area of all monomeric units of a particular type in a solute.

A_{mi} represents the non-polar van der Waals surface area of some ith group in a molecule.

A_u represents the total non-polar van der Waals surface area of a molecule.

A_v represents the total non-polar van der Waals surface area of a solvent molecule.

B is a constant in eqn. 32 (see ref. 13 for a more detailed discussion).

B_g is a constant in eqn. 2 (see ref. 2 for a more extensive discussion).

C_E represents the non-polar contact surface area of all end groups in a molecule.

C_F represents the non-polar contact surface area of all functional groups in a molecule.

C_g is a constant in eqn. 2 (see ref. 2 for a more extensive discussion).

C_l represents the total non-polar contact surface area of branching groups in a molecule.

C_m represents the non-polar contact surface area of a monomer unit in a molecule.

C_M represents the combined non-polar contact surface area of all monomeric units of the same type in a molecule.

C_{M+E} represents the total non-polar constant surface area of all end groups and monomeric units in a molecule.

$C_T = C_{spt} + C_u$, which is the total non-polar surface area that is solvated when a solute desorbs from a column.

C_{spt} represents the non-polar contact surface area on the support surface determined by contact with a solute molecule.

C_u represents the non-polar contact surface of a solute molecule.

C_v represents the non-polar contact surface area of a solvent molecule.

C_α is a constant in eqn. 18 that accounts for solvent functional-group contribution to solvent contact surface area (C_v).

C_β is a constant in eqn. 19 that accounts for functional-group contributions in the solvent to C_{spt}.

C_γ is a constant in eqn. 20 that accounts for functional-group contributions from the solvent to C_u.

$C_\delta = S_{cs}/S_{cu}$

$C_\varepsilon = nr/q$

$C_\mu = C_\beta + C_\gamma$

$C_\eta = C_\varepsilon/C_\delta$

$C_\rho = (C_\gamma C_\varepsilon/C_\delta) + C_\beta$

$C_\sigma = C_\eta + 1$

$C_\omega = C_\rho - C_\mu$

$C_\psi = C_F + C_1$

$C_\lambda = A - id - (BC_\psi/C_m)$

$C_\theta = (avf_u/m) + C_\lambda$

$d = \log 1/[D_0]$ when $[D_0]$ is a constant.

D_g is a constant in eqn. 2 (see ref. 2 for a more extensive discussion).

$[D_0]$ represents desorbing agent concentration in moles/liter.

f is a geometric factor representing the fraction of the total non-polar surface area of a molecular or group that contacts the surface of a reversed-phase support.

F is fractional contribution of functional groups to A_u.

f_e is the geometric factor for an end-group substituent in a molecule.

f_f is the geometric factor for a particular functional group.

f_i is the geometric factor for a branching group in a molecule.

f_m is the geometric factor for a monomeric unit in a molecule.

f_{mi} is the geometric factor for an ith monomeric unit.

f_u is the geometric factor for a molecule.

f_v is the geometric factor for a particular solvent.

$g = a/m$

$i = S_{cu}C_\omega/C_v$

I is a constant which is equal to $K_1\varphi[L_d]^n$.

J is a constant that shows the relationship between incremental changes in $\log I$ and Z. $J = d(\log I)/dZ$.

k' is the capacity factor of a solute, where $k' = K_d\varphi$.

k_w is the extrapolated value of the capacity factor of a solute in water (ref. 1).

K_D designates the distribution coefficient of a solute, where $K_D = [S_b]/[S_m]$.

K_1 is a formation constant in eqn. 5.

$(K_1)_A$ represents the formation constant for some solute A.

$(K_1)_B$ represents the formation constant for some solute B.

L_d designates desorbing agent adsorbed on the alkylsilane bonded phase.

m is a constant in eqn. 44.

n is the number of alkyl residues onto which a solute is absorbed.

n_e is the number of end groups in a molecule.

n_f is the number of functional groups in a molecule.

n_i is the number of branching points in a molecule.

n_m is the number of monomeric units of the same type in a molecule.

$n_{(m+e)}$ is the combined number of end groups and monomeric units in a molecule.

n_{mi} is the number of ith monomeric units in a molecule.

P designates the octanol–water partition coefficient of a solute.

q is the number of solvent molecules displaced from the contact surface area of the solute when it adsorbs to a reversed-phase support.

r is the number of solvent molecules displaced from a single alkylsilane when solute absorbs.

$s = S_{cu}C/C_v$

S is the slope of eqn. 1 (see ref. 1 for a more extensive discussion).

$[S_b]$ designates solute concentration on the stationary phase in moles/m^2.

$[S_b]_A$ represents the concentration of solute A in the stationary phase.

$[S_b]_B$ represents the concentration of solute B in the stationary phase.

S_{cs} is a constant representing support surface coverage by solvent. Values of 1, 2 and 4 for S_{cs} represent 100, 50 and 25% surface coverage, respectively.

S_{cu} is a constant representing coverage of the contact surface area of a solute molecule by solvent. Values of 1, 2 and 4 for S_{cu} represent 100, 50 and 25% coverage respectively.

$[S_m]$ designates solute concentration in the mobile phase in moles/liter.

$[S_m]_A$ represents the concentration of solute A in the mobile phase.

$[S_m]_B$ represents the concentration of solute B in the mobile phase.

S_s is the slope of the plot of log k' vs. v_f according to eqn. 1.

v is a constant in eqn. 44.

V_f is the volume fraction of organic solvent in the mobile phase.

Z is the number of solvent molecules required to displace a protein from the reversed-phase surface.

φ represents the phase ratio of the support which in the case of RPC is the volume of the stationary phase divided by the volume of the mobile phase.

Φ_b designates the volume fraction of organic solvent B in the mobile phase.

REFERENCES

1 L. R. Snyder, J. W. Dolan and J. R. Gant, *J. Chromatogr.*, 165 (1979) 3.
2 P. J. Schoenmakers, H. A. H. Billiet, R. Tijssen and L. de Galan, *J. Chromatogr.*, 149 (1978) 519.
3 Cs. Horváth, W. Melander and I. Molnár, *J. Chromatogr.*, 125 (1976) 129.
4 P. Jandera, H. Colin and G. Guiochon, *Anal. Chem.*, 54 (1982) 435.
5 X. Geng and F. E. Regnier, *Proc. Am. Soc. Pept. Chem.*, (1984) 727.
6 X. Geng and F. E. Regnier, *J. Chromatogr.*, 296 (1984) 15.

7 B. Grego, F. Lambrou and M. T. W. Hearn, *J. Chromatogr.*, 266 (1983) 89.
8 M. T. W. Hearn and B. Grego, *J. Chromatogr.*, 266 (1983) 75.
9 J. P. Larmann, J. J. DeStefano, A. P. Goldberg, R. W. Stout, L. R. Snyder and M. A. Stadalius, *J. Chromatogr.*, 255 (1983) 163.
10 L. R. Snyder, M. A. Stadalius and M. A. Quarry, *Anal. Chem.*, 55 (1983) 1413A.
11 A. W. Adamson, *Physical Chemistry of Surfaces,* 4th ed., Wiley, New York, 1982.
12 A. Bondi, *J. Phys. Chem.*, 68 (1964) 441.
13 L. R. Snyder and J. J. Kirkland, *Introduction to Modern Liquid Chromatography*, 2nd ed., Wiley, New York, 1979, p. 580.
14 S. H. Yalkowsky and S. C. Valvani, *J. Med. Chem.*, 19 (1976) 727.
15 R. M. McCormick and B. L. Karger, *Anal. Chem.*, 52 (1980) 2249.
16 E. H. Slaats, W. Markovski, J. Fekete and H. Poppe, *J. Chromatogr.*, 207 (1981) 299.
17 K. Jinno and A. Ishigaki, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 5 (1982) 668.
18 H. Colin, A. M. Krstulovic, M. F. Gonnard, G. Guiochon, L. Yun and P. Jandera, *Chromatographia*, 17 (1983) 9.
19 W. Th. Nauta and R. F. Rekker, *The Hydrophobic Fragmental Constant*, Elsevier, Amsterdam, 1977.