SOLVOPHOBIC INTERACTIONS IN LIQUID CHROMATOGRAPHY WITH NONPOLAR STATIONARY PHASES[*]

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SUMMARY

Solute interaction with nonpolar stationary phases in liquid chromatography is examined on the basis of the solvophobic theory. The chromatographic process is viewed as a reversible association of the solute with the hydrocarbonaceous ligands of bonded phases. A detailed analysis of the effect of the solvent on this process yields an expression for the capacity factor with essentially no adjustable constants. The theory satisfactorily accounts for the factors affecting solute retention under a wide range of experimental conditions. It makes possible the characterization of the solvophobic (eluent) strength of mixed solvents having different composition and the evaluation of the various solvophobic forces representing incremental values of the logarithm of the capacity factor. The wide applicability of nonpolar stationary phases (reversed phases) in liquid chromatography is demonstrated by the rapid separation of biogenic acids and bases on octadecylsilica columns with neat aqueous elements.

INTRODUCTION

The beginning of liquid chromatography (LC) with unpolar stationary phases is traced back to a suggestion made by Boscott[1] in 1947, but it was Boldingh[2] who first separated long-chain fatty acids on a column of rubber powder by using aqueous methanol and acetone in 1948. Subsequently, Howard and Martin[3] described the use of liquid paraffin and *n*-octane as stationary phases in liquid–liquid chromatography of fatty acids. They christened the technique "reversed-phase" chromatography in order to distinguish it from conventional partition chromatography. It has been generally assumed[4] that reversed-phase chromatography is restricted to the separation of unpolar substances such as lipids and hydrocarbons, although as early as in 1951 Martin and Porter employed a "reversed-phase" system for the fractionation of ribonuclease by column chromatography[5]. Thereafter, stationary phases less polar than the eluent have been widely employed by lipid chemists despite the relative instability of the columns and other technical difficulties.

In the middle of the sixties it was recognized that superior stationary phases

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can be obtained by covalently binding a suitable organic moiety to the surface of a rigid porous support. Although the first unpolar "bonded" phases were used in gas chromatography[6], Stewart and Perry[7] soon made the suggestion that the treatment of a siliceous support with octadecylchlorosilane would yield a useful stationary phase for LC. At that time these authors did not recognize yet the wide applicability of bonded phases in LC. Nonetheless, they envisioned "that a family of anchored phases offers the best (if not the only) present possibility for liquid–liquid chromatography of lipophilic mixtures without the necessity for precision thermostating and careful pre-equilibration of conventional "immiscible" phase pair". Since then, octadecyl-silica has become a widely used stationary phase mainly due to the work of Kirkland[8] and Majors[9], who have popularized pellicular and microparticulate bonded phases, respectively.

Today, "reversed-phase" chromatography is one of the most widely used techniques for the separation of a large variety of mixtures by high-performance LC. It is estimated that 60–80% of the analytical separations are carried out by using this technique. In most cases octadecylsilica, whose surface is schematically illustrated in Fig. 1, is the stationary phase and mixtures of methanol or acetonitrile with water are used as eluents. The chromatographic system is simple and the components of the mixed solvents are readily available in sufficient purity. The reproducibility of the method is very good and the columns are stable as long as the pH of the eluent is below seven. Hydrocarbonaceous ligands other than the octadecyl moiety can also be used in order to optimize the properties of the stationary phase for specific sepa-ration problems.

This work commenced with the observation that neat aqueous eluents, which do not contain organic solvents, could also be used for the separation of small, rel-atively polar biological molecules on octadecylsilica[10]. In the absence of an organic component from the eluent, however, the interaction between the solute and the hydro-carbonaceous moiety of the stationary phase has to be the sole cause of solute reten-
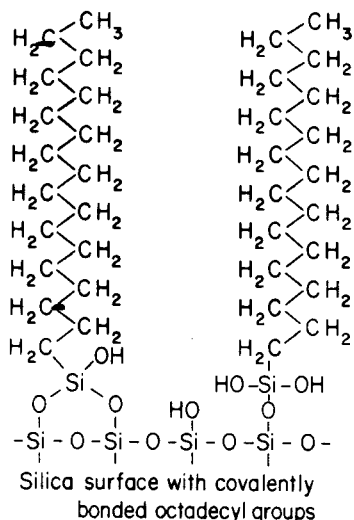

Silica surface with covalently bonded octadecyl groups

Fig. 1. Silica surface covered with a molecular fur of covalently bonded octadecyl groups.
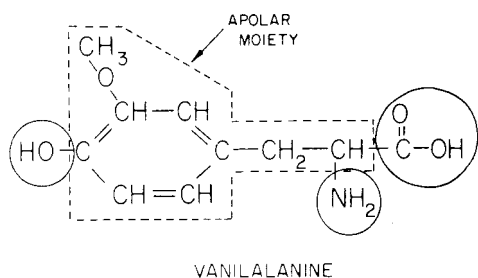
VANILALANINE

Fig. 2. Illustration of the polar groups (circles) and the nonpolar hydrocarbonaceous moiety in a typical solute.

tion. In other words, the chromatographic process is governed by the so-called hydrophobic effect[11]. It is recalled that the recently introduced hydrophobic affinity chromatography[12,13] has been successfully employed for the separation of biopolymers on the basis of hydrophobic interactions.

Fig. 2 shows the structure of vanilalanine, which exemplifies the substances employed in this study. It is seen that besides polar groups this compound possesses a substantial hydrocarbonaceous moiety that can enter into hydrophobic interactions with the octadecyl chains of the stationary phase. The phenomenon responsible for solute retention can be pictured as a reversible association process between the hydrocarbonaceous ligand anchored to the surface and the solute molecule, as shown in Fig. 3.

Other non-covalent interactions such as ionic and hydrogen bonding, which have been widely exploited in aqueous LC, are caused by a more or less strong attraction between the solute and the stationary phase. In contrast, hydrophobic interactions originate from a net repulsion between the water and the unpolar ligand as
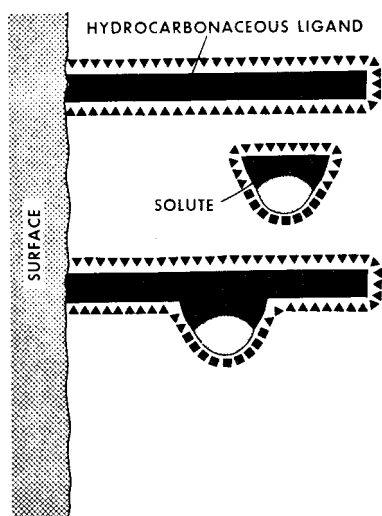

Fig. 3. Association of the solute with the hydrocarbonaceous function on the surface of the stationary phase.

well as the unpolar moiety of the solute. The tendency of water to reduce the nonpolar surface area of the molecules in contact with the solvent is then mainly responsible for their association. Fig. 4 schematically illustrates the interactions between a solute having ionic, hydrogen bonding, and hydrophobic functions and three types of corresponding stationary phases. The two top cases show complex formation with an ionic and hydrogen bonding stationary phase and suggest that the driving force for both coulombic and hydrogen bonding interactions is the attraction between the solute and the anchored ligand. In contradistinction, the driving force for the association with the hydrocarbonaceous ligand is the concommitant decrease in the nonpolar surface area exposed to the solvent. Somewhat vaguely we may say that in the case of hydrophobic bonding it is mainly the solvent that forces the molecules to associate rather than the attraction between them.
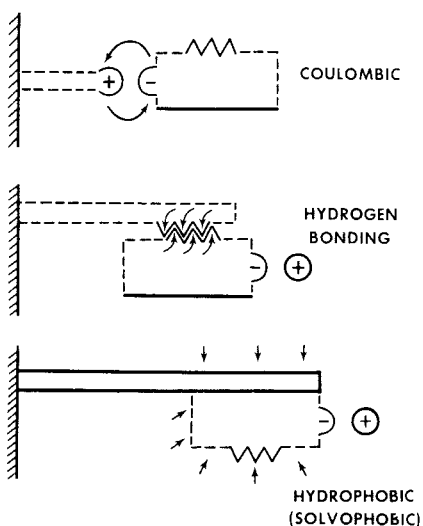

Fig. 4. Schematic illustration of the possible interactions between the solute and stationary phase in chromatography with aqueous and other polar eluents.

The hydrophobic effect plays a very important role in life sciences[14,15] and the subject has extensively been treated theoretically[16,17]. The statistical approach that relates this phenomenon to hypothetical water structures, however, is less suitable to interpret the chromatographic process. On the other hand, a quite general theory has been developed by Sinanoğlu[18] and Sinanoğlu and Abdulnur[19] to describe the effect of the solvent on chemical events. Their solvophobic theory requires essentially no adjustable constants and employs a relatively simple approach to treat the interaction between molecules having nonpolar moieties in polar solvents. In view of this exact theory solvophobic effects are not restricted to neat aqueous media. However, the very high cohesive density of water is responsible for the hydrophobic effect, which is the most pronounced solvophobic effect.

In an attempt to provide a theoretical framework that accounts for the governing factors in LC with nonpolar stationary phases, we adapted the solvophobic theory.

Treating solute retention as an association process, *vide supra*, we have been able to describe liquid–solid chromatography with bonded phases in a fundamental fashion and establish a basis for the use of this technique for physico-chemical measurements.

THEORY

In our present model the interaction between the solute and the stationary phase in solvophobic chromatography is considered as a reversible association of the solute molecules, S, with the hydrocarbonaceous ligands, L, at the surface. Accordingly, solute retention is governed by the equilibrium
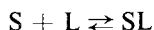
$$S + L \rightleftharpoons SL$$

where the complex SL is assumed to be formed by solvophobic interactions and the process is characterized by the equilibrium constant, K, which is defined by

$$K = \frac{[SL]}{[S][L]} \tag{1}$$

In order to evaluate K we employ the solvophobic theory put forward by Sinanoğlu *et al.* in a series of papers[18-25]. Unlike most other theories which deal with the hydrophobic effect, it is cast in terms of macroscopic solute and solvent properties and will be briefly reviewed here. Our treatment is restricted to unionized solutes, *i.e.*, ionic interactions are neglected.

Molecular associations in solution can be conceptually broken down into two processes. One is the interaction of the molecules, S and L, to yield SL in a hypothetical gas phase without any intervention by the solvent. The other more involved process entails the interactions of the associating species and the complex individually with the solvent proper. In our case the association in the gas phase is assumed to occur by van der Waals forces only and the free energy change of the process is denoted by $\Delta F_{vdw, assoc}$. The standard unitary free energy change for placing the species into the solution is expressed by the difference between the free energy change required for the creation of a cavity to accommodate the species j in the solvent, $\Delta F_{c,j}$, and that arising from the interaction between the species and the surrounding solvent molecules, $\Delta F_{i,j}$. Thus, the total standard unitary free energy change of the second process for each species is given by

$$\Delta F_j^0 = \Delta F_{c,j} + \Delta F_{i,j} + RT \ln (RT/P_0 V) \tag{2}$$

where the last term, which contains the mole volume of the solvent, V, and the atmospheric pressure, P_0, accounts for the entropy change arising from the change in "free volume". The overall standard unitary free energy change for the association in solution, ΔF_{assoc}^0, is given then by

$$\Delta F_{assoc}^0 = \Delta F_{vdw,assoc} + (\Delta F_{c,SL} + \Delta F_{i,SL}) - (\Delta F_{c,S} + \Delta F_{i,S}) - (\Delta F_{c,L} + \Delta F_{i,L}) - RT \ln (RT/P_0 V) \tag{3}$$

where the subscripts S,L and SL refer to the three species involved.

Sinanoğlu[18] expressed the free energy change of the cavity formation for species j as

$$\Delta F_{c,j} = \kappa_j^e A_j \gamma (1 - W) N \tag{4}$$

where A_j is the molecular surface area of species j, γ is the surface tension, N is Avogadro's number, and

$$W \equiv \left(1 - \frac{\kappa_j^s}{\kappa_j^e}\right)\left(\frac{d \ln \gamma}{d \ln T} + \frac{2}{3}\mathscr{A}_j T\right) \tag{5}$$

$\mathscr{A}_j$ is the liquid coefficient of thermal expansion for the species. κ_j^e expresses the ratio between the energy required for the formation of a suitably shaped cavity with a surface area A_j in the solvent and the energy required to expand the planar surface of the solvent by the same area, which is approximately given by $A_j \gamma$. κ_j^s is the corresponding function for the entropy production associated with making the cavity. Halicioğlu and Sinanoğlu have calculated and tabulated the κ values for a number of pure solvents[21]. The κ_j^e values for species j can be estimated by

$$\kappa_j^e = 1 + (\kappa^e - 1)(V/V_j)^{2/3} \tag{6}$$

where κ^e is evaluated for the neat solvent, and V and V_j are the mole volumes of the solvent and the species j, respectively. A similar relationship has been proposed for κ_j^s also. Both κ_j^e and κ_j^s approach unity as the size of solute molecules with respect to the size of solvent molecules increases.

The second term in eqn. 2, which expresses the interaction of species j with the solvent, has been assumed to be the sum of a van der Waals component, $\Delta F_{vdw,j}$, and an electrostatic free energy term, $\Delta F_{e.s.,j}$, so that

$$\Delta F_{i,j} = \Delta F_{vdw,j} + \Delta F_{e.s.,j} \tag{7}$$

According to Sinanoğlu the van der Waals term is given by

$$\Delta F_{vdw,j} = - f(\xi,l)\Delta_j D_j DB_j \tag{8}$$

where

$$\Delta_j = 1.35\, I_j I/(I_j + I) \tag{9}$$

with the ionization potentials I and I_j for the solvent and species j, respectively, and D_j is the Clausius–Mosotti function of the species given by

$$D_j = \frac{n_j^2 - 1}{n_j^2 + 2} \tag{10}$$

where n_j is the refractive index of the species j. D for the solvent is expressed in a similar way.

The function $-f(\xi,l)B_j$ has the approximate analytical form

$$-f(\xi,l)B_j \approx \frac{0.564 \times 27}{8\pi} (Q' + Q'') \tag{11}$$

Q' and Q'' are dimensionless functions that can be obtained by integrating the effective pair potential between the solvent and solute molecules over the total volume. Because $Q'' < 0.1Q'$, Q'' will be neglected and we evaluate Q' from the following relationship

$$Q' = v_j \left[\frac{\bar{\sigma}^6}{(\bar{R}-l)^9} \left(\frac{t^2}{11} + \frac{t}{5} + \frac{1}{9} \right) - \frac{1}{(\bar{R}-l)^3} \left(\frac{t^2}{5} + \frac{t}{2} + \frac{1}{3} \right) \right] \tag{12}$$

where

$$t = l/(\bar{R} - l) \tag{13}$$

$\bar{R}$ is the arithmetic mean diameter of the two molecules, l is the mean Kihara parameter, and $\bar{\sigma}$ is the mean London parameter given by

$$\bar{R} = \frac{1}{2}(R_j + R) \tag{14}$$

$$l = \frac{1}{2}(l_j + l) \tag{15}$$

and

$$\bar{\sigma} = \frac{1}{2}(\sigma_j + \sigma) \tag{16}$$

All of these core size parameters can be evaluated for species j from the molecular volume, v_j, by the use of the following semiempirical relationships

$$R_j = 1.74 \left(\frac{3v_j}{4\pi} \right)^{1/3} \tag{17}$$

$$l_j = 1.74 \left(\frac{3v_j}{4\pi} \right)^{1/3} \frac{0.24 + 7\omega_j}{3.24 + 7\omega_j} \tag{18}$$

and

$$\sigma_j = \left(\frac{3v_j}{4\pi} \right)^{1/3} \frac{4.64}{3.24 + 7\omega_j} \tag{19}$$

where ω_j is the acentric factor of j and can be evaluated for polar substances from that of nonpolar compounds of similar geometry. The core size parameters for the solvent R, l and σ are evaluated in a similar way. Eqns. 8 through 19 enable us to calculate $\Delta F_{vdw,j}$ for various solute–solvent combinations.

The second term on the right-hand side of eqn. 7 has been approximated[18] as

$$\Delta F_{e.s,j} = -\frac{N}{2} \frac{\mu_j^2}{v_j} \mathcal{D}\mathcal{P} \tag{20}$$

where μ_j is the static dipole moment of j and $\mathscr{D}$ is a function of the static dielectric constant of the solvent, ε, as given by

$$\mathscr{D} = \frac{2\,(\varepsilon - 1)}{2\varepsilon + 1} \tag{21}$$

On the other hand, $\mathscr{P}$ depends on the polarizability of the species j, α_j, as

$$\mathscr{P} = \frac{1}{4\pi\varepsilon_0 \left(1 - \mathscr{D}\,\dfrac{\alpha_j}{v_j}\right)} \tag{22}$$

where ε_0 is the permittivity constant. The variation of $\mathscr{P}$ with solvent properties is negligible.

After substituting and combining eqns. 3, 4, 7 and 20, we obtain for the overall process that the standard unitary free energy change is given by

$$\Delta F^0_{\text{assoc}} = \Delta F_{\text{vdw,assoc}} + \Delta F_{\text{vdw,SL}} - \frac{N}{2}\,\frac{\mu^2_{\text{SL}}}{v_{\text{SL}}}\,\mathscr{D}\mathscr{P} + N\kappa^e_{\text{SL}}\,A_{\text{SL}}\,\gamma\,(1 - W_{\text{SL}}) -$$

$$\Delta F_{\text{vdw,S}} + \frac{N}{2}\,\frac{\mu^2_{\text{S}}}{v_{\text{S}}}\,\mathscr{D}\mathscr{P} - N\kappa^e_{\text{S}}\,A_{\text{S}}\,\gamma\,(1 - W_{\text{S}}) - \Delta F_{\text{vdw,L}} + \frac{N}{2}\,\frac{\mu^2_{\text{L}}}{v_{\text{L}}}\,\mathscr{D}\mathscr{P} -$$

$$N\kappa^e_{\text{L}}\,A_{\text{L}}\,\gamma\,(1 - W_{\text{L}}) - RT \ln \frac{RT}{P_0\,V} \tag{23}$$

In the chromatographic system of interest the solvent molecules are significantly smaller than the solute molecules and the size of the hydrocarbonaceous ligand, usually an octadecyl moiety, is much greater than that of the solute. Then the following assumptions appear to be quite reasonable

$$W_{\text{SL}} = W_{\text{S}} = W_{\text{L}} = 0 \tag{24}$$

$$\Delta F_{\text{vdw,SL}} = \Delta F_{\text{vdw,L}} \tag{25}$$

$$\mu_{\text{SL}} = \mu_{\text{S}} \tag{26}$$

$$\mu_{\text{L}} = 0 \tag{27}$$

and

$$\kappa^e_{\text{SL}} = \kappa^e_{\text{L}} = 1 \tag{28}$$

For convenience the molecular volume of the complex is assumed to be a multiple of the molecular volume of the solute

$$v_{\text{SL}} = \lambda v_{\text{S}} \tag{29}$$

where λ is the proportionality factor and the total surface area of the complex is expressed as

$$A_{\text{SL}} = A_{\text{S}} + A_{\text{L}} - \Delta A \tag{30}$$

where ΔA is the contact surface area of the associated species. With these assumptions eqn. 23 can be written as

$$\Delta F^0_{\text{assoc}} = \Delta F_{\text{vdw,assoc}} - \Delta F_{\text{vdw,s}} + \frac{N(\lambda - 1)}{2\lambda} \frac{\mu^2_s}{v_s} \mathscr{D}\mathscr{P} - N\Delta A\gamma -$$

$$N(\kappa^e_s - 1) A_s\gamma - RT \ln \frac{RT}{P_0 V} \qquad (31)$$

By substituting κ^e_s from eqn. 6 into eqn. 31 we obtain that

$$\Delta F^0_{\text{assoc}} = \Delta F_{\text{vdw,assoc}} - \Delta F_{\text{vdw,s}} + \frac{N(\lambda - 1)}{2\lambda} \frac{\mu^2_s}{v_s} \mathscr{D}\mathscr{P} - N\Delta A\gamma -$$

$$\frac{NA_s}{V_s^{2/3}} (\kappa^e - 1) V^{2/3} \gamma - RT \ln \frac{RT}{P_0 V} \qquad (32)$$

The thermodynamic equilibrium constant, which governs solute retention in the chromatographic process, is K and according to our model it is related to the free energy change by

$$\ln K = -\frac{\Delta F^0_{\text{assoc}}}{RT} \qquad (33)$$

The measured chromatographic parameter is the capacity factor, k, which is related to K by

$$\ln k = \ln K + \varphi \qquad (34)$$

where φ is a characteristic constant (the logarithm of the so-called phase ratio) for a given column. In view of eqns. 32–34, the capacity factor can be evaluated by the following expression

$$\ln k = \varphi - \frac{\Delta F_{\text{vdw,assoc}}}{RT} + \frac{\Delta F_{\text{vdw,s}}}{RT} - \frac{N(\lambda - 1)}{2\lambda} \frac{\mathscr{P}\mu^2_s}{RTv_s} \mathscr{D} + \frac{N\Delta A\gamma}{RT} +$$

$$+ \frac{NA_s\gamma}{RTV_s^{2/3}} (\kappa^e - 1) V^{2/3} + \ln \frac{RT}{P_0 V} \qquad (35)$$

Alternatively we can derive an expression similar to eqn. 35 without assuming that the solute molecule and the complex are very large in comparison to the solvent molecules. Following Sinanoğlu and assuming that the solute, ligand, and complex all have spherical shape we can calculate the surface area of the species by

$$A_j = 4.836 \, v_j^{2/3} = 4.836 (V_j/N)^{2/3} \qquad (36)$$

Combining eqns. 4 and 6 with the assumption that $W = 0$, we obtain that

$$\Delta F_{c,j} = NA_j\gamma + 4.836N^{1/3} (\kappa^e - 1) V^{2/3}\gamma \qquad (37)$$

The combination of eqns. 3, 7, 20 and 37 with the assumptions stated in eqns. 24–30 yields for the unitary standard free energy change of the association process the following expression

$$\Delta F^0_{assoc} = \Delta F_{vdw, assoc} - \Delta F_{vdw,S} + \frac{N(\lambda - 1)}{2\lambda} \frac{\mu_S^2}{v_S} \mathcal{D}\mathcal{P} - N\Delta A\gamma -$$

$$4.836 N^{1/3} (\kappa^e - 1) V^{2/3} \gamma - RT \ln \frac{RT}{P_0 V} \qquad (38)$$

In view of eqns. 33 and 34, the capacity factor is then calculated by

$$\ln k = \varphi - \frac{F_{vdw, assoc}}{RT} + \frac{F_{vdw,S}}{RT} - \frac{N(\lambda - 1)}{2\lambda} \frac{\mu_S^2}{v_S RT} \mathcal{D}\mathcal{P} + \frac{N\Delta A\gamma}{RT} +$$

$$+ \frac{4.836 N^{1/3} (\kappa^e - 1) V^{2/3} \gamma}{RT} + \ln \frac{RT}{P_0 V} \qquad (39)$$

Eqn. 39 can also be obtained directly from eqn. 35 with the relationship between the molecular area and volume stated in eqn. 36. As seen, eqns. 35 and 39 are equivalent when the relationship between the molecular surface and volume in eqn. 36 is applicable.

The theoretical expression for the capacity factor as presented in eqns. 35 or 39 has been derived for $P_0 = 1$ atm in the last term. In LC the average pressure in the column is usually much higher, therefore, the pressure dependence of the various terms is of interest. An examination of the effect of pressure shows that most terms, including the last term expressing the entropy of mixing, are fairly indifferent to changes in pressure provided the liquid is incompressible.

Nevertheless κ^e, which is related to the internal energy change associated with the vaporization of the solvent, merits special consideration. If the surface tension and the mole volume of the solvent are not affected by pressure changes we can use the expression derived by Halicioğlu[24]

$$\kappa^e = \frac{N^{1/3} \Delta E_{vap}}{V^{2/3} \gamma \left(1 - \frac{d \ln \gamma}{d \ln T} - \frac{2}{3} \mathcal{A}T\right)} \qquad (40)$$

where ΔE_{vap} is the heat of vaporization, to estimate the effect of pressure on κ^e. In order to do so it suffices to estimate the pressure dependence of ΔE_{vap}. Literature data indicate that the heat of evaporation increases slightly with the pressure. Our calculation for solvents of chromatographic interest have indicated that κ^e increases less than 20% from its atmospheric value when the pressure increases to 60 atm. It has also been found that the relative change in κ^e with the pressure is about the same for solvents such as water, methanol, and acetonitrile. The development of methods to calculate κ^e values for conditions of chromatographic interest is currently under investigation and will be the subject of subsequent communication.

The canonical expression for the capacity factor as given in eqn. 39 can be further simplified in certain special cases. For a given solute when the solvent composition is changed at constant temperature and flow-rate with a fixed column, we as-

sume that the solute and ligand properties as well as the $\Delta F_{vdw,assoc}$ term are invariant. Hence, eqn. 39 can be written as

$$\ln k = A + B\mathscr{D} + C\gamma + D(\kappa^e - 1) V^{2/3}\gamma + E + \ln(RT/P_0 V) \tag{41}$$

where A and C may be regarded as constants and can be determined experimentally. B, D, E and the last term are also constant and can be estimated. The meaning of the constants is given by

$$A = \varphi - \frac{\Delta F_{vdw,assoc}}{RT} \tag{42}$$

$$B = \frac{1}{RT} \frac{1-\lambda}{2\lambda} \frac{\mu_S^2}{v_S} N\mathscr{P} \approx \frac{1}{4\pi\varepsilon_0 RT} \frac{1-\lambda}{2\lambda} \frac{\mu_S^2}{v_S} \frac{N}{1-(\alpha_S/v_S)} \tag{43}$$

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

$$C = N\Delta A/RT \tag{44}$$

$$D = 4.836 N^{1/3}/RT \tag{45}$$

$$E = \Delta F_{vdw,S}/RT \tag{46}$$

When the same eluent and column are used, the capacity factor of different solutes may be obtained at a fixed temperature and flow-rate from the following expression

$$\ln k = A' + B' \frac{1-\lambda}{2\lambda} \frac{\mu_S^2}{v_S} \frac{1}{1-(\alpha_S/v_S)} + C'\Delta A \tag{47}$$

where the constants A', B', and C' are given by

$$A' = \varphi - \frac{\Delta F_{vdw,assoc}}{RT} + \frac{\Delta F_{vdw,S}}{RT} + \frac{4.836 N^{1/3}(\kappa^e - 1) V^{2/3} \gamma}{RT} + \ln \frac{RT}{P_0 V} \tag{48}$$

$$B' = N\mathscr{D}/RT \tag{49}$$

and

$$C' = N\gamma/RT \tag{50}$$

Chromatographic experiments are frequently carried out at different temperatures. In order to estimate the effect of temperature on the capacity factor we recall that the van't Hoff equation expresses the standard enthalpy change, ΔH_{assoc}, for the solute–stationary phase interaction by

$$\frac{d(R \ln K)}{d(1/T)} = -\Delta H^0_{assoc} \tag{51}$$

We get ΔH_{assoc} from eqn. 39 assuming that φ is temperature independent, as follows

$$-\Delta H^0_{assoc} = -\Delta H_{vdw.assoc} + \Delta H_{vdw,S} + \frac{1-\lambda}{2\lambda} \frac{N\mu_S^2 \mathscr{P}\mathscr{D}}{v_S} \left(1 - \frac{d \ln \mathscr{D}}{d \ln T} + \mathscr{A}_S T\right) +$$
$$+ N\Delta A\gamma \left(1 - \frac{d \ln \gamma}{d \ln T} - \frac{2}{3}\mathscr{A}_S T\right) +$$
$$+ 4.836 N^{1/3} (\kappa^e - 1) V^{2/3} \gamma \left[1 - \frac{d \ln \gamma}{d \ln T} - \frac{2}{3}\mathscr{A}_S T - \frac{d \ln (\kappa^e - 1)}{d \ln T}\right] -$$
$$- RT (1 - \mathscr{A}T) \qquad (52)$$

Eqn. 52 implies that van't Hoff plots for the capacity factor would not yield straight lines. Nevertheless our chromatographic data show that ΔH_{assoc} can be fairly constant over a relatively wide range of temperature for reasons discussed later.

MATERIALS, EQUIPMENT AND PROCEDURES

A Perkin-Elmer Model 601 liquid chromatograph with a Rheodyne Model 7010 sampling valve with a 20-μl loop, a Perkin-Elmer Model LC 55 variable-wavelength detector, and a Perkin-Elmer Model R-56 recorder were used throughout the study. The detector was operated at 254 nm. All experiments were carried out with Partisil 1025 ODS (Whatman, Clifton, N.J., U.S.A.) columns, 25 × 0.46 cm I.D., under isocratic elution conditions at 25 °C. Reagent-grade chemicals for the preparation of the buffers were obtained from Fisher Scientific (Pittsburgh, Pa., U.S.A.). Methanol and acetonitrile were "distilled in glass" from Burdick and Jackson Labs. (Muskegon, Mich., U.S.A.). Distilled water was prepared with a Barnstead distilling unit in our laboratory. The sample compounds were purchased from Aldrich (Milwaukee, Wisc., U.S.A.) and Sigma (St. Louis, Mo., U.S.A.).

Capacity factors have been evaluated from the chromatograms in the usual way[26]. The mobile phase hold-up times were measured by injecting NaNO$_3$ together with the sample components and the retention time of the small peak at the front was taken as t_0. The concentration of the tracer was sufficiently low not to affect the retention of the early peaks. The retention times were measured from the distance between the injection point and the peak maximum on the chromatogram.

In experiments with neat aqueous eluents the pH was adjusted with phosphate buffer and Na$_2$SO$_4$ was added to maintain an ionic strength of about 3. The eluents in the experiments with water–methanol and water–acetonitrile mixtures were acidified by 0.1 M phosphoric acid in order to suppress the ionization of the acidic solutes. In the calculations the phosphoric acid was ignored. The composition of the mixed solvents is expressed in per cent (v/v) of the organic component defined by the number of milliliters of organic solvent, Y, which was mixed with $100 - Y$ ml of water. The following five substances have been found to elute with a conveniently measurable k value over the full composition range: benzoic acid, o-toluic acid, p-toluic acid, cinnamic acid, and 1-naphthoic acid. The k values were measured with the neat aqueous eluent, which was assumed to have the properties of pure water, with mixed solvents containing 10–90 % (v/v) of methanol or acetonitrile in 10 % increments as well as with the neat organic solvents. These measurements were performed with a single column whose properties did not change in the course of experiments.

For each eluent the relevant thermodynamic properties have been calculated. The term $\Delta F_{vdw,s}$ has also been evaluated for each solute and solvent combination shown in Fig. 17 according to the procedure outlined in eqns. 8–19. Table I shows the source of literature data for calculating the various thermodynamic functions. The ionization potentials of the compounds shown in Fig. 17 were estimated as 8 eV. Their acentric factor was calculated as 0.26 with toluene as the homomorph. The κ^e values of the neat solvents were taken from the literature[21] or estimated. The values used in this study were 1.277, 1.778, and 0.90 for water, methanol, and acetonitrile, respectively.

TABLE I

LITERATURE SOURCES FOR THE PROPERTIES OF SOLVENT MIXTURES AS SHOWN BY THE REFERENCE NUMBERS

Property	Reference	
	Water–methanol	Water–acetonitrile
Surface tension (γ)	29	30
Density (ϱ)	29, 31	32
Dielectric constant (ε)	33	34
Refractive index (n)	29	30
Acentric factor (ω)	21, 35	21, 35

The molecular surface areas were estimated from the group surface increments given by Bondi[27] with the appropriate crowding corrections[28]. The total surface area, TSA, has been evaluated as the sum of the group area increments; the hydrocarbon-aceous surface area, HSA, was obtained by summing the area increments of carbon and hydrocarbon groups only. Dipole moments were obtained from monographs[36,37].

The average mole volume of mixed solvents, V, has been evaluated, depending on the nature of the literature data, by one of the following expressions

$$V = \left[\varrho \left(\frac{w_1}{M_1} + \frac{w_2}{M_2} \right) \right]^{-1} \tag{53}$$

or

$$V = \frac{1}{\varrho} \left[X_2 \left(M_2 - M_1 \right) + M_1 \right] \tag{54}$$

where ϱ is the density of the mixture, X is the mole fraction, w is the weight fraction, and M is the molecular weight. The subscripts 1 and 2 refer to water and the organic solvent, respectively.

On the graphs the composition of mixed solvents is expressed by per cent (v/v), which is generally used in chromatographic practice. The values have been calculated by one of the following expressions

$$\text{Per cent (v/v) of component 2} = \frac{100 \, w_2 \, \varrho_2}{w_2 \left(\varrho_1 - \varrho_2 \right) + \varrho_2} \tag{55}$$

or

$$\text{Per cent (v/v) of component 2} = \frac{100 \, X_2 \, \varrho_1 / M_1}{\dfrac{\varrho_2}{M_2} + X_2 \left(\dfrac{\varrho_1}{M_1} - \dfrac{\varrho_2}{M_2} \right)} \tag{56}$$

Eqn. 41 was used to analyze the data obtained with mixed solvents. From the properties of the solutes and solvents, the terms $B\mathscr{D}$, E and $\ln (RT/P_0 V)$, have been exactly calculated over the full range of solvent composition. For pure solvents the term $D \, (\kappa^e - 1) \, V^{2/3} \gamma$ was also calculated with the κ^e values stated above, and, from the experimental data measured with neat eluents, the constants A and C were evaluated. With the A and C values so obtained we estimated the magnitude of κ^e for mixed solvents from the experimental data by using known values of E, V and γ. The validity of the assumption $W = 0$ (eqn. 24) was tested; it was found that the values obtained with the exactly calculated W (eqn. 5) deviated less than 4% from the values obtained by making the above assumption. All calculations were carried out on a PDP 11 minicomputer using BASIC language.

RESULTS AND DISCUSSION

Hydrophobic chromatography

The chromatograms depicted in Figs. 5–7 illustrate that relatively polar substances can readily be separated on octadecylsilica columns with neat aqueous eluents of appropriate pH. As solute retention is caused by hydrophobic interactions between the hydrocarbonaceous ligands in the stationary phase and the unpolar moiety of the solute molecules, the technique that employs neat aqueous eluents is accurately termed hydrophobic chromatography.
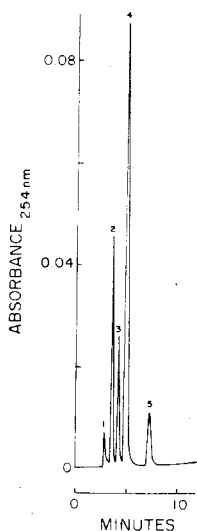

Fig. 5. Chromatogram of biogenic amines and acids. Column, Partisil 1025 ODS; eluent, 1.0 M Na_2SO_4 in 0.1 M H_3PO_4–KH_2PO_4 buffer, pH 2.55; flow-rate, 1.0 ml/min; inlet pressure, 1000 p.s.i.; temperature, 25 °C. 1 = 3,4-Dihydroxymandelic acid; 2 = norepinephrine; 3 = octopamine; 4 = norphenylephrine; 5 = tyrosine.
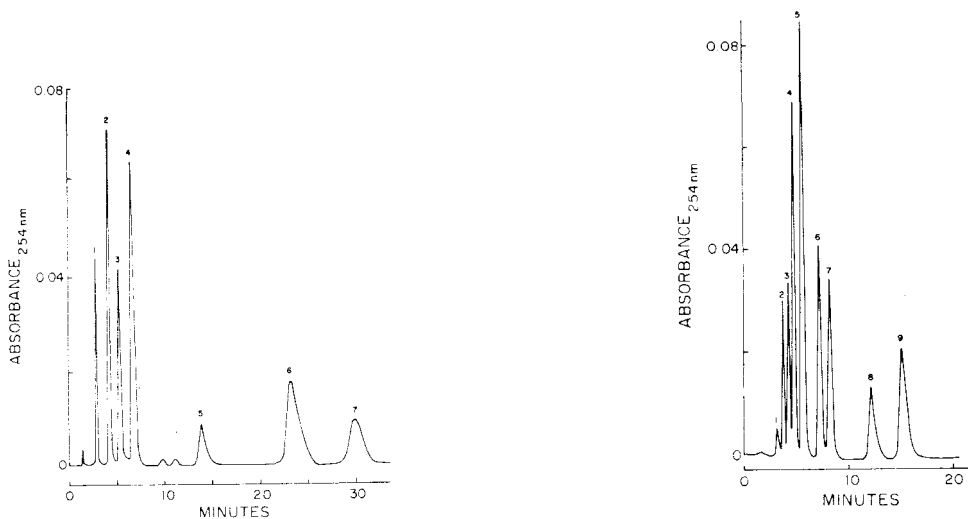
Fig. 6. Chromatogram of acidic and basic catecholamine metabolites. Column, Partisil 1025 ODS; eluent, 1.0 M Na$_2$SO$_4$ in 0.1 M H$_3$PO$_4$–KH$_2$PO$_4$ buffer, pH 2.1; flow-rate, 2 ml/min; inlet pressure, 2000 p.s.i.; temperature, 25 °C. 1 = Normetanephrine; 2 = vanillinmandelic acid; 3 = metanephrine; 4 = 3-methyldopamine; 5 = paranephrine; 6 = homovanillic acid; 7 = homovanillic alcohol.

Fig. 7. Chromatogram of mandelic acid derivatives. Column, Partisil 1025 ODS; eluent, 1.0 M Na$_2$SO$_4$ in 0.1 M H$_3$PO$_4$–KH$_2$PO$_4$ buffer, pH 4.4; flow-rate, 1.0 ml/min; inlet pressure, 1000 p.s.i.; temperature, 25 °C. 1 = 3,4-Dihydroxymandelic acid; 2 = norepinephrine; 3 = 4-hydroxymandelic acid; 4 = 3-hydroxymandelic acid; 5 = 3-methoxy-4-hydroxymandelic acid; 6 = 4-hydroxyphenyl-methylaminoethanol; 7 = tyramine; 8 = metanephrine; 9 = 3-O-methyldopamine.

The relatively high column efficiency and peak symmetry may by due to the fact that in Partisil 1025 ODS the surface concentration of the hydrocarbonaceous moiety is relatively low, as indicated by the 4–6 % carbon content of the dry stationary phase. Therefore, it is assumed that the silica surface closely resembles the representation in Fig. 1. On the other hand, in octadecylsilicas of high carbon content, the pore surface of the sorbent is likely to be coated by an organosiloxane polymer film. The advantage of the molecular fur configuration depicted in Fig. 1 is that there are silanol groups, at the surface which facilitate wetting by the neat aqueous eluent. Thus, interfacial mass transfer resistance is minimized. In addition, neither peak symmetry nor column efficiency is impaired since the solute molecules are not illaqueated in the entangled chains of the polymer film.

It has been observed that the ionization of the solutes can have a dramatic effect on the retention and both the capacity factors and the relative retention values are strongly influenced by the pH of the eluent. Whereas the foregoing theoretical treatment does not deal with the ionization of the solute explicitly, the subject will be discussed in a subsequent communication[38].

Solvophobic chromatography

For relatively nonpolar solutes the hydrophobic interactions with an apolar stationary phase such as octadecylsilica are so strong that they cannot be eluted with
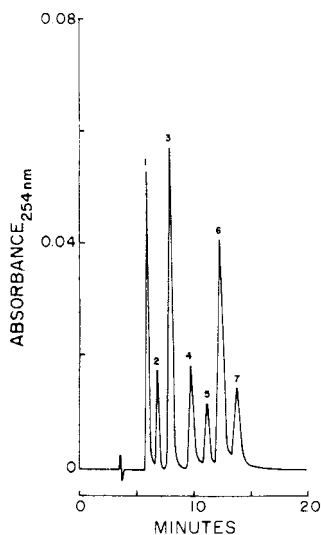
Fig. 8. Chromatogram of aromatic acids. Column, Partisil 1025 ODS; eluent 30% (v/v) acetonitrile and 70% (v/v) 0.05 M phosphate buffer, pH 2.7; flow-rate, 1 ml/min; inlet pressure, 1000 p.s.i.; temperature, 25 °C. 1 = Benzoic acid; 2 = 2-bromobenzoic acid; 3 = 2-iodobenzoic acid; 4 = 4-nitrocinnamic acid; 5 = naphthoic acid; 6 = 4-iodobenzoic acid; 7 = 3-chlorocinnamic acid.

neat aqueous eluents at ambient temperature. By the use of mixed solvents, however, the capacity factors can be reduced to any desired value at least for low-molecular-weight solutes. Fig. 8 illustrates the separation of aromatic acids on octadecylsilica by elution with an acetonitrile–water mixture. With neat aqueous eluents the retention of the uncharged solutes would be too strong and the chromatographic system would be impractical.

This is in agreement with the general practice in chromatography using unpolar stationary phases and, therefore, the most commonly used eluents are mixed solvents. The elution of large solute molecules on a column containing octadecylsilica of high carbon content may require the employment of neat organic solvents. Under such conditions the term solvophobic chromatography appears to be most appropriate because it correctly conveys the nature of the fundamental solute–stationary phase interaction underlying the chromatographic process. Hydrophobic chromatography, of course, is a particular case of solvophobic chromatography when a neat aqueous eluent is employed with a nonpolar stationary phase.

Test of theory

Solvophobic chromatography is probably the least complicated of all liquid–solid chromatographic processes in which the solute interacts with the stationary phase. Yet, the equations derived from the theory for the capacity factor are quite involved, as seen in eqns. 41 and 47, despite the simplifying assumptions.

Nevertheless under certain conditions some of the terms in these equations remain fairly constant so that further simplification is possible. First, we examine the dependence of the capacity factor on the molecular dimensions of the solute at a fixed eluent composition. Going back to eqn. 47, we see that the two van der Waals terms have been lumped together in the leading constant A'. Whereas these terms de-

pend on solute properties, for closely related solutes they are expected to vary at the same extent and the changes in the two terms very nearly cancel each other because of their opposite signs. Therefore A' can be considered constant for solutes having commensurable molecular dimensions. According to eqn. 47 the observed variation of the capacity factor with solute properties depends on the last two terms. The second term entails the dipole moment, the molecular volume, and the polarizability of the solute as well as the volume change occurring upon binding the solute to the ligand. The third term is essentially the product of the contact area in the complex and the surface tension of the eluent.
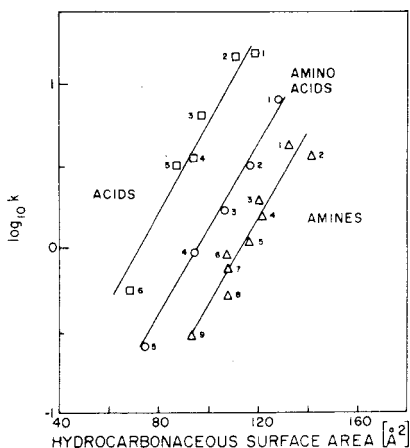

Fig. 9. Graph illustrating the relationship between the logarithm of the capacity factor and the hydrocarbonaceous surface area of different classes of solutes in hydrophobic chromatography. Column, Partisil 1025 ODS; eluent, 1.0 M Na$_2$SO$_4$ in 0.1 M phosphate buffer, pH 2.05; flow-rate, 1.0 ml/min; inlet pressure, 1000 p.s.i.; temperature, 25 °C. Acids: (1) homovanillic acid, (2) phenylacetic acid, (3) 4-hydroxyphenylacetic acid, (4) mandelic acid, (5) 3,4-dihydroxyphenylacetic acid, (6) 3,4-dihydroxymandelic acid; amino acids: (1) tryptophan, (2) phenylalanine, (3) tyrosine, (4) 3,4-dihydroxyphenylalanine, (5) 3,4-dihydroxyphenylserine; amines: (1) phenylethylamine, (2) 3-O-methyldopamine, (3) phenylethanolamine, (4) tyramine, (5) normetanephrine, (6) dopamine, (7) norphenylephrine, (8) octopamine, (9) norepinephrine.

In Fig. 9 the logarithm of the measured capacity factors is plotted against the hydrocarbonaceous surface area of three groups of closely related aromatic solutes: carboxylic acids, amino acids, and amines. The k values were obtained with a neat aqueous phosphate buffer at the same conditions. It is seen that each family of compounds yields a linear plot, the slopes of which are nearly identical. The straight lines suggest that for each family the contact area is directly proportional to the hydrocarbonaceous surface area (HSA) of the solutes. Consequently, the second term in eqn. 47 is about the same for all members of a family. The identity of the slopes is expected because if ΔA is proportional to the HSA of the solutes, then the slope depends strictly upon the surface tension which was constant in the experiments. The data show that the contact area is about 35% of HSA.

For closely related substances we may expect that the second term of eqn. 47 is fairly constant and in this case eqn. 47 can be simplified to

$$\ln k = A'' + \frac{N}{RT}\Delta A\gamma \tag{57}$$

where A' and the second term in eqn. 47 are lumped into A''. Indeed, eqn. 57 predicts the linear behavior shown in Fig. 9. The relative displacement of the lines representing the different families can be accounted for by the differences mainly in the second term in eqn. 47, which expresses the electrostatic effect and influences the value of A'' in eqn. 57. Fig. 9 demonstrates that the intercept, the value of A'', is the greatest for the acids and the smallest for the bases. It is recalled that the sign of the second term in eqn. 47 is negative so that its absolute value would be smaller for the acids and the greatest for the bases in agreement with the intercepts in Fig. 9. The dipole moment whose squared value appears in the numerator of the second term of eqn. 47, is approximately 1 debye for the acids and 15–20 debyes for the amino acids. Thus, the displacement of the lines can readily be explained by the difference in the average dipole moments for the two families. On the other hand, the bases carry a net charge at the eluent pH; therefore, the low value of the intercept can be explained by a repulsive effect corresponding to the dipole effect discussed above.

Fig. 9 implies that the contact area between the solute and the ligand is proportional to the hydrocarbonaceous surface area of the substances investigated. Such dependence of the capacity factor on the molecular surface area has been frequently observed in chromatography with a wide variety of homologous series and usually reported as a linear relationship between log k and the carbon number[39,40]. This manifestation of Traube's rule arises from the proportionality between the number of carbon atoms and the molecular surface area in homologues.

In our case, the three families do not represent genuinely homologous series; yet, with the use of the hydrocarbonaceous surface area instead of the carbon number, Traube's rule can be broadly applied in solvophobic chromatography, as demonstrated in Fig. 9. From this it follows that for a group of compounds, which have essentially the same properties with respect to the second term in eqn. 47, but do not represent a single homologous series, the plot of the log k against HSA or under circumstances against TSA should yield a single straight line. For instance, we expect that a plot of log k vs. carbon number for primary, secondary and tertiary aliphatic alcohols would give three straight lines having the same slope but different intercepts. A plot of the same log k values against the molecular surface area, however, should yield a single straight line.

For a given solute the effect of changing eluent composition has been expressed by eqn. 41. When only the surface tension changes, eqn. 41 can be simplified to

$$\ln k = A''' + B''\gamma \tag{58}$$

where A''' is the sum of all terms which do not contain the surface tension and

$$B'' = \frac{N\Delta A + 4.836N^{1/3}(\kappa^e - 1)V^{2/3}}{RT} \tag{59}$$

Eqn. 58 predicts a linear dependence of log k on the surface tension of the eluent for a given solute provided κ^e and the mole volume of the eluent remain constant. Fig. 10 illustrates the surface tension of some aqueous salt solutions and mixed solvents as a function of the composition. In view of the data eqn. 58 would predict

that the capacity factor increases with the salt concentration of the eluent in hydro-phobic chromatography and decreases with the water concentration when mixed solvents are employed as eluents. This is in agreement with the general experience.
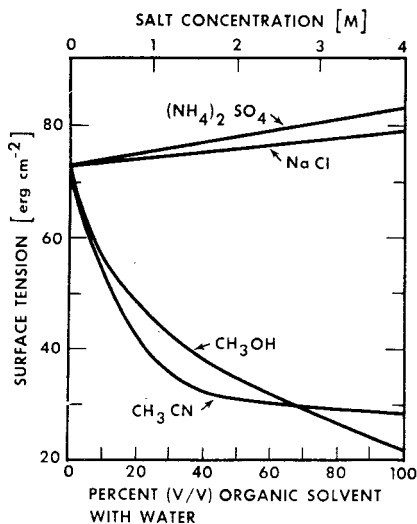

Fig. 10. Surface tension as a function of the composition in mixed solvents and salt solutions. Water–methanol and water–acetonitrile systems represent the mixed solvents.

The surface tension of inorganic salt solutions in water is to a good approxim-ation a linear function of the salt concentration and can be expressed by

$$\gamma = \gamma_0 + \tau m \tag{60}$$

where γ_0 is the surface tension of pure water ($\gamma_0 = 72.0$ dyne cm^{-1} at 25 °C), m is the molal salt concentration, and τ is a coefficient which depends on the nature of the salt. In Table II the τ values are listed for a number of common inorganic salts.

According to eqn. 60 the log k values should linearly increase with the salt concentration as long as eqn. 58 holds. Indeed, as seen in Fig. 11, plots of log k against KCl concentration in the eluent yield straight lines for a number of aromatic acids and bases of relatively complex chemical structure. The slopes are similar, indicating similar contact areas for these closely related substances. The intercepts depend on the van der Waals terms and the electrostatic interaction term in view of eqn. 48. At the eluent pH most species carry a net charge and this can greatly influence the value of A''' in eqn. 58, which determines the magnitude of the intercepts. It is interesting to note that the intercepts decrease with the hydrocarbonaceous surface area, HSA, of the solutes and only homovanillic acid (HSA = 119 Å^2), which does not have an ionized amino group at the eluent pH, is an exception. On the other hand, the correlation between the elution order and total surface area, TSA, of the solutes is by far not so satisfactory.

The results show that eqn. 58 is a useful approximation as long as the change in the surface tension of the eluent does not affect the value of the terms lumped to-

TABLE II

COEFFICIENT τ IN EQN. 60 THAT EXPRESSES THE INCREASE IN SURFACE TENSION
OF AQUEOUS SALT SOLUTIONS WITH THE MOLALITY OF THE SALT

Salt	τ
KCl	1.50
NaCl	1.64
Na_2HPO_4	2.03
Mg_2SO_4	2.06
$(NH_4)_2SO_4$	2.17
Na_2SO_4	2.74
K_2SO_4	2.80
Na_3PO_4	2.88
Na_3-citrate	3.12
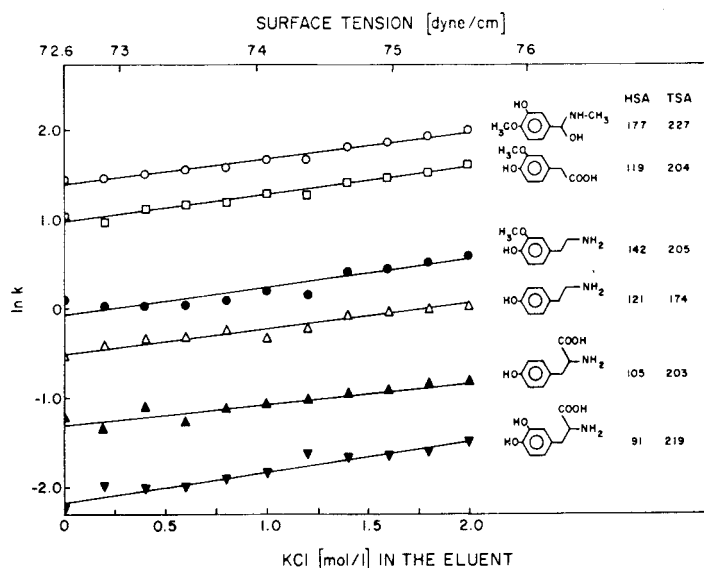
Fig. 11. Plot illustrating the effect of salt concentration on the capacity factor. The concentration of
KCl in a 0.05 M KH$_2$PO$_4$ solution was varied. The upper scale shows the surface tension of the
eluent. The hydrocarbonaceous surface area (HSA) and the total surface area (TSA), of the solute
molecules are shown. Column, Partisil 1025 ODS; flow-rate, 1 ml/min; temperature, 25 °C.

gether in the constant A'''. The results depicted in Fig. 12 demonstrate, however,
that with mixed solvents this is no longer the case. Whereas the plots of ln k against
γ for water–methanol and water–acetonitrile mixtures show that the capacity factor
decreases with the surface tension, the dependence is strongly nonlinear. This behav-
ior, however, is not unexpected as the mole volume of the solvent mixtures is a
function of the composition and κ^e is also likely to change. Consequently, B'' in
eqn. 58 is expected to vary over such a wide range of solvent composition.

    In order to take these factors into account and express the capacity factor as
a function of the eluent composition for mixed solvents, we have to use eqn. 41 and
subject the theory to a much more exacting test than before. With the exception of
the leading constant all terms of eqn. 41 are dependent on the properties of the eluent.
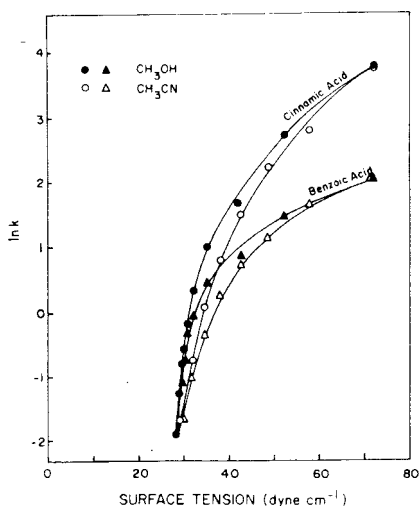
Fig. 12. Plots illustrating the dependence of solute retention of the surface tension of mixed solvents
The capacity factors of cinnamic and benzoic acid were measured in water–methanol and water–
acetonitrile mixtures. Column, Partisil 1025 ODS; flow-rate 1.0 ml/min; temperature, 25 °C.

In order to illustrate the effect of composition on the key eluent properties for water–
methanol and water–acetonitrile mixture, κ^e, $\mathscr{D}$, γ and $\ln(RT/P_0V)$, have been
calculated in terms of reduced energy units with appropriate scaling factors over the
full composition range. As a result their variation with composition could be conve-
niently depicted in Figs. 13 and 14. It is noted that due to the scaling factors employed
the actual magnitude of the functions is obscured and only their relative change
with the solvent composition is illustrated.

 For a given solute the magnitude of solvophobic interactions is largely depen-
dent on these four solvent properties, which embody the solvophobic strength of the
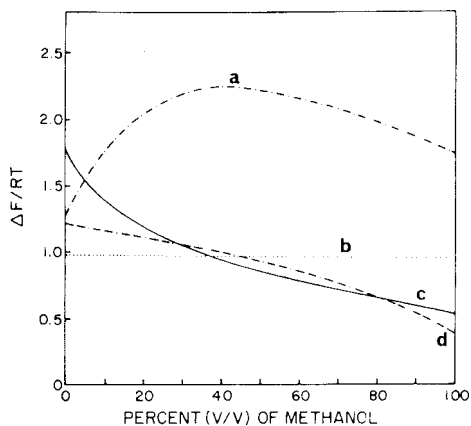solvent. It is seen in Figs. 13 and 14 that the individual properties do not change with

Fig. 13. Graph illustrating the properties of methanol–water mixtures at 25 °C as a function of
composition. The properties are expressed in normalized energy units and are represented by the curves
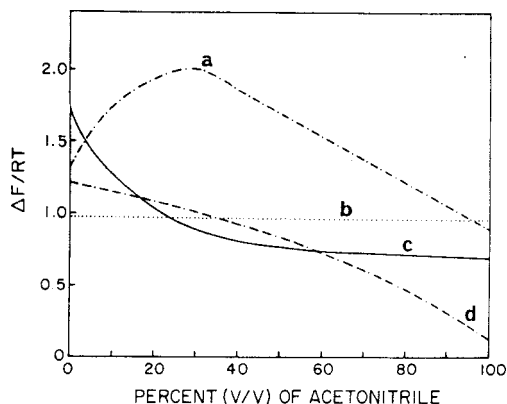(a) κ^e, (b) $\mathscr{D}$, (c) $A\gamma/RT$ with $A = 1$ Å^2, and (d) $\ln(RT/P_0V) - 7$.

Fig. 14. Graph illustrating the properties of acetonitrile–water mixtures at 25 °C as a function of the composition. The properties are expressed in dimensionless energy units and are represented by the curves (a) κ^e, (b) $\mathscr{D}$, (c) $A\gamma/RT$ with $A = 1$ Å^2, and (d) $\ln(RT/P_0V) - 7$.

varying solvent composition in the same way. Whereas both the surface tension and $\ln(RT/P_0V)$ decrease with increasing concentration of the organic component in both mixtures, κ^e goes through a maximum and $\mathscr{D}$ is practically constant. The behavior of κ^e and $\ln(RT/P_0V)$ as seen in Figs. 13 and 14 readily explains the failure of eqn. 58 manifested in Fig. 12.

The effect of the solvophobic strength of the eluent on the capacity factor is illustrated in Figs. 15 and 16. The ordinate is a $\ln k$ scale, which expresses the magnitude of the experimental capacity factor, $\ln k_{obs}$, and that of the individual terms in eqn. 41, which represent incremental $\ln k_i$ values. The actual value of $\ln k$ is the sum of the increments so that

$$\ln k_{obs} = \Sigma \ln k_i \tag{61}$$

Thus, Figs. 15 and 16 give a graphical representation of the various terms, except the leading constant, A, of eqn. 41, and their dependence on the eluent composition for a given solute which is in this case o-toluic acid. The relative magnitude and composition dependence of the $\ln k_i$ values have been found quite similar for the other solutes investigated: benzoic acid, p-toluic acid, cinnamic acid, and 1-naphthoic acid.

The terms of eqn. 41 have been evaluated by the following procedure. First, the value of $\Delta F_{vdw,s}/RT$ (term E of eqn. 41) was calculated for each solute and neat solvent combination according to eqns. 8–19 and the terms $D(\kappa^e - 1)V^{2/3}\gamma$ and $\ln(RT/P_0V)$ were calculated for the neat solvents. The value of $B\mathscr{D}$ was calculated with the properties of the solute and the neat solvent. The estimated value of λ ranged from 3–4.4. With these constants and the known γ values as well as with the observed capacity factors, the column and solute parameters A and C were calculated from eqn. 41. Subsequently the appropriate properties of the two kinds of solvent mixtures having different compositions and the corresponding $\Delta F_{vdw,s}/RT$ terms were calculated. Assuming that A, B, and C are independent of the solvent composition, we then evaluated by using eqn. 41 again the term $D(\kappa^e - 1)V^{2/3}\gamma$ as a function of the composition for both water–methanol and water–acetonitrile mixtures.
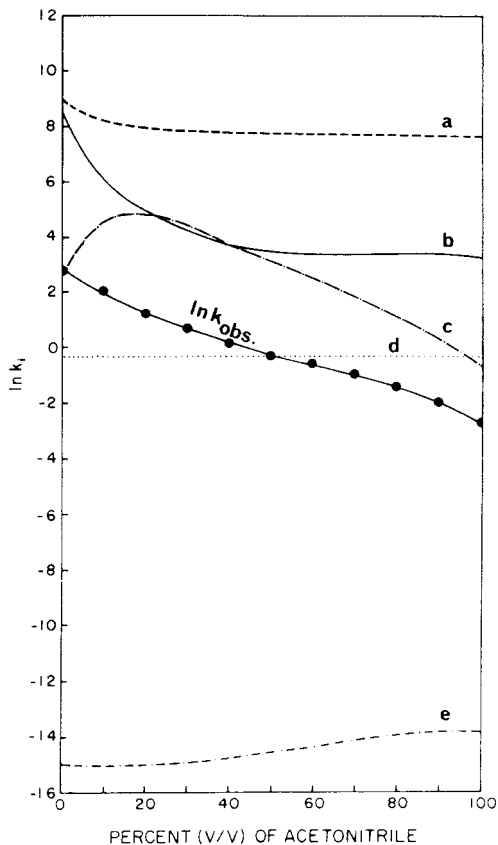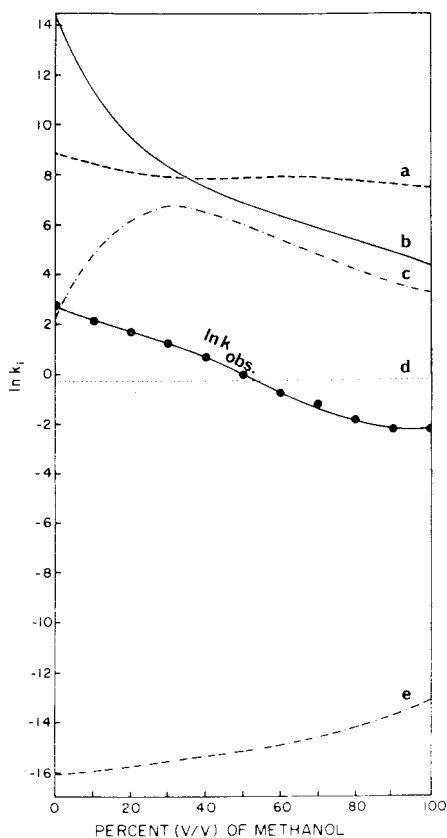
Fig. 15. Graph illustrating the logarithm of the calculated capacity factor increments, ln k_i, and the logarithm of the capacity factor, ln k_{obs}, measured for o-toluic acid on an octadecylsilica column with water–methanol eluent systems of different composition at 25 °C. The ln k_i values were evaluated by eqn. 41, so that the curves represent the individual terms as follows: (a) ln (RT/P_0V), (b) $C\gamma$, (c) $D(\kappa^e - 1)V^{2/3}\gamma$, (d) $B\mathcal{D}$, and (e) E. The value of term A is -7.00.

Fig. 16. Graph illustrating the logarithm of the calculated capacity factor increments, ln k_i, and the logarithm of the capacity factor, ln k_{obs}, measured for o-toluic acid on an octadecylsilica column with water–acetonitrile eluent systems of different composition at 25 °C. The ln k_i values were evaluated by eqn. 41 so that the curves represent the individual terms as follows: (a) ln (RT/P_0V), (b) $C\gamma$, (c) $D(\kappa^e - 1)V^{2/3}\gamma$, (d) $B\mathcal{D}$, and (e) E. The value of term A is -6.60.

It is seen in Figs. 15 and 16 that the solvophobic forces, illustrated by the curves representing all the terms but A of eqn. 41, show a similar dependence on the solvent composition with both eluent systems. The van der Waals force increases with the concentration of the organic component. This effect is largely due to the increase in the apparent solvent radii, which determine the value of Q' in eqn. 12, although the changing values of the average ionization potential and the Clausius–Mosotti function also affect the shape of curve (e) in Figs. 15 and 16. The electrostatic term, $B\mathcal{D}$, is remarkably insensitive to solvent effects; in fact, it can be regarded invariant with the solvent composition when the solute is uncharged. Of course, this term, which orginates from Onsager's reaction field[41] ,would have a stronger functional dependence

upon the dielectric constant of the medium if the solutes were ionized and consequent-
ly would have a greater effect on the capacity factor with changing solvent composi-
tion. As shown by curve (a) in Figs. 15 and 16 the term representing the entropy of
mixing changes about that much as the van der Waals term, but in the opposite
direction, over the full range of composition. Consequently, the two solvophobic
forces counterbalance each other.

    The solvophobic forces that have the greatest influence on the observed capacity
factor are those which are dependent on the surface tension, *i.e.*, the term $C\gamma$ shown
by curve (b) and $D (\kappa^e - 1) V^{2/3}\gamma$ shown by curve (c) in Figs. 15 and 16. The change in
the measured capacity factor with the solvent composition is almost entirely deter-
mined by the corresponding variation of the two dominant solvophobic forces. In
this regard the role of κ^e is of great significance. This parameter has been defined as
the ratio of the energy required to create a cavity for a solvent molecule to the energy
required to extend the planar surface of the liquid by the surface area of the mole-
cule[21,24]. The results in Figs. 15 and 16 show that the term containing κ^e first rapidly
increases with the concentration of the organic component while the surface tension
sharply decreases then, after reaching a maximum, decreases slowly. In the case of
water–acetonitrile mixtures, this decrease accounts almost completely for the de-
crease in the capacity factor with the water content at high acetonitrile concentra-
tions. The behavior of κ^e of mixed solvents has not yet been investigated by others. In
our view, the rapid increase in κ^e when organic solvent is added to water initially is
due to abrupt changes in the liquid structure in the circumambient region of the
cavity.

    As mentioned before, the variation of the individual solvophobic forces with
the solvent composition for the other four solutes has been found similar to that shown
in Figs. 15 and 16. Of course, the terms A and B in eqns. 41 are different for each solute.
It is recalled that A depends on the column parameters and entails the free energy
change of the association process in the gas phase, whereas B is a function of solute
properties and λ. The values of these constants as calculated by the aforementioned
procedure are listed in Table III. It is seen that the A values have the expected trend
because they entail the van der Waals term for the gas phase association which should
increase with the size of the solutes. Consequently the value of A increases as the cal-
culated molecular volumes increase and the concomitant λ values decrease. Never-
theless, the sum $A + E$ is fairly constant because the effect of molecular size in both
van der Waals terms is essentially eliminated. The magnitude of B is largely deter-

TABLE III

CONSTANTS OF EQN. 41 OBTAINED FOR THE FIVE SOLUTES WITH THE TWO MIXED
SOLVENT SYSTEMS

Solute	A^*	$A + E^{**}$	B	λ
Benzoic acid	−10.2	−24.0	−0.3	4.38
Cinnamic acid	−7.1	−22.7	−0.7	3.75
p-Toluic acid	−7.8	−22.9	−0.9	3.55
o-Toluic acid	−6.8	−23.4	−0.3	3.53
1-Naphthoic acid	−1.8	−21.1	−0.3	3.00

    * Average values for the two solvent systems.
    ** The sum was calculated with E values for neat water.

mined by the dipole moment and the effect of λ is relatively minor because $(\lambda - 1)/2\lambda$ varied only between 0.33 and 0.39 in the range of λ shown in Table III.

The constant C in eqn. 41 is proportional to the area of the contact surface in the solute–ligand complex, ΔA. It is expected that for such closely related molecules as the five aromatic acids in question, ΔA is proportional to the total surface area of the solute molecules. Indeed, there is a linear relationship between C and TSA, as shown in Fig. 17. From the slope of a straight line, which goes through the points, we calculated that ΔA is about 20% of the total surface area. This fraction is significantly smaller than the 35% found earlier from the slopes in Fig. 9. This discrepancy, of course, can be explained by the fact that in Fig. 9 the abscissa is the hydrocarbon-aceous surface area, whereas in Fig. 17, the total surface area of the solute molecule was used. It is possible, however, that the slopes in Fig. 9 were increased by an area-dependent van der Waals effect which was excluded from C by the method by it was calculated. The dashed line in Fig. 17 is intended to illustrate the surmised dependence of C on TSA with a ligand such as that used in the experiments. The line suggests that with decreasing size of the solute molecules, an increasing fraction of the solute surface is in contact with the ligand when the complex is formed, *i.e.*, the slope of the C *vs.* TSA curve increases.

The temperature dependence of solute retention in solvophobic chromato-graphy is determined by the enthalpy of association as long as the structure of the stationary phase is unaffected by the changing temperature. As shown by eqn. 52, the explicit temperature dependence of ΔH^0_{assoc} implies that van't Hoff plots of the observed capacity factors would be curved. A close examination of the temperature-dependent terms of eqn. 52 shows, however, that in most cases the deviation from lin-earity is relatively small. We estimated the variation of the temperature-dependent terms for a typical solute and water. The results showed that the expected change in the enthalpy over a temperature range of 50° is less than 5%. Consequently, the ent-halpy change in a temperature interval of chromatographic interest is probably small
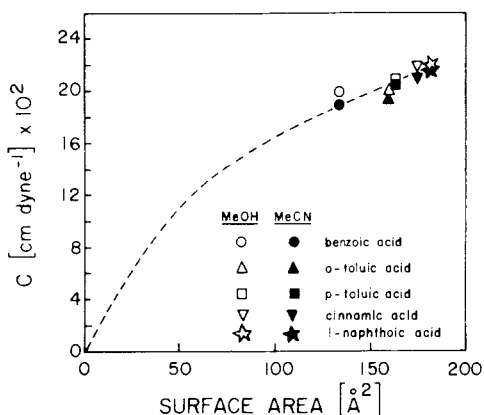

Fig. 17. Graph illustrating the relationship between the coefficient C in eqns. 41 and 44, which is proportional to the contact area of the associating species, and the total molecular surface area of the solutes indicated. The coefficient C for each solute was evaluated from the k values measured with either methanol–water or acetonitrile–water eluent systems over the full range of solvent composition. Column, Partisil 1025 ODS; flow-rate, 1 ml/min; temperature, 25 °C.

enough to yield linear van't Hoff plots. The validity of the foregoing assessment is clearly seen in Fig. 18. The average value of ΔH^0_{assoc} for the catecholamine derivatives is about -3 kcal mole^{-1} which is somewhat higher than those reported in the literature[42] for solutes of comparable size in solvophobic chromatography with methanol–water. This is readily explained by the higher κ^e value for the mixed solvent than for pure water in view of eqn. 52.

It has been postulated[11] that hydrophobic interactions are strengthened with increasing temperature in contradistinction to the general experience in solvophobic chromatography and the results presented in Fig. 18. According to eqn. 52, ΔH^0_{assoc} could be negative if its value would be dominated by the fourth and/or the fifth term. The fourth term increases with the contact area; consequently, it could be large when both the ligand and the solute are large molecules. The magnitude of the fifth term is comparable to the other terms in eqn. 52. If they are relatively small, however, the fifth term, because of its negative sign, could make ΔH^0_{assoc} negative. The occurrence of such a situation in chromatographic practice is unlikely under conditions investigated in this study.

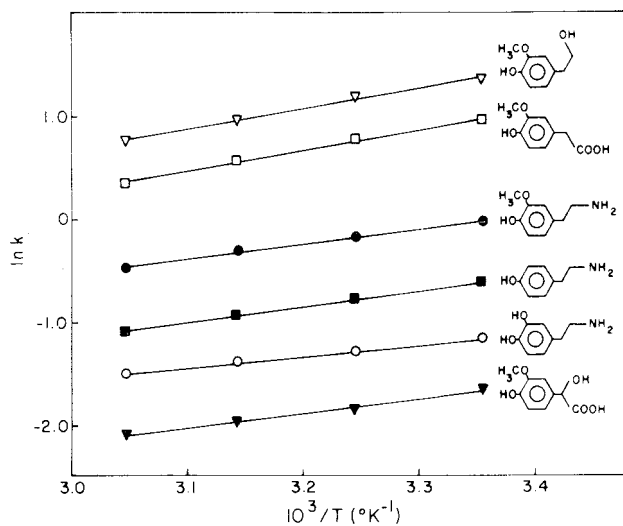Fig. 18. Van't Hoff plots of the capacity factors measured with biological substances. Column, Partisil 1025 ODS; eluent, 0.05 M KH$_2$PO$_4$; flow-rate, 1 ml/min.

CONCLUSIONS

The application of the solvophobic theory to LC with nonpolar stationary phases enabled us to carry out a detailed theoretical analysis of the factors responsible for solute retention and to interpret our experimental results obtained in a wide range of conditions.

The solvophobic strength of the eluent has been characterized by four functions of the solvent properties, among which the surface tension plays a paramount role in this type of chromatography. Taking into account the solute properties, we have calculated the solvophobic forces which determine the magnitude of the capacity factor in a given column for a variety of solute and solvent mixtures. Our theory corrob-

orates the semiempirical results of other workers[43,44] and offers a catholic framework for predicting and interpreting chromatographic behavior.

The chromatographic process has been viewed in this study as a reversible association of the solute with the hydrocarbonaceous ligands covalently bound to the surface of the stationary phase. The theory, however, can be readily applied to liquid–liquid chromatography or to describe solvophobic interactions with other unpolar stationary phases such as graphite, which has been used in gas chromatography[45] and recently was successfully employed in LC as well[40]. On the other hand, the association model may facilitate a rigorous theoretical treatment of solute interaction with other bonded phases whose ligand may contain polar or even ionic functions.

Owing to the cardinal role of hydrophobic interactions in life processes, there is a need for a physico-chemical method to quantitatively measure the hydrophobicity of biologically active substances. In the light of our present understanding, solvophobic chromatography could be a very useful tool to obtain such information. Moreover, stationary phases with hydrophobic surfaces of different configuration could be used as probes to map the detailed hydrophobic profile of biologically interesting compounds.

We have seen that in LC the magnitude of solute retention is determined by the energy balance of the solute–stationary phase, the eluent–stationary phase and the solute–eluent interactions. Solvophobic chromatography is dominated by the last two interactions and solvation of the solute solely accounts for the relative retention. Conversely, in the other types of LC, excluding exclusion chromatography, the interaction of the solute with the stationary phase plays the predominant role in effecting solute retention. In practice, the imbalance of the interaction energies is sufficiently great to warrant a clear distinction between solvophobic chromatography and the other techniques. From this perspective Howard's and Martin's suggestion[3] to call it "reversed-phase" chromatography may not have been entirely infelicitous even if this attribute does not express the essential physico-chemical phenomenon that governs the chromatographic process.

NOTE ADDED IN PROOF

The exploitation of the solvophobic effect in chromatographic separations is, of course, not restricted to the use of nonpolar stationary phases. Dr. M. Lederer pointed out that our historical introduction could have included as examples "salting-out" chromatography[46] and "solubilization" chromatography[47]. In both cases polystyrene-type ion exchangers were used for the separation of nonionic substances on the basis of solvophobic interactions between the solutes and the hydrocarbonaceous matrix of the ion-exchange resins. For instance, in "salting-out" chromatography alcohols, aldehydes, ketones and esters were separated by adsorption of the sample onto the resin matrix from saturated ammonium sulfate followed by elution with progressively diluted salt solutions. In "solubilization" chromatography hydro-

organic mixtures were used as the eluent to separate fatty acids, ethers and hydro-carbons on ion-exchange columns. Naturally, our theoretical approach —*mutatis mutandi*— can be extended to interpret these and other chromatographic separations where solvophobic interactions play a predominant role.

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