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HYDROPHOBIC-DISPERSIVE PARTITION COEFFICIENT IN ALKYL-BONDED REVERSED-PHASE SYSTEMS WITH WATER–METHANOL MOBILE PHASES

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SUMMARY

Based on a recently proposed model of monomeric alkyl-bonded silica, the hydrophobic-dispersive partition coefficient is considered as a complex function of the polarity of the solute and of its molecular geometry, of the eluent composition and of the amount of the alkyl ligates bonded. An equation for the coefficient is derived by following the thermodynamic approach and using the solubility parameter concept; it involves energy terms reflecting the solute interactions with the reversed-phase system. A variety of experimental data is used for evaluation of the enthalpy effects governing the retention of different solutes in RP-18–water–methanol systems. The results are correlated with changes in mobile phase composition and the amount of alkyl ligates in the packings. Conclusions are drawn concerning the rôle of hydrophobic bonding and its significance to the retention.

INTRODUCTION

Recently, a model for monomeric alkyl-bonded silica was proposed[1], accounting for the availability of unreacted surface silanols together with the anchored alkyl ligates. In binary aqueous–organic mobile phases the silanols appear to be hydrated, while the hydrocarbonaceous moieties are partially or fully solvated by molecules of the organic modifier. Generalizing the numerous studies discussed in refs. 1 and 2, it was suggested that the solute retention in an alkyl-bonded reversed-phase (RP) system is governed (in absence of solute dissociation) by mixed silanophilic (SPH) and hydrophobic-dispersive (HD) mechanisms. An appropriate retention equation was derived[2], written in the form

$$E = N_{AH}K_{SP} + W_{AL}K_{HD} \qquad (1)$$

where E is the so-called integral retention effect, representing the net retention volume per gram of packing[3], K_{SP} and K_{HD} are the partial partition coefficients related to the SPH and HD mechanisms, respectively, N_{AH} is the number of moles of the accessible surface silanols and W_{AL} is the mass of the alkyl ligates, both N_{AH} and W_{AL} being expressed per gram of packing.

As already mentioned[2], the hydrophobic-dispersive partition coefficient, K_{HD}, is a complex function of a variety of factors such as the polarity and molecular geometry of the solute, the eluent composition and the amount of the bonded alkyl moieties. In the commonly used RP-18 systems, W_{AL} is large and varies within the range 0.1–0.25 g g^{-1}, corresponding to a carbon content of 9–20%. Because of the strong shielding effect of the alkyl chains on the silanols, W_{AL} is usually enough to prevent a contribution from the SPH mechanism. Then, the first term on the right-hand side of eqn. 1 can be cancelled and the behaviour of the experimentally observed capacity ratio, k, wil parallel that of K_{HD}.

A number of studies have dealt with the dependence of k (or ln k) on the eluent composition[4–10]. Linear[4,9] and quadratic[8,10] equations have been proposed to represent this dependence in the case of binary aqueous–organic eluents. Attention has been paid also to the stationary phase effects in RP systems, including the interactions of the solute with silanols, alkyl ligates and the solvating molecules[11,12]. However, the absence of comprehensive publications dealing with the rigorous interpretation of the dependence of k on the amount of ligates is quite surprising, especially when this dependence has been demonstrated experimentally[13–15].

In the present paper an attempt is made to derive an expression for K_{HD}, which encompasses all factors governing its complex character. Following a thermodynamic approach, an equation is obtained that combines the energy terms for the various interactions in the RP system with an appropriate function of W_{AL}. The possibility of an approximate evaluation of the HD interactions is considered.

THEORETICAL

Characteristic dependences relevant to the stationary phase

It is commonly accepted that the effect of a modifier on a stationary phase is mainly due to solvation of the alkyl ligates by oriented modifier molecules extracted from the aqueous–organic eluent. Recent studies by McCormick and Karger[16] and Yonker *et al.*[17,18] showed that the stationary phase is also mechanically associated with molecules of the eluent. However, it is arguable whether all the stagnant mobile phase components can be considered as stationary phase ingredients in an alkyl-bonded silica packing.

Following the above mentioned model[1,2], a stationary phase will be considered as a system consisting of modifier-solvated alkyl chains bonded to the silica surface, whose unreacted silanols are hydrated. Each alkyl ligate is surrounded by a limited space which is filled by solvating organic molecules, as well as by free (non-solvating) molecules of the eluent, providing the hydrating water molecules do not enter this "solvation space". Obviously, the volume and molecular capacity of this space are dependent on the length of the alkyl chain and its configuration, on the amount of ligates in the packing and on the mobile phase composition.

With pure organic modifier as the eluent, the solvation space has its maximum capacity. For example, in a RP-18–methanol system it is supposed that the methyl groups of the solvating methanol molecules are oriented towards alkyl chains and from an approximate monolayer around them. The capacity of this layer can be expressed in terms of the number of solvating molecules that is equivalent to the ratio n_O/n_A, where n_O and n_A are the numbers of moles of the solvating molecules and the ligates, respectively, per gram of packing. Taking into account the molecular geometries of the C_{18} alkyl chain and methanol[19], it can be shown that the maximum solvation space capacity in pure methanol, *i.e.*, n_O/n_A, does not exceed 36 molecules. There is evidence that in pure acetonitrile or tetrahydrofuran the solvation space could be formed by more than one monolayer of solvating molecules[18]. Hence, this should be reflected in the different approaches to evaluating the maximum capacity of the solvation space.

As shown later, the present study involves characteristic dependences relevant to the stationary phase. Unfortunately, it was not possible to derive them rigorously, following any theoretical approach. They were determined by using the experimental data of Yonker *et al.*[17] concerning the stagnant amount of water–methanol mobile phase in a RP-18 packing. To obtain the important relationship between n_O/n_A and the eluent composition at a given W_{AL}, the initial volume of extracted methanol (data from ref. 17) was reduced at each mobile phase composition by the volume of the available non-solvating molecules. The latter was calculated by multiplying the volume of water present in the stagnant mobile phase with a factor equal to the methanol-to-water volume ratio of the corresponding eluent. Having the volume of the solvating molecules per gram of packing and the carbon content of the stationary phase (19.8% according to ref. 17), simple calculations allowed the data to be represented in n_O/n_A values, which were correlated with the water weight fraction, ψ, of the eluent. Assuming this dependence holds for a relatively large W_{AL} range, the following equation was proposed (see Appendix I)

$$n_O/n_A = \Psi A \cdot \exp(BW_{AL}^2 + CW_{AL}) \tag{2}$$

wherein

$$\Psi = (1 - \psi)/(1 + \psi) \tag{3}$$

The constants in eqn. 2; $A = 29.97$, $B = 21.35$ and $C = -10.02$, were estimated by using data for RP-18 packings of different W_{AL} prepared by Hennion *et al.*[15]. All calculations were performed by the method of rigorous least squares adjustment applicable to non-linear problems[20,21]. The good fitness of eqn. 2 to the recalculated data from ref. 17 is illustrated by curve 1 in Fig. 2 and will be discussed later. It is interesting that A represents the average miximum capacity of the ligate solvation space for a RP-18–methanol system. As seen, $A < 36$ and the difference, $(36 - A)$, can be referred to as the average number of the non-solvating methanol molecules present in this space.

The volume fraction of the solvating molecules, φ_S', can be defined as

$$\varphi_S' = V_M^s/V^s \tag{4}$$

where V_M^s and V^s are the volumes of the solvating methanol molecules and the total solvation space, respectively, both per gram of packing at constant ψ and W_{AL}. Obviously, to calculate φ'_S in the case of different ψ and W_{AL} values, it is essential to know V^s_M and V^s as functions of these quantities. While V^s_M can be expressed as

$$V^s_M = n_A \bar{V}_M \Psi A \cdot \exp(BW^2_{AL} + CW_{AL}) \quad (5)$$

where $\bar{V}_M$ is the molar volume of methanol, no explicit function is obtainable for V^s.

Assuming as a first approximation that the relationship between V^s and ψ at each W_{AL} parallels that between the total volume of stagnant mobile phase, V_{ES} (per gram of packing), and ψ. Then, an useful plot of V_{ES}/n_A *vs.* ψ can be obtained by recalculating the experimental data from ref. 17. Such a plot is presented in Fig. 2 and discussed later. Further, it can be written

$$V^s = V^s_{max}(V_{ES}/V^{max}_{ES}) \quad (6)$$

where V^s_{max} and V^{max}_{ES} are the corresponding maximum values of V^s and V_{ES} at ψ = 0. It is obvious that

$$V_{ES}/V^{max}_{ES} = (V_{ES}/n_A)/(V^{max}_{ES}/n_A)$$

where V_{ES}/n_A (at given ψ) and V^{max}_{ES}/n_A (at $\psi = 0$) are obtainable from the plot of V_{ES}/n_A *vs.* ψ. Moreover, according to eqn. 3, in pure methanol, $\Psi = 1$ and therefore V^s_{max} can be expressed as

$$V^s_{max} = n_A \bar{V}_M A' \cdot \exp(BW^2_{AL} + CW_{AL}) \quad (7)$$

where $A' = 36$. Then, the combination of eqns. 4–7 gives

$$\varphi'_S = \Psi \, (A V^{max}_{ES}/A' V_{ES}) \quad (8)$$

and hence the volume fraction of the solvating molecules can be approximated.

Thermodynamic considerations

The aqueous–organic mobile phase in a RP system typically represents a mixture of two liquids of non-limited mutual miscibility. By ascribing to the mixture the properties of a single liquid, we can consider it under chromatographic conditions as a solvent, in which the solute forms an infinitely diluted mobile phase solution. Then, the solute chemical potential in the eluent, μ_E, can be expressed as

$$\mu_E = \mu^0 + RT \cdot \ln \gamma_E X_E \quad (9)$$

where μ^0 is the bulk chemical potential of the pure solute, X_E is the solute mole fraction in the eluent, γ_E is the corresponding solute activity coefficient and R and T are the gas constant and the absolute temperature, respectively.

It is supposed that on partitioning the solute molecules enter the ligate solvation space, displacing preferentially the non-solvating free molecules of the eluent. As a result, no change in the mobile phase composition upon displacement is expected, and this has been confirmed experimentally by Scott and Kucera[22]. Once in the solvation space, the solute molecules form a layer-like solution, whose surface-to-volume ratio appears to be large. This enables us to consider it as a superficial stationary phase solution, in which the solute chemical potential, μ_S, can be presented as

$$\mu_S = \mu_S^0 + RT \cdot \ln \gamma_S X_S \tag{10}$$

where μ_S^0 is the chemical potential of the pure solute as a layer and X_S and γ_S are the corresponding solute mole fraction and activity coefficient in the stationary phase.

Taking into account that the superficial stationary phase solution immediately comes into contact with the mobile phase solution and following the approach of Lucassen-Reynders[23,24], it can be shown that μ_S is related to μ_E by

$$\mu_E = \mu_S + \bar{A}(\sigma_E - \sigma_S) \tag{11}$$

where the term $\bar{A}(\sigma_E - \sigma_S)$ accounts for the excess surface energy change caused by the difference between the surface tensions of both the mobile phase (σ_E) and the stationary phase (σ_S) solutions. Here $\bar{A}$ represents the so-called partial molar area, *i.e.*, the interfacial surface area per mol of solute. Further, the combination of eqns. 9÷11 leads to

$$RT \cdot \ln (X_S/X_E) = RT \cdot \ln(\gamma_E/\gamma_S) + (\mu^0 - \mu_S^0) - \bar{A}(\sigma_E - \sigma_S) \tag{12}$$

where $(\mu^0 - \mu_S^0)$ represents the difference between the chemical potentials of the pure solute in two different standard states, *i.e.*, the bulk and layer, respectively. In accordance with the common convention[25], the layer of the pure solute is considered to be extended to a surface area of value $\bar{A}$. Then

$$\mu^0 - \mu_S^0 = -\bar{A}\sigma \tag{13}$$

where σ is the solute surface tension and eqn. 12 can be rewritten in the form:

$$RT \cdot \ln(X_S/X_E) = RT \cdot \ln(\gamma_E/\gamma_S) - \bar{A}(\sigma_E - \sigma_S + \sigma) \tag{14}$$

Bearing in mind the nature of the HD interactions[9,25,26], the last term of eqn. 14 can be considered as an energy gain due to the hydrophobic bonding. Therefore, it will be designated further by ΔH_H and interpreted as a partial molar excess surface enthalpy effect[25], *i.e.*:

$$\Delta H_H = \bar{A}(\sigma_E - \sigma_S + \sigma) \tag{15}$$

After introducing ΔH_H into eqn. 14, the latter can be rearranged in the form:

$$\ln(X_S/X_E) = \ln(\gamma_E/\gamma_S) - \Delta H_H/RT \tag{16}$$

As follows from eqn. 1, K_{HD} is defined by:

$$K_{HD} = \frac{\text{number of moles of solute interacting with the stationary phase per gram of alkyl ligates}}{\text{number of moles of solute dissolved in unit volume of mobile phase}}$$

On the other hand

$$X_S/X_E = n_S^s n_E/(n_A + n_O)n_E^s \tag{17}$$

where n_S^s and n_E^s are the numbers of moles of solute present in the stationary and mobile phases, respectively, $(n_A + n_O)$ represents the number of moles of stationary phase (including those of the ligates, n_A, and of the solvating molecules, n_O) and n_E is the number of moles of eluent. By rearranging eqn. 17 and multiplying both its sides by $\bar{V}_E/M_A$, where $\bar{V}_E$ is the molar volume of the eluent and M_A is the molecular mass of the ligate, it can be shown that

$$\begin{aligned}(X_S\bar{V}_E/X_EM_A)\,(1 + n_O/n_A) &= n_S^s(n_E\bar{V}_E)/(n_AM_A)n_E^s \\ &= n_S^sV_E/W_An_E^s \equiv K_{HD}\end{aligned} \tag{18}$$

where V_E and W_A are the total volume of the eluent and the total mass of the ligates, respectively. Taking into account the previous discussion about the dependence of n_O/n_A on ψ and W_{AL}, eqn. 18 can be rewritten in the form

$$K_{HD} = (X_S\bar{V}_E/X_EM_A)\,[1 + \mathrm{f}(\psi,W_{AL})] \tag{19}$$

where, in case of a water–methanol mobile phase, $\mathrm{f}(\psi,W_{AL})$ is defined by eqns. 2 and 3. Further, a combination of eqns. 16 and 19 gives:

$$K_{HD} = (\bar{V}_E/M_A)\,[1 + \mathrm{f}(\psi,W_{AL})]\,(\gamma_E/\gamma_S)\exp\left(-\Delta H_H/RT\right) \tag{20}$$

Eqn. 20 represents K_{HD} as a function of both ψ and W_{AL} in an incomplete form. As shown by Horváth *et al.*[27], the molar volume, $\bar{V}_E$, of a binary aqueous–organic eluent can be evaluated from the expression

$$\bar{V}_E = 1/[\psi/M_w + (1 - \psi)/M_o]\,\rho_E \tag{21}$$

where ρ_E is the eluent density and M_w and M_o are the corresponding molecular masses of water and organic modifier in the mobile phase. Thus, $\bar{V}_E$ depends on ψ. On the other hand, γ_E and γ_S are also functions of ψ, while ΔH_H appears to depend on both ψ and W_{AL}. Hence, to be practically employed, in eqn. 20 these quantities need to be either known or defined explicitly.

Unfortunately, it is difficult to find data for the above quantities as regards different solutes in actual RP systems. There are also not many approaches to define them as explicit functions. An useful and simple approach is the regular solution theory developed by Hildebrand and Scott[28] on the basis of the so-called solubility parameter concept. The latter has been applied by some authors[10,29,30] to retention problems in RP systems, but contradictory conclusions have been drawn concerning its adaptability.

It must be mentioned, however, that in all cases of treating the retention by the solubility parameter concept, no attention has been paid to the following evidence: (i) the solute molecules form with the stationary phase a superficial rather than a bulk solution, in which the surface energy change plays an important rôle, and (ii) the solute concentration in the stationary phase solution is sufficiently high, that within the zone spreading the solution cannot be considered as infinitely diluted.

According to the regular solution theory[28,31], the solute activity coefficient in an infinitely diluted mobile phase solution is determined by

$$\gamma_E = \exp\,[\bar{V}(\delta - \delta_E)^2 \varphi_E^2 / RT]$$

where $\bar{V}$ is the solute molar volume, δ and δ_E are the solubility parameters of the solute and the eluent, respectively, and φ_E is the volume fraction of the eluent in the mobile phase solution. In the case of infinite dilution, $\varphi_E \approx 1$, hence:

$$\gamma_E = \exp\,[\bar{V}(\delta - \delta_E)^2 / RT] \tag{22}$$

For a binary eluent mixture

$$\delta_E = \varphi_o \delta_o + (1 - \varphi_o)\delta_w \tag{23}$$

where δ_o and δ_w are the solubility parameters of the organic modifier and water, respectively[31,32]; φ_o is the volume fraction of the organic modifier calculable from

$$\varphi_o = (1 - \psi)/[\psi/\rho_w + (1 - \psi)/\rho_o]\,\rho_o \tag{24}$$

where ρ_w and ρ_o are the densities of water and the organic modifier respectively.

Let us assume now that the solubility parameter concept is applicable to the superficial stationary phase solution[31]. It then follows that

$$\gamma_S = \exp\,[\bar{V}(\delta - \delta_S)^2 \varphi_S^2 / RT] \tag{25}$$

where φ_S is the volume fraction of solvating molecules in the superficial solution, referred to the entire solvation space and δ_S represents the stationary phase solubility parameter defined as:

$$\delta_S = \varphi_S' \delta_o + (1 - \varphi_S')\delta_E \tag{26}$$

Note that φ_S and φ_S' must be distinguished. As stated above, φ_S' is the volume fraction of solvating molecules in the solvation space before the solute molecules enter it. In the case of a water–methanol mobile phase, φ_S' is calculable from eqn. 8.

Another problem is how to evaluate φ_S. This quantity depends not only on both ψ and W_{AL}, but also on the molecular dimensions of the solute. Taking account of the steric hindrance, it can be shown (see Appendix II) that φ_S is related to φ_S' by

$$\varphi_S = \varphi_S' (1 - p) \tag{27}$$

where p is the probability of the solute molecule penetrating among the alkyl ligates. The simplest way of evaluating p is from the relationship

$$p = (\bar{d}_l - \bar{d}_m)/\bar{d}_l \tag{28}$$

where $\bar{d}_l$ is the average chain-to-chain distance determined as the square root of the mean accessible silica surface area around a ligate[2]. The parameter $\bar{d}_m$ represents an averaged linear dimension of the solute molecule. It is obtained from those molecular dimensions which are smaller than $\bar{d}_l$ and, hence, enable either the full or partial penetration of the solute molecule. Using available data[19], $\bar{d}_m$ can easily be evaluated. Thus, the combination of eqns. 27 and 28 leads to:

$$\varphi_S = \varphi_S' \bar{d}_m/\bar{d}_l \tag{29}$$

Thus, by combining eqns. 25–29, it is possible to evaluate γ_S. It must be mentioned, however, that eqn. 25 enables the determination of only a "fraction" of the activity coefficient in the superficial stationary phase solution. This fraction corresponds to that part of the solution consisting of both the solute and the solvating molecules. Hence, it is assumed that the non-solvating eluent molecules, present in the solution, do not play a significant rôle in the formation of the solution, although their influence upon the intermolecular interactions is taken into account by δ_S in enq. 26. Therefore, the γ_S value obtained by using eqn. 25 might be interpreted as "directly contributing" to the solute retention.

To proceed further, we introduce the right-hand sides of eqns. 22 and 25 into eqn. 20. Then the latter acquires the more common and complete form:

$$K_{HD} = \frac{\bar{V}_E}{M_A}[1 + f(\psi, W_{AL})] \exp\left\{\frac{\bar{V}[(\delta-\delta_E)^2 - (\delta-\delta_S)^2\varphi_S'^2] - \Delta H_H}{RT}\right\} \tag{30}$$

As stated above, ΔH_H depends on both ψ and W_{AL} and it would be desirable to know this dependence. However, it is certainly difficult to define ΔH_H as an explicit function of these quantities, although eqn. 15 shows a probable way. Hence, eqn. 30 cannot serve for predicting K_{HD}. Nevertheless, insofar as it enables the evaluation of ΔH_H on the basis of experimentally obtained K_{HD} values, a rearrangement in the form

$$\Delta H_H = \bar{V}[(\delta-\delta_E)^2 - (\delta-\delta_S)^2\varphi_S'^2] - RT \cdot \ln\left\{\frac{K_{HD}M_A}{[1 + f(\psi, W_{AL})]\bar{V}_E}\right\} \tag{31}$$

will be quite useful because, as shown below, $-\Delta H_H$ can also be conventionally interpreted as the energy of the HD interactions between the solute molecules and the alkyl ligates.

EXPERIMENTAL

Materials

LiChrosorb Si 100 of mean particle size 7 μm and octadecyltrichlorosilane (both from E. Merck, Darmstadt, F.R.G.) were used for preparing a RP-18 packing. Eluent mixtures were made up from LiChrosolv grade methanol (E. Merck) and twice distilled water. Additional solvents employed were tetrachloromethane and *n*-heptane (E. Merck), both of LiChrosolv grade.

Twelve solutes of well specified solubility parameters[31,33], benzene, toluene, ethylbenzene, *o*-xylene, *m*-xylene, 1-propanol, 1-butanol, 2-propanol, 2-butanol, 2-butanone, 3-pentanone and *m*-cresol, were selected for the chromatographic experiments. All were obtained from Poly Science (Niles, IL, U.S.A.) and were of minimum 99.5% purity. Deuterated water (E. Merck) was used as a non-retained species for determining the breakthrough time.

Apparatus

Experiments were performed with equipment assembled from a Series 2 liquid chromatograph, a Model LC-25 refractive index (RI) detector, a Model 2 calculating integrator (all from Perkin-Elmer, Norwalk, CT, U.S.A.) and a Servogor Model RE 512 recorder (Goerz Electro, Austria). A polished bore stainless-steel column blank of 250 mm × 4.6 mm I.D. obtained from Perkin-Elmer was used for preparing the column. Both the detector and the column were thermostatted at 25°C by circulating water.

Procedures

The RP-18 packing was prepared by using the procedure of Evans *et al.*[34], suitably modified in the laboratory to allow more complete silica derivatization at room temperature. A bonded phase containing 15.04% carbon was obtained, as compared to that obtained with the conventional method (4–6%). The column was packed via the balanced-density slurry method performed in a home-made device. To determine the packing mass, a combination of weight measurements and a chromatographic determination of the column dead volume in pure methanol was employed.

Eluent mixtures were prepared gravimetrically. Prior to mixing, both methanol and water were thoroughly degassed under vacuum. Portions of each liquid were weighed with an accuracy of ± 0.01 g and mixed. During the experiments the eluents were thermostatted at 25°C and continuously stirred magnetically for additional degassing. At each composition the eluent density was measured at 25°C via the conventional picnometric method.

Before the flow-rate measurements, the column was equilibrated with approximately 200 ml of the corresponding eluent mixture. To avoid column contamination, the packing was washed in all cases prior to changing the mobile phase composition. This was performed by pumping solvents through the column in the order: methanol, tetrachloromethane, *n*-heptane, tetrachloromethane, methanol. Eluent flow-rates were measured by use of a precise burette thermostatted at 25°C. For determining the extra-column volume, five replicate injections of ^2H_2O were made into pure methanol with the column removed.

Samples were prepared by dissolving the selected solutes in methanol at typical concentrations of 1–3 μl ml^{-1}. Three to five successive injections of 3–5 μl from each sample were made to estimate the average solute retention time. In the case of asymmetrical peaks, the finite concentration technique developed by Conder[35] was used for determining the retention.

The solute integral retention effects, E (eqn. 1), were calculated and reduced by the corresponding silanophilic contributions, $E_{SP} = N_{AH}K_{SP}$, according to the procedure described in ref. 2. Only net hydrophobic-dispersive retention effects, $E_{HD} = W_{AL}K_{HD}$, were taken into account when evaluating the solute K_{HD} values.

RESULTS AND DISCUSSION

Stationary phase model and characteristic dependences

Fig. 1 represents an attempt to visualize the model of an alkyl-bonded RP system as discussed above. The mobile phase is a water–methanol mixture. Possible interactions of two different molecules, *n*-hexane and phenol, with the stationary phase are also illustrated.

Following eqn. 2, curve 1 in Fig. 2 demonstrates the dependence of n_O/n_A on ψ at constant W_{AL}. It is in good agreement with the recalculated experimental data[17]. The ratio V_{ES}/n_A is represented as a function of ψ by curve 2 in Fig. 2, and this dependence is employed to evaluate the volume fraction, φ'_S. For both curves 1 and 2, the points at $\psi = 0.241$ corresponding to water–methanol (20:80%, v/v) as mobile

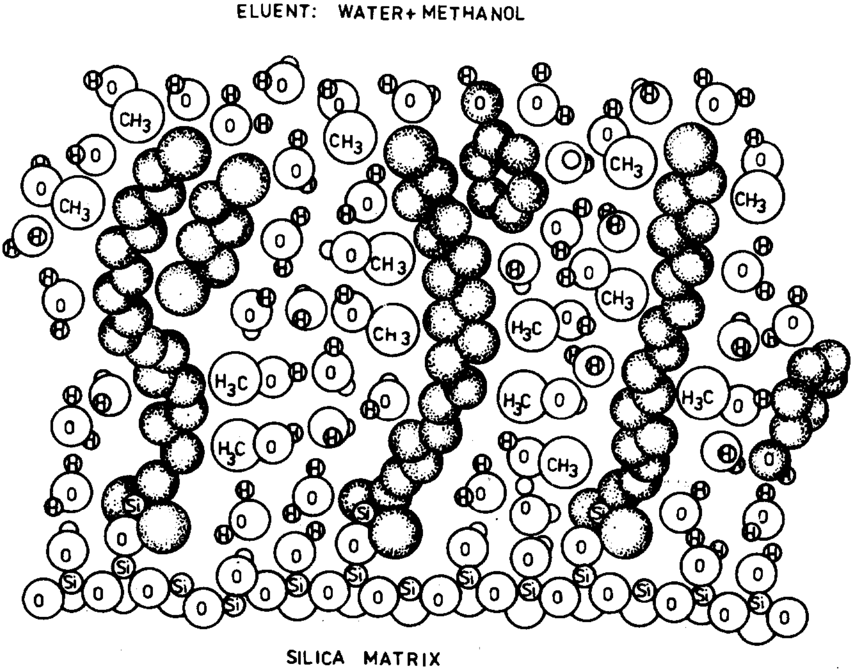

Fig. 1. Model of a C_{18} alkyl-bonded RP system. Alkyl ligates and solute molecules are depicted as shaded circles. Eluent: water–methanol mixture. Solutes: *n*-hexane and phenol.

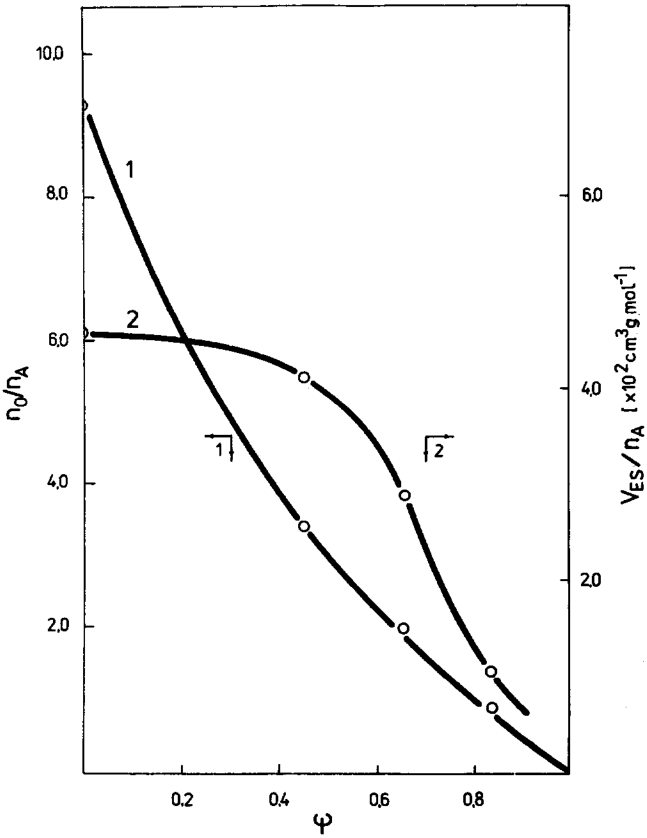

Fig. 2. Dependences of n_O/n_A (1) and V_{ES}/n_A (2) on ψ at W_{AL} = 0.233 g g^{-1}. The points were obtained by recalculating data from ref. 17 for a RP-18–water–methanol system.

phase[17] are omitted, their relatively large deviations probably being due to incorrect determination of V_{ES}. Both n_O/n_A and V_{ES}/n_A decrease at different rates with increasing ψ. However, this is readily explained in terms of the expected ligate shrinkage caused by the water enrichment of the mobile phase.

The dependence of n_O/n_A on W_{AL} (eqn. 2) is demonstrated in Fig. 3 by a family of curves for different ψ values. Each curve is drawn through a set of points calculated for the corresponding experimental values of W_{AL}[2,15,17]. It follows from eqns. 2 and 3, that at ψ = 1, n_O/n_A = 0 in the full range of W_{AL}. For each ψ, the ratio n_O/n_A decreases with increasing W_{AL} and tends to a minimum at W_{AL} = 0.234 g g^{-1}, which is nearly equal to the amount of ligates on the RP-18 packing used in ref. 17. With further increase in W_{AL} a slight increase in n_O/n_A is observed in accordance with eqn. 2. To explain this, it should be noted that a W_{AL} value of 0.268 g g^{-1} represents almost the maximum amount of ligates that can be bonded to the silica (carbon content 23%). The mean chain-to-chain distance in this case is 0.24 nm, which is approximately half the average diameter of methanol molecules. Although methanol molecule penetration among the ligates is strongly inhibited, solvation can occur around

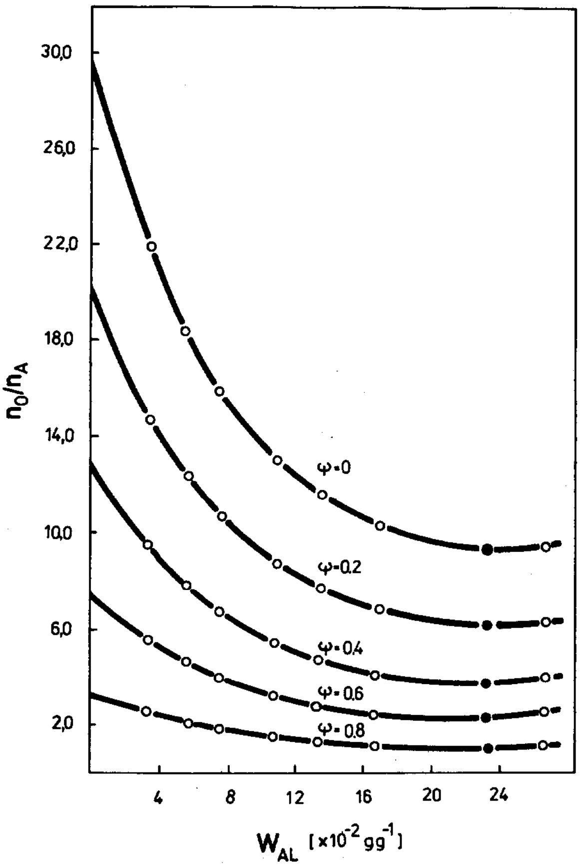

Fig. 3. Dependence of n_O/n_A on W_{AL} at different ψ values according to eqn. 2. All points correspond to RP-18 packings of various W_{AL}. ○, Data from refs. 2 and 15; ●, data from ref. 17. Eluents: water–methanol mixtures.

the end methyl groups of the chains, the latter being able to form a relatively compact layer-like structure. Thus, the existence of a complete solvating layer over this structure appears to be the reason for the slight increase in the n_O/n_A ratio.

Values of all quantities depending on ψ and characterizing both the mobile and the stationary phases of the RP-18 system studied are presented in Table I. As expected, the mobile phase molar volume, $\bar{V}_E$, decreases whereas the solubility parameter, δ_E, increases with increasing ψ. A similar behaviour of the stationary phase solubility parameter, δ_S is observed; however, the rate of increase is lower than that of δ_E in the ψ range of 0.1–0.9. In comparison with the moderately decreasing volume fraction, φ'_S, the ratio n_O/n_A decreases quickly with increasing ψ. This is compatible with the relatively large ligate content of the packing, W_{AL} = 0.1762 g g^{-1}, the average chain-to-chain distance, $\bar{d}_1$, varying within the range 0.2–0.5 nm.

TABLE I

CHARACTERISTICS OF A RP-18–WATER–METHANOL SYSTEM AS FUNCTIONS OF THE MOBILE PHASE COMPOSITION

Parent silica: Si 100; surface area 363 $m^2 g^{-1}$. RP-18 packing: carbon content, 15.04%; mass in the column, 1.9980 g; W_{AL} = 0.1762 g g^{-1}.

ψ	*Mobile phase characteristics*			*Stationary phase characteristics*			
	ρ_E* (g cm^{-3})	$\bar{V}_E$** (cm^3 mol^{-1})	δ_E*** (cal cm^{-3})$^{\frac{1}{2}}$	n_O/n_A§	φ'_S§§	δ_S§§§ (cal cm^{-3})$^{\frac{1}{2}}$	$\bar{d}_1$ (nm)
0.00	0.7865	40.74	14.50	9.949	0.833	14.50	0.494
0.10	0.8155	36.45	15.23	8.140	0.692	14.72	0.479
0.20	0.8415	32.95	15.99	6.633	0.570	15.14	0.461
0.30	0.8673	29.95	16.78	5.357	0.471	15.71	0.441
0.40	0.8907	27.43	17.61	4.264	0.395	16.38	0.418
0.50	0.9123	25.28	18.48	3.316	0.336	17.14	0.391
0.60	0.9310	23.46	19.39	2.487	0.297	17.94	0.359
0.70	0.9497	21.84	20.35	1.756	0.283	18.70	0.319
0.80	0.9660	20.44	21.36	1.105	0.281	19.43	0.267
0.90	0.9824	19.18	22.42	0.524	0.254	20.41	0.195
1.00	0.9971	18.07	23.53	0.000	0.242†	23.53	–

* Determined picnometrically at 25°C.

** Calculated from eqn. 21.

*** Calculated from eqn. 23.

§ Calculated from eqn. 2.

§§ Calculated from eqn. 8.

§§§ Calculated from eqn. 26.

† Extrapolated value corresponding to the volume fraction of water molecules present in the solvation space.

Energy terms governing retention

Let us rewrite eqn. 30 in the form

$$K_{HD} = \Phi \cdot \exp[(\Delta H_E - \Delta H_S - \Delta H_H)/RT] \tag{32}$$

where:

$$\Phi = (\bar{V}_E/M_A)[1 + f(\psi, W_{AL})]$$

According to the solubility parameter concept[32], $\Delta H_E = \bar{V}(\delta - \delta_E)^2$ and $\Delta H_S = \bar{V}(\delta - \delta_S)^2 \varphi_S^2$ are the partial molar excess enthalpies of mixing in the mobile and the stationary phases, respectively. From eqn. 32, the solute retention is governed by three energy terms: ΔH_E, ΔH_S and ΔH_H. Their calculated values depending on ψ are presented in Table II together with the experimental values of K_{HD} for the selected solutes. The solutes are additionally characterized by the values of their molar volume, $\bar{V}$, solubility parameter, δ, and average molecular linear parameter, $\bar{d}_m$. Because of unfavourable retention, K_{HD} values are not listed for the aromatic hydrocarbons with $\psi > 0.5$; for the oxygen-containing solutes with $\psi = 0$, for 3-pentanone with $\psi = 1$ and for *m*-cresol with $\psi > 0.8$.

TABLE II

HYDROPHOBIC-DISPERSIVE PARTITION COEFFICIENT AND ENERGY TERMS FOR SELECTED SOLUTES AS FUNCTIONS OF THE MOBILE PHASE COMPOSITION

Dimensions of quantities: $\bar{V}$ (cm^3 mol^{-1}); δ (cal cm^{-3})$^{\frac{1}{2}}$; $\bar{d}_m$ (nm); K_{HD} (cm^3 g^{-1}); ΔH_E, ΔH_S and ΔH_H (kcal mol^{-1}).

Solute and its characteristics		*K_{HD} and energy terms*	*Water weight fraction of the mobile phase, ψ*										
			0.00	*0.10*	*0.20*	*0.30*	*0.40*	*0.50*	*0.60*	*0.70*	*0.80*	*0.90*	*1.00*
Benzene		K_{HD}	1.073	2.299	4.314	7.567	13.52	23.99					
$\bar{V}$	89.41	ΔH_E	2.55	3.29	4.17	5.19	6.38	7.77					
δ	9.16	ΔH_S	0.837	0.667	0.566	0.507	0.482	0.487					
$\bar{d}_m$*	0.340	ΔH_H	2.01	2.30	2.73	3.32	4.03	4.90					
Toluene		K_{HD}	1.533	3.264	6.545	12.16	23.58	46.60					
$\bar{V}$	106.84	ΔH_E	3.31	4.24	5.33	6.58	8.05	9.74					
δ	8.93	ΔH_S	1.29	1.02	0.863	0.768	0.726	0.729					
$\bar{d}_m$*	0.370	ΔH_H	2.11	2.68	3.34	4.17	5.12	6.24					
Ethylbenzene		K_{HD}	1.828	4.087	8.680	17.46	36.95	81.10					
$\bar{V}$	123.08	ΔH_E	3.94	5.03	6.29	7.76	9.47	11.4					
δ	8.84	ΔH_S	1.74	1.38	1.16	1.03	0.972	0.959					
$\bar{d}_m$*	0.394	ΔH_H	2.18	2.97	3.85	4.87	6.02	7.37					
o-Xylene		K_{HD}	2.083	4.479	9.327	18.16	37.70	80.85					
$\bar{V}$	121.21	ΔH_E	3.59	4.61	5.82	7.22	8.86	10.8					
δ	9.06	ΔH_S	1.39	1.11	0.939	0.838	0.796	0.801					
$\bar{d}_m$*	0.370	ΔH_H	2.09	2.78	3.56	4.50	5.58	6.85					
m-Xylene		K_{HD}	2.157	4.626	9.775	19.49	41.24	90.31					
$\bar{V}$	123.48	ΔH_E	3.90	4.98	6.24	7.71	9.41	11.4					
δ	8.88	ΔH_S	1.52	1.20	1.01	0.901	0.851	0.853					
$\bar{d}_m$*	0.370	ΔH_H	2.26	3.03	3.87	4.88	6.02	7.36					

1-Propanol		K_{HD}	0.454	0.985	1.601	2.180	3.065	4.201	6.012	8.468	13.27	32.02
$\bar{V}$	75.19	ΔH_E	0.699	1.09	1.59	2.22	2.98	3.91	5.02	6.34	7.88	9.69
δ	12.18	ΔH_S	0.203	0.202	0.208	0.207	0.209	0.220	0.255	0.311	0.329	0.567
$\bar{d}_m$*	0.448	ΔH_H	1.13	0.893	0.935	1.21	1.61	2.17	2.85	3.71	4.74	5.50
1-Butanol		K_{HD}	0.715	1.542	2.537	4.008	6.119	10.01	16.30	26.23	45.41	112.8
$\bar{V}$	91.99	ΔH_E	1.21	1.77	2.47	3.32	4.35	5.58	7.04	8.76	10.8	13.1
δ	11.60	ΔH_S	0.375	0.354	0.345	0.329	0.319	0.326	0.370	0.444	0.461	0.767
$\bar{d}_m$*	0.448	ΔH_H	1.20	1.16	1.40	1.84	2.46	3.22	4.17	5.33	6.77	7.96
2-Propanol		K_{HD}	0.449	0.883	1.470	2.004	2.526	3.525	4.939	6.773	10.72	27.01
$\bar{V}$	76.97	ΔH_E	1.11	1.59	2.19	2.93	3.81	4.86	6.11	7.57	9.30	11.3
δ	11.44	ΔH_S	0.397	0.343	0.312	0.294	0.283	0.287	0.324	0.387	0.400	0.659
$\bar{d}_m$*	0.490	ΔH_H	1.35	1.32	1.49	1.89	2.48	3.16	3.99	5.00	6.20	7.08
2-Butanol		K_{HD}	0.670	1.401	2.327	3.525	5.262	8.140	12.73	19.85	34.38	88.09
$\bar{V}$	92.39	ΔH_E	1.59	2.23	3.00	3.94	5.06	6.38	7.94	9.76	11.9	14.3
δ	11.08	ΔH_S	0.586	0.495	0.440	0.406	0.384	0.383	0.428	0.508	0.519	0.839
$\bar{d}_m$*	0.490	ΔH_H	1.40	1.53	1.89	2.45	3.19	4.09	5.15	6.44	7.99	9.27
2-Butanone		K_{HD}	0.630	1.182	1.868	2.628	3.667	5.495	8.674	14.27	28.29	105.6
$\bar{V}$	90.1	ΔH_E	3.17	4.03	5.04	6.22	7.59	9.17	11.0	13.1	15.5	18.2
δ	9.3	ΔH_S	1.25	0.999	0.823	0.706	0.626	0.593	0.636	0.728	0.717	1.07
$\bar{d}_m$*	0.475	ΔH_H	2.36	2.93	3.68	4.61	5.70	6.90	8.23	9.75	11.5	12.9
3-Pentanone		K_{HD}	0.931	1.775	2.912	4.570	6.988	11.68	20.22	37.29	80.49	
$\bar{V}$	106.41	ΔH_E	4.05	5.11	6.34	7.78	9.44	11.4	13.6	16.1	19.0	
δ	9.06	ΔH_S	1.61	1.28	1.05	0.891	0.786	0.740	0.790	0.902	0.884	
$\bar{d}_m$*	0.475	ΔH_H	2.65	3.49	4.49	5.65	7.01	8.49	10.1	12.0	14.2	
m-Cresol		K_{HD}	0.789	1.948	3.502	6.278	11.47	22.93	47.03	94.62		
$\bar{V}$	104.7	ΔH_E	2.65	3.51	4.53	5.75	7.18	8.84	10.8	13.0		
δ	10.2	ΔH_S	0.669	0.586	0.544	0.536	0.559	0.553	0.604	0.703		
$\bar{d}_m$*	0.387	ΔH_H	2.28	2.52	3.08	3.79	4.72	5.76	7.05	8.59		

* Evaluated at $\psi = 0$ in correspondence with the chain-to-chain distance, $\bar{d}_1$, of the RP-18 packing in Table I.

In terms of the solubility parameter concept, the greater the enthalpy of mixing in an infinitely diluted solution, the smaller is the solute solubility in the corresponding solvent. As this holds for the mobile phase solution in a RP system, the increase in ΔH_E with increasing ψ agrees well with the decreased solubility of the solutes in the water-enriched eluent mixtures. With a predominant methanol content of the mobile phase, ΔH_E for the alcohols is smaller than that for the aromatic hydrocarbons, thus reflecting the higher solubility of the former in methanol. Ketones exhibit ΔH_E values comparable with those for the hydrocarbons, while for *m*-cresol has ΔH_E values intermediate between those for alcohols and hydrocarbons.

As to the enthalpy of mixing in the superficial stationary phase solution, two important factors leading to lower values should be mentioned. First, the stationary phase solution is usually concentrated, which is a premise for higher solute solubility due to strong solute–solute interactions. Secondly, the presence of passive alkyl ligates (favouring steric hindrance) lowers the overall energy of the stationary phase solution and, hence, also the solute partial molar enthalpy of mixing, ΔH_S. This is the main factor responsible for the decrease in ΔH_S when ψ varies between 0 and 0.5. When $\psi > 0.5$, the low solute solubility in the water-enriched stationary phase layer becomes responsible for the gradual increase in ΔH_S.

As seen from Table II, for each solute the partial molar enthalpy, ΔH_H, increases with increasing ψ. This corresponds to the reinforcement of hydrophobic bonding upon water enrichment of the mobile phase. At constant eluent composition, both the polarity and the area of contact of the solute molecule with the ligates determine the extent of the hydrophobic effect. Usually, this effect is stronger for less polar molecules and for those having a large non-polar moiety. Since the solubility parameter, δ, is a measure of the solute polarity, a certain correlation between ΔH_H and δ is observed. The smaller the solubility parameter, the greater is the hydrophobic effect and *vice versa*. This correlation holds for most solutes within the full range of ψ values in our study. Deviations are observed for the solute pairs toluene–*o*-xylene, ethylbenzene–*m*-xylene and 1-butanol–2-propanol and a brief explanation of their behaviour is given below.

Toluene ($\delta = 8.93$) is insignificantly less polar than *o*-xylene ($\delta = 9.06$) and the enthalpy effects of solvophobic bonding for both solutes, *i.e.*, their ΔH_H values at $\psi = 0$, correlate well with the corresponding δ values, the difference being negligible. At $\psi \geqslant 0.1$ however, ΔH_H for *o*-xylene becomes greater than that for toluene. This is due probably to *o*-xylene exhibiting a greater surface area than that of toluene. As expected, with equal or slightly different solute polarities, the contact area plays an important rôle.

Analogously, because of the weaker steric hindrance, the more compact molecule of *m*-xylene ($\delta = 8.88$) exhibits a greater effective contact area in comparison with that of ethylbenzene ($\delta = 8.84$). This is especially evident, when molecules of approximately equal polarities are able to penetrate among the ligates, *i.e.*, when $\psi < 0.5$. With further increase in ψ, however, the penetration is avoided and then, in agreement with the correlation observed, ΔH_H for ethylbenzene tends to become greater than that for *m*-xylene.

An interesting subject of debate involves the variations of ΔH_H for both 1-butanol ($\delta = 11.60$) and 2-propanol ($\delta = 11.44$). With methanol-enriched eluent mixtures, ΔH_H for 2-propanol is greater than that for 1-butanol, thus obeying the correlation with δ. When the water content of the mobile phase becomes noticeable ($\psi \geqslant 0.6$), an inversion occurs, so that the slightly more polar 1-butanol exhibits greater ΔH_H values in comparison with the less polar 2-propanol. To explain this behaviour, let us compare the $\bar{d}_m$ values of both solutes (Table II) with the chain-to-chain distances, $\bar{d}_1$, at ψ within the range of 0.1–0.5 (Table I). Even with methanol-enriched eluents, the full penetration of these molecules among the ligates is prevented. A partial penetration, however, seems possible, thus the difference between the effective contact areas of the molecules should be insignificant. Although, in comparison with 2-propanol, 1-butanol exhibits *a priori* a greater molecular area, at $\psi \leqslant 0.5$ the two solutes are subject to approximately equal steric conditions and, hence, the main factor responsible for their hydrophobic bonding appears to be the solute polarity. With water-enriched mobile phases, however, the molecules of both solutes interact under the conditions of a stationary phase layer. Then, in the absence of steric hindrance, the size of the area of effective contact becomes more substantial for the hydrophobic effect than the solute polarity.

Further, let us compare the variations of the hydrophobic-dispersive partition coefficient with those of the corresponding enthalpy effects for the solutes under the same chromatographic conditions. It is evident from Table II that there is no dominant dependence of K_{HD} on the any of the energy terms which would be capable of explaining the deviations observed. Obviously, K_{HD} is a characteristic quantity that incorporates the influence of the three ΔH terms, thus describing quantitatively the solute retention in a RP system. Comparing all ΔH values for a solute, it can be concluded that the strongest interactions arise in the mobile phase, in accord with accepted opinion[12,27]. However, it is of interest to discuss the rôle of the hydrophobic bonding and its significance to the retention.

It is well known that the hydrophobic effect is connected with the repulsive forces between a solute and the water molecules in a mobile phase. As a consequence, the solute molecules are constrained to associate with the alkyl ligates. The energy gain, ΔH_H, that accompanies this process can be considered to have "originated" from the mobile phase and to be "introduced" into the stationary phase. This enables us to interpret the hydrophobic bonding as a result of the so-called HD interactions between the solute molecules and the ligates[1,2]. Conventionally, we can ascribe a negative sign to the ΔH_H value, *i.e.*, $-\Delta H_H$, and consider it as a partial molar energy of the HD interaction. It should be borne in mind, however, that this "interaction energy" does not follow from the reduction of the internal energy of both the stationary and the mobile phases. Finally, it is apparent that the hydrophobic bonding "compensates" energetically the weak interactions associated with the stationary phase. In other words, it increases the solute concentration in the stationary phase, thus realizing the retention itself.

Dependence of retention and hydrophobic bonding on the amount of alkyl ligates

Experimental data of Hennion *et al.*[15] enabled us to study the retention behaviour as well as the hydrophobic bonding of the polyaromatic hydrocarbons and the hydroxy-aromatic compounds as a function of the amount of ligates. Solute K_{HD} values obtained with RP packings of different W_{AL} were taken from ref. 2. Two water–methanol eluent mixtures (30:70 and 60:40, v/v, respectively) have been used in the experiments. The values of all the mobile and stationary phase characteristics,

TABLE III

MOBILE AND STATIONARY PHASE CHARACTERISTICS DEPENDENT ON THE MOBILE PHASE COMPOSITION

Calculated according to the experimental conditions of Hennion *et al.*[15]

Eluent	ψ	*Mobile phase*			*Stationary phase*	
		$\bar{V}_E$ (cm^3 mol^{-1})	ρ_E (g cm^{-3})	δ_E (cal cm^{-3})$^{\frac{1}{2}}$	φ'_S	δ_S (cal cm^{-3})$^{\frac{1}{2}}$
Water–methanol (30:70)	0.352	28.60	0.8793	17.21	0.415	16.09
Water–methanol (60:40)	0.655	22.53	0.9419	19.92	0.289	18.35

dependent only on ψ, are presented in Table III. Those stationary phase characteristics which depend on both ψ and W_{AL} are given in Table IV.

A serious problem connected with the calculation of the enthalpy effects in eqn. 32 was the absence of data for the solubility parameters. Usually, δ values are given for liquid compounds at 25°C[31,33]. Except for benzene, the solutes listed in Table V are solids under normal conditions and no data for their solubility parameters were available. To use the experimental information, we attempted to evaluate δ following the recommendations reviewed in ref. 33. The method of the so-called molar attraction constants[36] was employed in the case of polyaromatic hydrocarbons. As it is inapplicable to the hydroxy-aromatic compounds, their δ values were evaluated by using Hildebrand's empirical equation[28], mainly applied to liquids. Obviously, there is no guarantee of the reliability of the calculated values, however, they enabled us to estimate approximately the enthalpy effects and hence to shed some light on the dependence of both the retention and the hydrophobic bonding upon the amount of alkyl ligates.

All solutes listed in Table V are characterized by the values of $\bar{V}$, δ and $\bar{d}_m$. Since the hydrocarbons and the hydroxycompounds were eluted with different mobile

TABLE IV

STATIONARY PHASE CHARACTERISTICS DEPENDENT OF BOTH THE MOBILE PHASE COMPOSITION AND THE AMOUNT OF ALKYL LIGATES

Calculated according to the experimental conditions of Hennion *et al.*[15].

Amount of alkyl ligates, $W_{AL} \cdot 10^{-2}$ (g g^{-1})	ψ = 0.352		ψ = 0.655	
	n_O/n_A	$\bar{d}_1$ (nm)	n_O/n_A	$\bar{d}_1$ (nm)
3.39	10.48	2.10	4.559	2.08
5.62	8.750	1.53	3.806	1.49
7.62	7.578	1.21	3.296	1.16
11.0	6.177	0.829	2.687	0.746
13.5	5.480	0.619	2.384	0.521
16.9	4.861	0.467	2.114	0.383
26.8	4.540	0.203	1.974	0.158

phases (ψ = 0.352 and 0.655, respectively), they are presented as two separate groups together with the corresponding data and the calculated results.

The dependence of K_{HD} on W_{AL} for the solutes studied is conveniently visualized in Figs. 4 and 5. For each compound there is an obvious tendency for K_{HD} to increase with increasing W_{AL} up to a definite value, beyond which K_{HD} decreases. As seen from Fig. 4, the maxima for hydrocarbons lie within a narrow W_{AL} range between 0.16 and 0.19 g g^{-1}. For the hydroxycompounds, however (Fig. 5), the maxima appear at different W_{AL} values. Hence, the observed phenomena seem well compatible with the solute molecular structure and dimensions, which to a large extent determine the possibility of penetration among the ligates.

As is evident from Table V, the solubility parameters of the polyaromatic hydrocarbons increase slightly with the number of rings. The rise in polarity is undoubtedly connected with the increased effect of conjugation in the molecules. Nevertheless, their δ values remain substantially lower than those of the hydroxy-aromatic compounds, whose polarity is determined by the presence of hydroxyl groups. Therefore, a difference is observed in the solubilities of both types of solutes in the mobile phase. With increasing δ, the enthalpy of mixing of the hydroxy-compounds with the eluent, ΔH_E, decreases, corresponding to a higher solubility. On the contrary, except for anthracene, increase in δ for the hydrocarbons leads to a greater ΔH_E value, in agreement with their lower solubility, as soon as the number of rings rises. Obviously, the different nature of the polarity is responsible for the observed phenomenon. In terms of the solubility parameter concept, this is due to the higher energy of cavity formation necessary for the large hydrocarbon molecules.

As expected, the enthalpy of mixing with the stationary phase, ΔH_S, is small

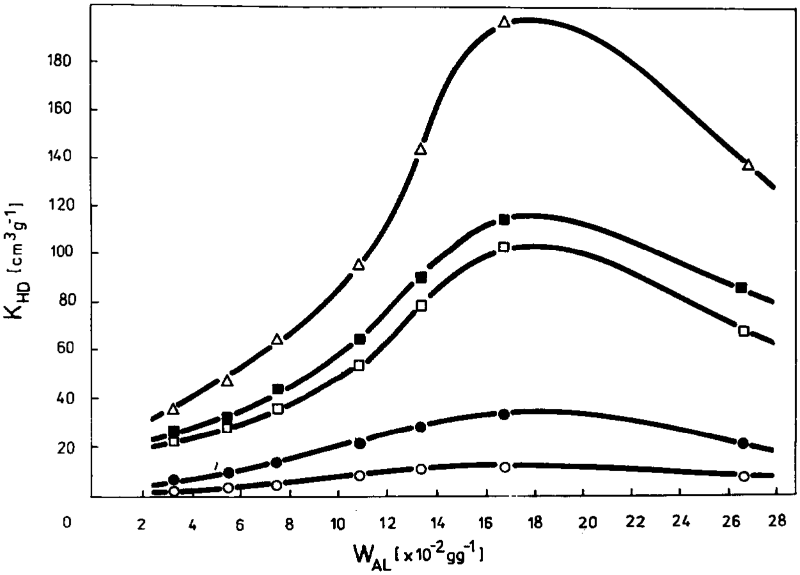

Fig. 4. Dependence of K_{HD} on W_{AL} for polyaromatic hydrocarbons under the experimental conditions of Hennion *et al.*[15]: RP-18 packings; eluent mixture, water–methanol, ψ = 0.352. ○, Benzene; ●, naphthalene; □, phenanthrene; ■, anthracene; △, pyrene.

TABLE V

ENERGY TERMS FOR POLYAROMATIC HYDROCARBONS AND HYDROXY-AROMATIC COMPOUNDS AS FUNCTIONS OF THE AMOUNT OF ALKYL LIGATES

Calculated according to the experimental conditions of Hennion *et al.*[15]. Dimensions of quantities as in Table II.

ψ	*Solute*	*Solute characteristics*			*Energy terms*		*Amount of alkyl ligates,* $W_{AL} \cdot 10^{-2}$ *(g g⁻¹)*						
		$\bar{V}$	δ	$\bar{d}_m$*	ΔH_E**		*3.39*	*5.62*	*7.62*	*11.0*	*13.5*	*16.9*	*26.8*
0.352	Benzene***	89.41	9.16	0.538	5.79	ΔH_S	0.048	0.091	0.146	0.312	0.558	0.738	0.738
						ΔH_H	5.54	5.18	4.82	4.13	3.65	3.35	3.66
	Naphthalene	111.50	9.38	0.662	6.84	ΔH_S	0.086	0.162	0.258	0.551	0.863	0.863	0.863
						ΔH_H	5.82	5.39	5.03	4.36	3.83	3.65	3.93
	Phenanthrene	152.34	9.56	0.761	8.92	ΔH_S	0.147	0.276	0.442	0.941	1.12	1.12	1.12
						ΔH_H	7.04	6.71	6.34	5.53	5.05	4.82	5.03
	Anthracene	143.40	9.66	0.743	8.17	ΔH_S	0.128	0.240	0.384	0.819	1.02	1.02	1.02
						ΔH_H	6.25	5.92	5.53	4.77	4.25	4.11	4.25
	Pyrene	163.11	9.70	0.801	9.20	ΔH_S	0.167	0.314	0.502	1.07	1.15	1.15	1.15
						ΔH_H	7.08	6.66	6.21	5.31	4.94	4.69	4.87
0.655	Hydroquinone	83.42	13.8	0.596	3.12	ΔH_S	0.012	0.023	0.038	0.092	0.144	0.144	0.144
						ΔH_H	1.44	1.16	0.975	0.885	0.875	0.951	1.25
	Resorcinol	87.40	13.3	0.539	3.83	ΔH_S	0.013	0.024	0.040	0.097	0.186	0.186	0.186
						ΔH_H	1.80	1.58	1.41	1.22	1.16	1.23	1.53
	5-Methylresorcinol	97.88	12.8	0.569	4.96	ΔH_S	0.019	0.037	0.061	0.146	0.252	0.252	0.252
						ΔH_H	2.67	2.39	2.19	1.94	1.81	1.85	2.21
	Phenol	89.38	11.3	0.519	6.64	ΔH_S	0.023	0.045	0.074	0.180	0.368	0.371	0.371
						ΔH_H	3.72	3.47	3.21	2.84	2.50	2.48	2.84

* Evaluated at ψ values corresponding to the chain-to-chain distance, $\bar{d}_1$, of the RP-18 packing having $W_{AL} = 3.39 \cdot 10^{-2}$ g g⁻¹.

** Not dependent on the amount of alkyl ligates.

*** Employed as a reference compound when considering the influence of condensed rings.

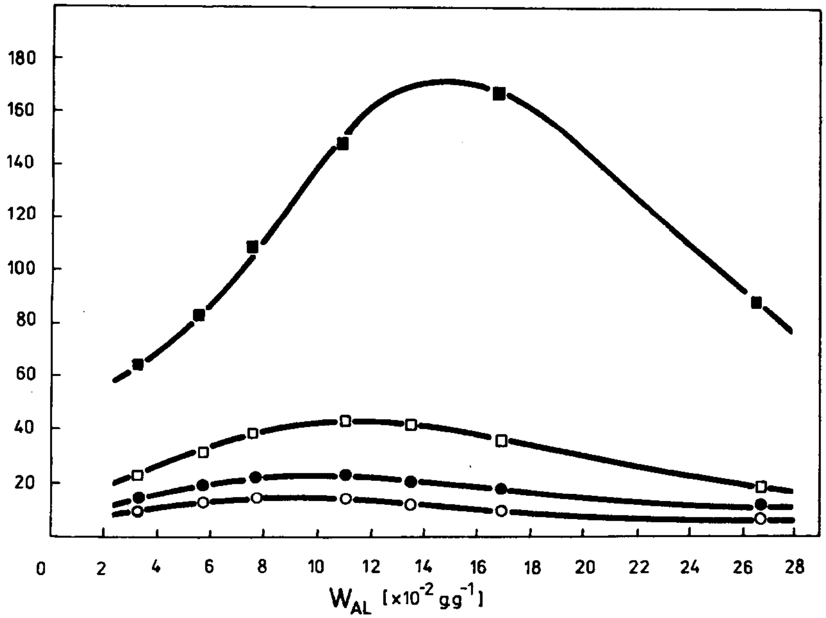

Fig. 5. Dependence of K_{HD} on W_{AL} for hydroxy-aromatic compounds under the experimental conditions of Hennion *et al.*[15]: RP-18 packings; eluent mixture, water–methanol, ψ = 0.655. ○, Hydroquinone; ●, resorcinol; □, 5-methylresorcinol; ■, phenol.

for all the solutes. It tends to increase slightly as W_{AL} increases to a definite value. Obviously, the restricted penetration of the solute molecules among the ligates leads to a lowering their solubility in the stationary phase. As soon as full penetration of the molecules becomes impossible, their interactions with the stationary phase layer remain invariable, corresponding to the constant ΔH_S values obtained with large amount of ligates.

Comparing the enthalpy effects of hydrophobic bonding for the polyaromatic hydrocarbons, it is seen that ΔH_H generally increases with the number of rings, in spite of the increased polarity. This is due to the enlargement of the molecular surface area capable for contact with the ligates. Since the molecules of phenanthrene and anthracene exhibit equal areas, their hydrophobic bonding is governed by the polarity. Therefore, the ΔH_H values for anthracene (δ = 9.66) always remain lower than those for phenanthrene (δ = 9.56).

An interesting exception is the large molecule of pyrene (δ = 9.70). Contrary to initial expectations, its ΔH_H values are generally lower than those for phenanthrene. However, one should not forget that the hydrophobic effect is favoured by the contact area between the solute molecule and the ligate, rather than by the whole solute molecular surface area. It may well be that the fourth ring contributes negligibly to the extent of the contact area realized with a molecule having three rings. Moreover, the penetration of pyrene into the stationary phase appears to be sterically more hindered than that of phenanthrene. Therefore, we consider the ΔH_H values obtained as quite reasonable.

As for the hydroxy compounds, their ΔH_H values correlate well with the corresponding solubility parameters. The more polar the solute, the weaker is the effect of hydrophobic bonding. Nevertheless, in comparison with the hydrocarbons, these solutes have been eluted with a more polar mobile phase, and their ΔH_H values are smaller. Hence, this is also evidence of the importance of both the polarity and the contact area for the hydrophobic effect.

As is seen from Table V, the molar enthalpy of hydrophobic bonding decreases for all the solutes with an initial increase in the amount of ligates. This is in agreement with the decreased concentration of the free eluent molecules in the ligate solvation space and especially that of water. As soon as the solute penetration becomes impossible at a definite value of W_{AL}, the molecules start to associate with the end methyl groups of the chains. Then, further increase in W_{AL} leads to a more compact ligate layer structure, thus increasing the probability for a better contact with the solute molecules. As a result, ΔH_H also starts to increase in correspondence with the conditions favourable for hydrophobic bonding.

The behaviour of ΔH_H is illustrated in Fig. 6. For hydrocarbons the minimum ΔH_H value conforms to the maximum of the hydrophobic-dispersive partition coefficient, K_{HD}. An analogous situation is observed for the hydroxy compounds. However, comparing Figs. 5 and 6, it is seen that for the different solutes the ΔH_H minimum generally appears at a slightly higher W_{AL} value than the corresponding maximum of K_{HD}. A probable explanation of this discrepancy could be looked for in the inaccuracy of the calculated solubility parameters, especially since Hildebrand's equation tends to give increased values for δ.

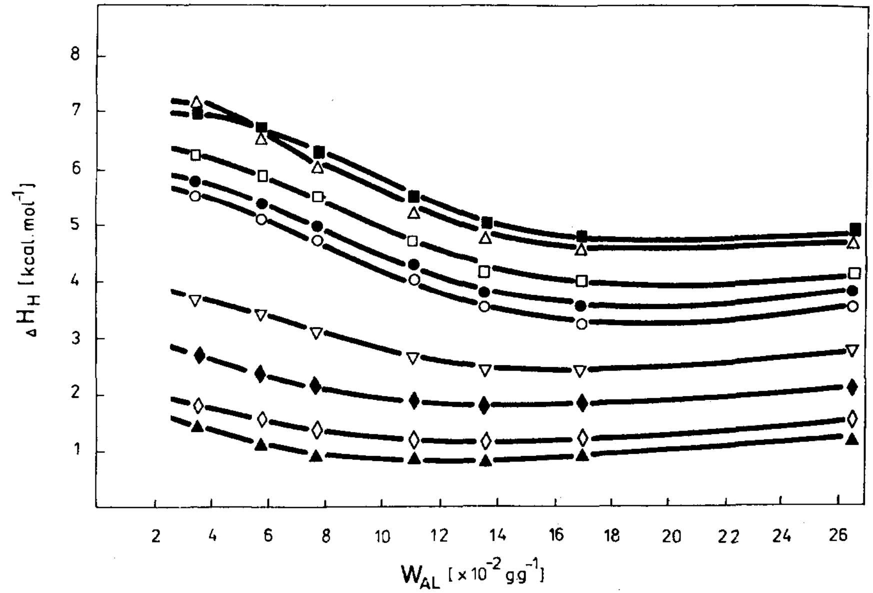

Fig. 6. Dependence of ΔH_H on W_{AL} for solutes chromatographed under the experimental conditions of Hennion *et al.*[15]. Polyaromatic hydrocarbons: ○, benzene; ●, naphthalene; □, anthracene; ■, phenanthrene; △, pyrene. Hydroxy-aromatic compounds: ▲, hydroquinone; ◇, resorcinol; ◆, 5-methylresorcinol; ▽, phenol.

In conclusion, it must be emphasized that the retention of even non-dissociated solutes in an alkyl-bonded RP system is an extraordinarily complex phenomenon governed by a variety of factors. The most important of them are:

(1) The nature of the solute expressed in terms of its molecular structure and dimensions, polarity, solubility in both the mobile and the stationary phases

(2) The mobile phase composition including the type of organic modifier and the water content

(3) The stationary phase formation determined by the structure, dimensions and amount of ligates causing the steric hindrance, as well as the composition of the extracted solvent filling the ligate solvation space.

The combined influence of all these factors determines the solute interactions with the RP system as a whole and, hence, the magnitude of the corresponding energy terms reflecting the solute behaviour in the system. If the so-called "side effects" occur, *e.g.*, equilibria connected with solute dissociation, then additional investigations are required in order to obtain insight into the nature of retention.

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APPENDIX I

The correlation between n_O/n_A and ψ at W_{AL} = const. can be approximated by the function

$$n_O/n_A = \Psi L(W_{AL}) \tag{A1}$$

where

$$\Psi = (1 - \psi)/(1 + \psi)$$

and $L(W_{AL})$ is a parameter dependent on W_{AL}. At W_{AL} = const., $L(W_{AL})$ must also be constant, as confirmed on the basis of data in ref. 17. The relative deviations of the calculated L values from the average L value were within ±1%.

Further, it was assumed that eqn. A1 holds for a relatively large W_{AL} range. An adequate approximation for $L(W_{AL})$ was achieved as follows.

(1) Since n_O is obviously dependent on both Ψ and n_A (where $n_A = W_{AL}/M_A$ and M_A is the molecular mass of the alkyl ligates), then, at Ψ = const., n_O will be a function only of W_{AL}. Hence:

$$n_O(W_{AL})/W_{AL} = (\Psi/M_A)L(W_{AL}) \qquad (\Psi = \text{const.}) \tag{A2}$$

Rearranging eqn. A2 in the form

$$L(W_{AL}) = (Q/W_{AL})n_O(W_{AL}) \tag{A3}$$

where $Q = M_A/\Psi$ is a constant, it becomes evident that $L(W_{AL})$ can be represented as a product of two functions: Q/W_{AL} and $n_O(W_{AL})$. The first one is an hyperbolic dependence, which tends to decrease, always remaining positive when W_{AL} increases. According to the accepted stationary phase model, $n_O(W_{AL})$ is expected to the positive and will increase with increasing W_{AL}. However, $n_O(W_{AL})$ cannot be linearly dependent on W_{AL}, because an increase in the surface ligate concentration will lead to a rapid decrease in the solvation space and, hence, to a non-proportional increase in n_O.

(2) On the other hand, at $W_{AL} = 0$, *i.e.*, $n_A = 0$, it follows that $n_O(W_{AL}) = 0$ (or $n_O = 0$), therefore $L(W_{AL})$ is undefined for $W_{AL} = 0$. However, for W_{AL} values very close to zero, the ratio n_O/n_A must be a real positive number, less but nearly equal to the maximum capacity of the solvation space at a given Ψ.

Summarizing the above considerations, the following conclusions can be made about the character of the $L(W_{AL})$ function at each Ψ = const.: (i) as a product of two positive functions, the first of which rapidly decreases while the second slowly increases with increasing W_{AL}, $L(W_{AL})$ will be a positive decreasing function, having a minimum; (ii) when W_{AL} tends to zero, $L(W_{AL})$ approaches its greatest value; (iii) with increasing W_{AL}, $L(W_{AL})$ decreases nearly in inverse proportion to W_{AL}, thus reaching its minimum at a definite W_{AL} value. Hence, a plot of $L(W_{AL})$ *vs.* W_{AL} is expected to show a nearly parabolic dependence, which can be approximated either by a second-degree polynomial or by an exponential function of the type $A \cdot \exp(BW_{AL}^2 + CW_{AL})$. Since the exponential function offers some advantages, it was preferred over the polynomial.

(3) It follows from eqn. A1 that n_O/n_A depends linearly on Ψ at each W_{AL}. Considering this dependence in a three-dimensional coordinate system (n_O/n_A *vs.* Ψ *vs.* W_{AL}) and using ordinary trigonometric rearrangements, it can be shown that at a reasonable constant n_O/n_A value, a definite value of Ψ corresponds to each W_{AL}. The Ψ value can be determined by solving a transcendental equation of the type $\Psi = \mathrm{f}[n_O/n_A, W_{AL}, \cos^2 \mathrm{arctg}\, \lambda\, (\Psi, W_{AL})]$, employing the conventional Newton iterative procedure. Thus, for a set of RP-18 packings of different W_{AL}[15], the corresponding Ψ values were calculated and a system of equations was obtained on the basis of

$$n_O/n_A = \Psi A \cdot \exp(BW_{AL}^2 + CW_{AL}) \tag{A4}$$

holding n_O/n_A the same as when determining the Ψ values. Further, the constants A, B and C were specified by using the method of rigorous least squares adjustment, applicable to non-linear problems[20,21].

APPENDIX II

In the case of a very low ligate concentration on the silica surface, we can consider the solute partitioning not to be sterically hindered. Then, it might be expected that the solute molecules are able to occupy the entire ligate solvation space, the solute volume fraction being equal to unity. Therefore, the following equation will hold

$$\lambda(1 - \varphi_E) = 1 \tag{A5}$$

remembering that $(1 - \varphi_E)$ is the solute volume fraction in the mobile phase solution, while λ is a proportionality parameter.

As far as eqn. A5 is valid for the entire solvation space, it must also be valid for that part of this space initially occupied only by the solvating molecules, φ'_S. Then:

$$\varphi'_S = \lambda(1 - \varphi_E)\,\varphi'_S \qquad \text{(A6)}$$

In other words, eqn. A6 reflects the fact that the volume fraction of the solute, which has displaced the solvating molecules, remains equal to their initial volume fraction, φ'_S.

In a real RP system the surface ligate concentration is usually sufficiently high to cause steric hindrance to the solute partitioning. Under these conditions, the superficial stationary phase solution is formed by both the solvating and the solute molecules, their volume fractions being φ_S and φ, respectively. Since the latter are referred to the solvation space, we may write

$$\varphi_S + \varphi = \varphi'_S$$

or

$$\varphi_S = \varphi'_S - \varphi \qquad \text{(A7)}$$

Hence, in the case of steric hindrance, instead of eqn. A6 we have

$$\varphi = \lambda(1 - \varphi_E)\varphi'_S p \qquad \text{(A8)}$$

where p is the probability of the solute molecules penetrating among the ligates. A combination of eqns. A5 and A8 yields

$$\varphi = \varphi'_S p$$

which, when introduced into eqn. A7, leads finally to eqn. 27, *e.g.*:

$$\varphi_S = \varphi'_S (1 - p)$$

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