Journal of Chromatography A, 656 (1993) 113–133
Elsevier Science Publishers B.V., Amsterdam

CHROM. 25 464

Revisionist look at solvophobic driving forces in reversed-phase liquid chromatography

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ABSTRACT

Based on the use of alkylbenzenes as test solutes, most of the free energy of retention in reversed-phase liquid chromatography (RPLC) is shown to arise from net attractive (exoergic) processes in the stationary phase, and not from net repulsive (endoergic) processes in the mobile phase. The classical view of the "passive" role of bonded phase ligands is challenged. However, it is also shown that variations in retention upon changing the mobile phase are dominated by alterations in the net processes in the mobile phase. Furthermore, it is shown that the free energy of transfer of a methylene group from the mobile phase to a bonded reversed phase over a wide range in mobile phase composition, is similar but not equal, to the free energy of transfer of a methylene group from the same mobile phase to pure bulk hexadecane. This observation is in accord with the partition model view of the mechanism of RPLC. Finally, by comparison of measured and computed activity coefficients, the regular solution theory is shown to be a grossly inadequate model of interactions in water and hydro-organic mixtures. It should not be used to model retention in aqueous mobile phases.

INTRODUCTION

Reversed-phase liquid chromatography (RPLC) is the most important mode of liquid chromatography [1]. Despite its importance and the numerous studies pertaining to the theory of retention in RPLC, there is still considerable uncertainty as to the mechanism of the overall process [2–10]. There are many areas of debate. For example, is retention in RPLC best described as an adsorption process or as a partitioning process? What is the driving force for retention? In which phase does the selectivity arise? This work focuses primarily on the second and third issues, but new data relevant to the first issue will be presented. However, our main goal is to examine the type and strength of the intermolecular interactions that are the origin of retention and selectivity. We emphasize at this point that our chief concern is with non-polar species. We believe that many of our views may not be applicable to polar solutes.

To be more specific about our goals, consider the case of gas–liquid and gas–solid chromatography. Here there is no question, that to a very good approximation, retention is driven by the processes in the stationary phase because at the temperatures and pressures used in gas chromatography, the mobile phase is nearly an ideal gas, and thus intermolecular interactions in it can be neglected [11]. In contrast, in liquid–solid chromatography on polar solids such as silica and alumina, the Snyder–Soczewinski displacement model applies (see ref. 12). A basic tenet of this model is that *interactions in the mobile phase are considered to be negligible with respect to the strength of interactions in the stationary phase*.

This paper addresses the questions: Where in RPLC do the dominant interactions of the solute reside? And can one neglect interactions in either phase, or are both important?

The solvophobic model of RPLC, as de-

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veloped by Horváth and coworkers [5–7], is physically highly detailed and very successful at predicting trends in retention upon changes in mobile phase composition, and in altering certain molecular properties of the solute principally its size. This model has led most workers to conclude that the stationary phase ligand plays a rather passive role in RPLC separations. In the past, one of us [13] stated that "... *the solvophobic theory of reversed-phase chromatography is essentially correct, that is, most of the free energy of transfer arises from processes taking place in the mobile phase*". We reached these conclusions based on studies of solute activity coefficients in solvents typical of the mobile phases used in RPLC. We were able to show that for a series of alkylbenzenes, the changes in chromatographic capacity factors with mobile phase composition were highly correlated with the measured mobile phase activity coefficients. By assuming that activity coefficients in the stationary phase were proportional to their values in bulk isopropanol, we were able to show that the retention data for all five non-polar solutes tested fell on a single curve of log k' *vs.* the mobile phase activity coefficient. This result was found to hold for four different organic modifiers. More recently, Zepeda *et al.* [3] reached a similar conclusion concerning the origin of selectivity in RPLC, based on studies of partial molar excess enthalpies and entropies of transfer. Thus, there seems to be a convergent view, to at least a crude first approximation, that the strength of the interactions in the stationary phase is small relative to those in the mobile phase.

We have now revised our view of the basic driving force involved in RPLC, based in part on new data, and in part on a new interpretation of data that have been accessible for some time. The purpose of this communication is to explain our revised view and, at the same time, to show that it is still consistent with conventional ideas as to what constitutes the "hydrophobic" effect [14]. Generally speaking, the hydrophobic effect relates to the increase in free energy and heat capacity that are observed when a non-polar moiety (atom, molecule or molecular fragment) is *transferred from a non-polar environment to water*. There is no question that this transfer is an extremely unfavorable process relative to the transfer of a non-polar species from one low-polarity medium, even an alcohol (see below), to a second low-polarity medium such as a hydrocarbon. Rather, we are concerned with the magnitudes of the individual free energies, in the mobile and stationary phases, relative to one another. Our current view is that the free energies in the stationary phase, that is the non-polar phase, are in fact larger than the free energies in the more polar mobile phase.

EXPERIMENTAL

HPLC-grade water, methanol, acetonitrile, isopropanol, and tetrahydrofuran (Fisher Scientific) were used throughout this work. Additional solvents used in this work were of the highest purity available: Deuterium oxide (99.9% isotope purity, Cambridge), *n*-octanol (99+%, anhydrous, Aldrich) *n*-hexadecane (99%, Aldrich). The alkylbenzene (benzene, toluene, ethylbenzene, *n*-propylbenzene and *n*-butylbenzene) and *n*-alkane (*n*-pentane, *n*-hexane, *n*-octane and *n*-nonane) solutes were of a purity which exceeded 99% (Aldrich) and were used without any further purification. The *n*-alcohol solutes: methanol (HPLC-grade, Fisher Scientific), ethanol (Chemistry Stores, University of Minnesota, >99.9% pure by GLC); *n*-propanol (HPLC-grade, Aldrich) and *n*-butanol (HPLC-grade, Aldrich) were all used without further purification. However, *n*-pentanol (99+%, Aldrich) and *n*-hexanol (98%, Aldrich) solutes required further purification; the procedure is described elsewhere [15].

All measurements were made at 25°C using head-space gas chromatography (HSGC) with the solutes at infinite dilution in the solvent of interest. Infinitely dilute solute concentrations were maintained throughout this work. This condition was confirmed by working where a linear relationship between the liquid- and gas-phase solute concentrations could be maintained. The details of the HSGC system and experimental methods have been presented in great detail elsewhere [15–17] and will not be reiterated here; however, since several different approaches were taken in making the measurements of this work, each method used will be briefly outlined below.

Gas–liquid partition coefficients in pure solvents

Gas–liquid partition coefficients of the *n*-alkane and *n*-alcohol solutes at infinite dilution were established by measuring the individual solute gas-phase concentrations above infinite dilute solutions of the solutes in the solvent of interest. The experiment was conducted in a water-jacketed cell (60 ml) which was thermostated to 25°C (±0.1°C). The highest concentration of a mixture of the solutes in the solvent was prepared gravimetrically. This solution was either added directly to the cell in the case of alkanes, or to an auto buret in the case of the alcohols. The solute concentration was varied by either successively diluting the solution with the solvent (for the alkanes), or by concentrating the solution with successive additions of the solute to the solution (for the alcohols). These additions were made by a computer controlled auto buret (Model ABU-11, Radiometer, Copenhagen, Denmark). Three replicate measurements of the equilibrated gas phase were made at each incremental addition of the solute mixture. The solute gas-phase concentration was calibrated using a large bulb of known volume (12 l) and adding a small volume of a mixture of the solutes of interest (typically less than 100 μl) using a gas-tight syringe (Dynatech Precision Sampling, Model C-160FN). The mass of solute added to the bulk was measured to the nearest 0.01 mg. Three replicate measurements of the gas-phase calibration standard were made before and after each run. A detailed discussion of the data analysis is given in refs. 17 and 18.

Gas–liquid partition coefficients of alkylbenzenes in hydro–organic solvents

The gas–liquid partition coefficient of the alkylbenzenes was determined in aqueous mixtures of methanol, acetonitrile, 2-propanol and tetrahydrofuran. The following volume fractions of the organic solvent component were studied: pure water, 10, 20, 30, 40, 50, 70% (v/v). These mixtures were prepared at a single solute concentration in a 500-ml volumetric flask with the solutes added gravimetrically and maintained at an infinitely dilute concentration. An aliquot of this solution was added to a water-jacketed cell and thermostated to 25°C (±0.1°C). Three replicate measurements of the equilibrated gas-phase above these solutions were made. The gas phase was calibrated in a similar manner as discussed above.

Hexadecane–hydro–organic partition coefficients of alkylbenzene

At each of the above prepared volume fractions of the hydro–organic solutions, a known amount of hexadecane was added to the cell via a computer-controlled buret. Three different buret volumes (0.25, 2.5 and 25 ml) were used throughout this measurement. Six increments of hexadecane were added at each solvent composition. The volume of hexadecane added in each increment was determined from the mass of hexadecane delivered and its density at 25°C. After each addition, the solution was allowed to equilibrate for 90 min and then three replicate analyses of the head-space were made. From the area counts before and after the addition of the hexadecane to the solution, it is a simple matter to calculate the hexadecane–hydro–organic partition coefficient. A detailed discussion of the data analysis for the calculation of the gas–hydro–organic and hexadecane–hydro–organic partition coefficients is given in ref. 17.

RESULTS AND DISCUSSION

As stated above, we measured the gas–liquid partition coefficients of a series of alkylbenzenes over the full range of mobile phase compositions for mixtures of water with methanol, acetonitrile, isopropanol and tetrahydrofuran. These results are shown in Figs. 1–4. The most remarkable result shown in these plots is that in all four types of mobile phases, there is a very narrow range in compositions at which the free energies of transfer, and thus the partition coefficients, of the five solutes are the same. The partition coefficients and compositions at the approximate

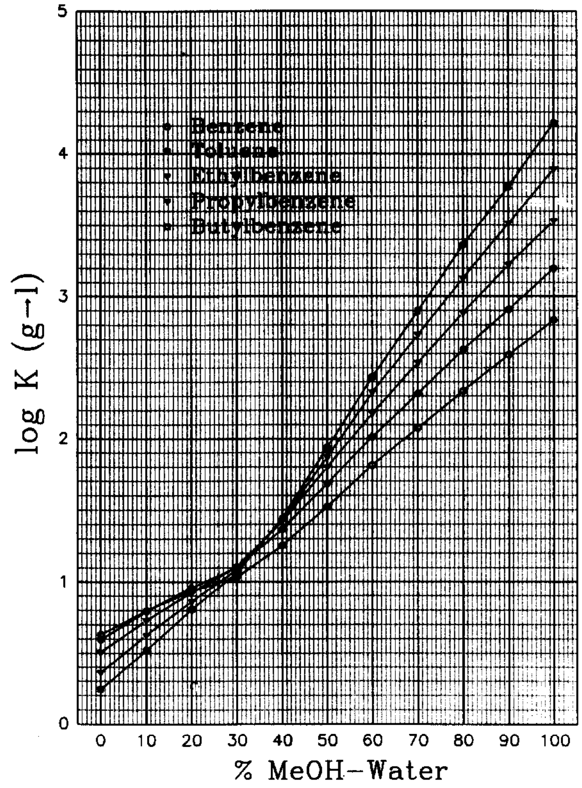

Fig. 1. Plot of log K for gas to liquid transfer to methanol–water *vs.* volume fraction of methanol.

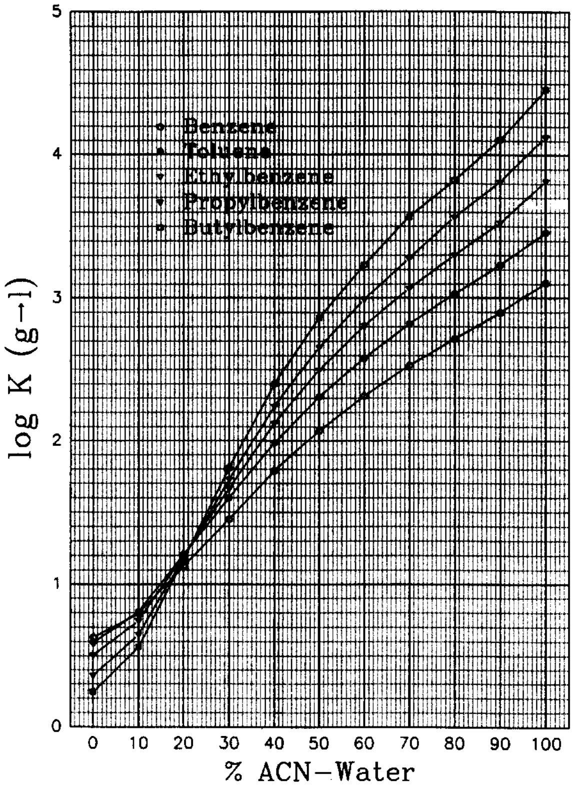

Fig. 2. Plot of log K for gas to liquid transfer to acetonitrile–water *vs.* volume fraction of acetonitrile (ACN).

intersection point are summarized in Table I. Note that because the transfer of benzene to pure water is more favorable than that of butylbenzene, and conversely the transfer of benzene to methanol is less favorable than the transfer of butylbenzene, it follows that the various curves must cross. That they do so "at" or "close to" a single composition is very interesting. Clearly, at this point the methylene group increment to the free energy of transfer of the parent molecule is zero for *all* solutes. It cannot be too strongly emphasized that the free energy of transfer of a solute represents a balance between solute–solvent interactions and the disruption of the solvent–solvent interactions, *i.e.* the "cavity" formation free energy that results when a solute is placed in the solvent. Thus the statement that the free energy of transfer of a methylene group from the mobile phase to the gas phase is zero does not mean that there are no solute–solvent interactions at this mobile phase composition. Rather we mean the net free energy or balance between exoergic solute–solvent interactions and endoergic disrupted solvent–solvent (cavity) interactions is zero at this composition.

It should be clear that if one attempted to do a hypothetical gas chromatographic separation of these alkylbenzenes with stationary phases whose compositions were as given in Table I, one would find co-elution of the solutes and a very poor separation. In contrast, it is equally clear that the isocratic separation of these alkylbenzenes by RPLC using the same mobile phases would be absolutely straightforward. The RPLC selectivity would be superb. *We conclude, that at least at some mobile phase compositions, a great deal of the non-polar selectivity observed in RPLC must be due to the net free energy of the solute in the stationary phase.* This raises the question of how much influence the stationary phase has on the overall retention, in addition to

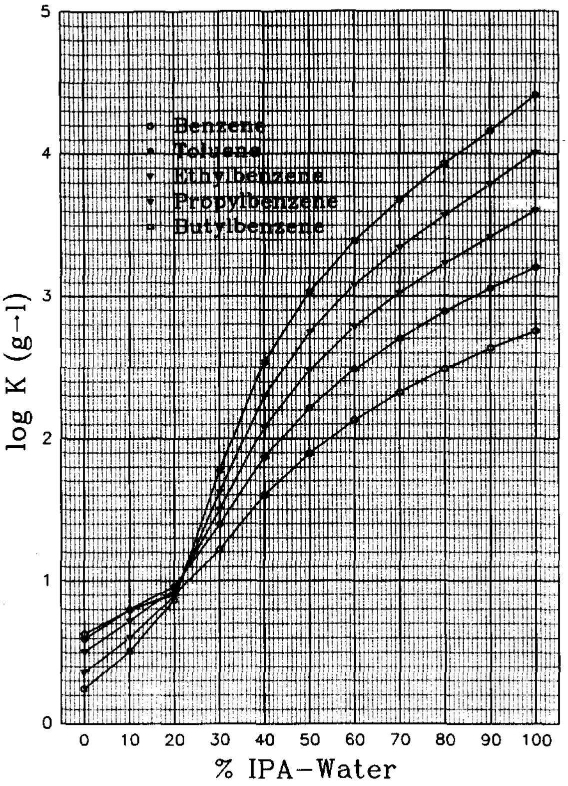

Fig. 3. Plot of log K for gas to liquid transfer to isopropanol–water *vs.* volume fraction of isopropanol (IPA).

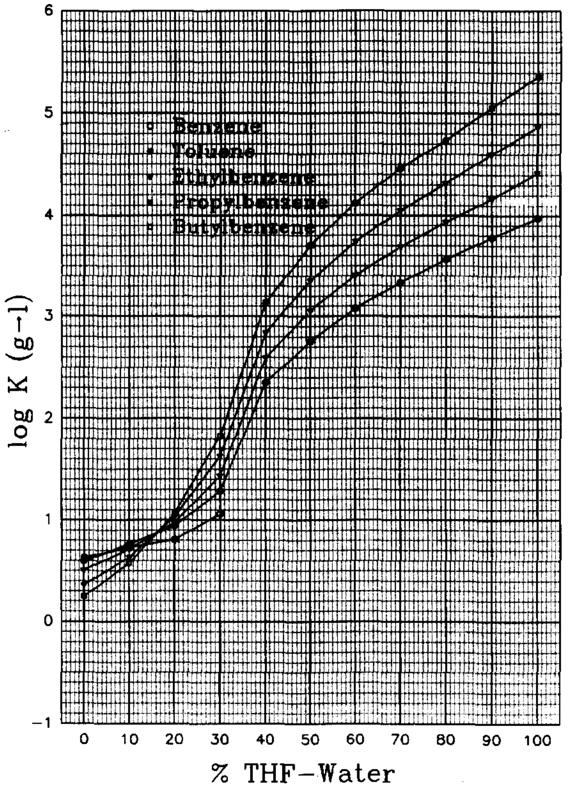

Fig. 4. Plot of log K for gas to liquid transfer to tetrahydrofuran–water *vs.* volume fraction of tetrahydrofuran (THF).

its strong influence on non-polar selectivity as established by the data given in Figs. 1–4.

The above question is also related to the following set of observations. First, in RPLC the slopes of plots of log k' *vs.* carbon number within a homologue series are very similar for different homologue series of solutes. The data of Tchapla *et al.* [8] are quite clear on this point (see Fig. 5). Second, slopes of the logarithmic gas to water partition coefficients *vs.* carbon number for a variety of homologous series of solutes differ from homologue series to homologue series (see Fig. 6) [15]. Because the mobile phase must contribute something to retention in RPLC these two results seem to be contradictory. The most obvious explanation for this seeming paradox is to postulate that the contribution of the net processes taking place in the mobile phase to the overall retention process is considerably smaller than is the contribution from the net processes taking place in the stationary phase and thus variations in the slope from homologue series to homologue series in the mobile phase contribution are overwhelmed by the contribution from the stationary phase. As shown by the data of Fig. 7 [18] which pertain to the transfer from the gas phase to hexadecane the slopes shown in Fig. 6, for gas to water transfer, are in fact considerably smaller. This

TABLE I

INTERSECTION POINTS FOR PLOTS OF LOG K *VS.* MOBILE PHASE COMPOSITION

Modifier	% Volume[a]	log K [b]
Methanol	33	1.20
Isopropanol	22	1.05
Acetonitrile	22	1.30
THF	16	0.87

[a] Volume percent modifier at point of intersection.

[b] Common value of log K at point of intersection.

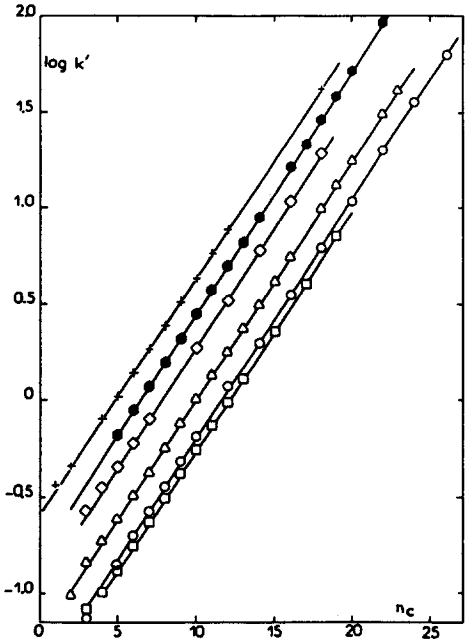

Fig. 5. Plot of log k' *vs.* carbon number (n_C) for reversed-phase liquid chromatography. The data are for a 90% methanol mobile phase and were taken from ref. 8 (© American Chemical Society). The various homologue series used were: + = *n*-phenylalkanes; ● = *n*-alkanes; ◇ = *n*-alkychlorides; △ = *n*-methyl esters of carboxylic acids; ○ = *n*-alcohols; □ = 2-*n*-alkanones.

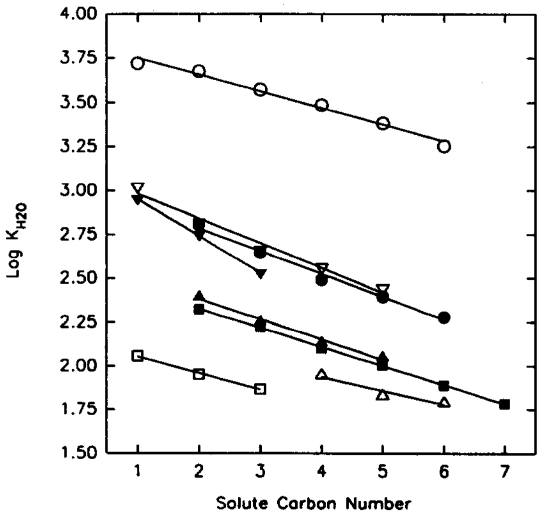

Fig. 6. Plot of log K for gas to water transfer (log K_{H_2O}) at 25°C. The data are from ref. 15. ○ = Alcohols; ● = ketones; ▽ = nitriles; ▼ = nitros; □ = formates; ■ = acetates; △ = isoacetates; ▲ = aldehydes.

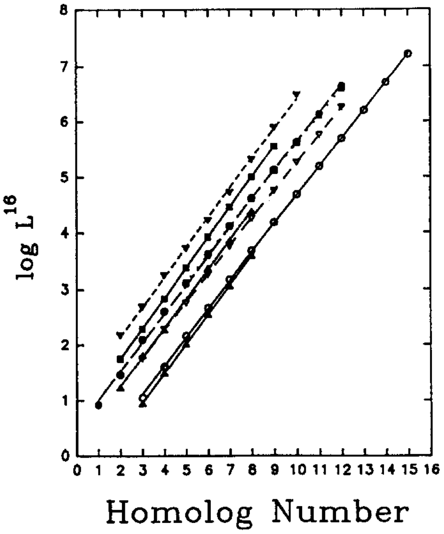

Fig. 7. Plot of log K for gas to hexadecane transfer (log L^{16}) at 25°C. The data were taken from ref. 18. ○ = Alkanes; ● = alcohols; ▽ = 2-ketones; ▼ = thiols; □ = cycloketones; ■ = carboxylic acids; △ = aldehydes; ▲ = alkenes.

reinforces our belief that for non-polar solutes the free energies in the stationary phase are very important.

The fact that the stationary phase contribution to the retention of a non-polar moiety in RPLC is more significant than the contribution from the mobile phase can be demonstrated more directly by considering the thermodynamic cycle shown in Fig. 8 and its consequences described below. Here the transfer of solute from one condensed phase (the mobile phase) to a second condensed phase (the stationary phase) is shown as taking place via an intermediary ideal gas phase. It is

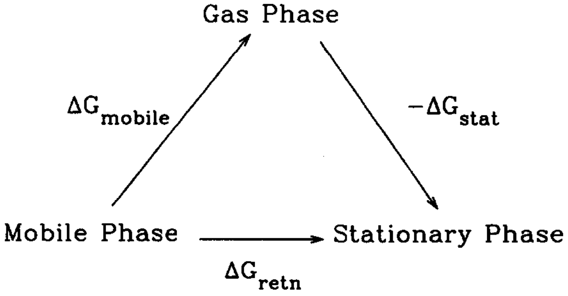

Fig. 8. Thermodynamic cycle representing the mobile to stationary phases transfer processes.

clear that the free energy of transfer from the mobile to the stationary phase, which corresponds to the retention process, can be written as:

$$\Delta G^0_{\text{retn}} = \Delta G^0_{\text{mobile}} - \Delta G^0_{\text{stat}} \tag{1}$$

where $\Delta G^0_{\text{mobile}}$ and ΔG^0_{stat} denote the free energy of transfer from the mobile to the gas phase and the free energy of transfer from the stationary to the gas phase, respectively. The processes corresponding to the three parts of the thermodynamic cycle are:

$$\mathrm{A_m} \leftrightharpoons \mathrm{A_s} \tag{2}$$

$$\mathrm{A_m} \leftrightharpoons \mathrm{A_g} \tag{3}$$

$$\mathrm{A_s} \leftrightharpoons \mathrm{A_g} \tag{4}$$

where A denotes the solute species. Each of these phase equilibria can be written as an equilibrium expression of the form:

$$K_{i,j} = [\mathrm{A}]_j/[\mathrm{A}]_i \tag{5}$$

where i and j denote a pair of phases, and [A] is the solute concentration in that phase. The corresponding free energy of transfer is given by an equation of the form:

$$-RT \ln K_{\text{transfer}} = \Delta G^0_{\text{transfer}} \tag{6}$$

In this work, we choose to use molar units for concentration and not mole fraction units as discussed more cogently by Ben Naim as well as others [19–22].

Since we have direct measurements of $K_{\text{m,g}}$ as shown in Figs. 1–4, and such K values for other species between water and the gas phase are available [15–17], or have been measured by HSGC (see Table II), we can readily evaluate one leg of the thermodynamic cycle. Unfortunately, we are stuck at this point. To compute any other part of the system requires some approximation. ΔG^0_{retn} can be obtained from measurements of the capacity factor (k') and knowledge of the phase ratio (ϕ) as follows [3]:

$$\Delta G^0_{\text{retn}} = -RT \ln k' + RT \ln \phi \tag{7}$$

However, determination of the phase ratio in RPLC is quite difficult, and furthermore, we would like to examine what happens when pure water is used as the mobile phase. Capacity factor data for non-polar solutes under such conditions are scarce and difficult to measure due to lack of reproducibility in measurements and hysteresis problems [23]. In contrast, it is an easy matter to compute the free energy of transfer of a methylene group since this corresponds to the difference in ΔG^0_{retn} for two homologues. Assuming that the same phase ratio can be applied to both homologues, we get:

$$\Delta G^0_{\text{retn,CH}_2} = -RT \ln k'_{n+1}/k'_n \tag{8}$$

where n and $n+1$ denote the number of methylene units in the pair of homologues. We will return to the issue of free energy of transfer of a methylene group in more detail below.

TABLE II

LN K VALUES IN WATER AND HEXADECANE

Solute	ln K	
	Water[a]	Hexadecane[b]
Benzene	1.45	6.45
Toluene	1.37	7.70
n-Ethylbenzene	1.17	8.67
n-Propylbenzene	0.82	9.72
n-Butylbenzene	0.56	10.79
Methanol	8.54	2.25
Ethanol	8.45	3.28
n-Propanol	8.21	4.57
n-Butanol	8.01	5.78
n-Pentanol	7.77	6.97
n-Hexanol	7.49	8.12

[a] Gas to water partition coefficient at 25°C.

[b] Gas to hexadecane partition coefficient at 25°C.

In contrast to the above situation, there is a great deal of data available for the transfer of both polar and non-polar solutes from hexadecane to the gas phase. Thus, if we use this data to compute ΔG^0_{stat}, in essence, we are forcing RPLC to act as a pure bulk partitioning process and not at all as an interfacial process. We do not doubt that the interfacial nature of the stationary phase is critically important in bonded phase RPLC. We adopt the present course in order to compare the rough magnitudes of the quantities defined by the cycle given in Fig. 8. We fully expect that the use of bulk hexadecane as a model will obscure a great deal of "fine structure" in the overall picture. We will return to this issue below and compare the free energy of transfer of a methylene group from the mobile phase to a conventional RPLC support to that for the present idealization. The use of pure hexadecane as a model of the strength of interactions in the bulk phase, rather than hexadecane equilibrated with methanol–water mixture, is not at all a bad approximation. The solubility of pure methanol in hexadecane is very low (*ca.* 0.003 mol fraction [24]), and we showed elsewhere [13,15] that it has virtually no effect at all on the gas to hexadecane transfer free energy. The amount of water that dissolves in hexadecane is even smaller [15].

The measured partition coefficients used in this work for transfer from water to the gas phase, and from hexadecane to the gas phase, are summarized in Table II. For our present purposes, we will summarize data within a homologue series by examining the contribution of a methylene group to the various free energies defined in Fig. 8. These results are plotted in terms of what we call thermodynamic "energy level diagrams," as shown in Fig. 9.

Examination of the data as presented in this fashion clearly shows that *the solvation process of a methylene group in hexadecane is very significant, and in fact, is larger in magnitude than is the solvation process in the mobile phase.* Here we use the term solvation process to indicate the net free energy resulting from the endoergic cavity formation process and the exoergic solute–solvent interaction. In classical liquid–liquid chromatography, where both the mobile and stationary phases are bulk liquids, we would now be certain that interactions in the stationary phase dominate the selectivity of a methylene group when a pure aqueous eluent is used.

RPLC is most commonly carried out with a mixed aqueous organic mobile phase. Inspection of Figs. 1–4 again leads us to a paradoxical conclusion if we ignore the importance of processes in the stationary phase. As seen with all four types of mobile phase modifiers, the pure organic modifiers do a far better job of discriminating the alkylbenzenes than does water. This is in stark contrast with all practical experience in RPLC. It is quite well known that

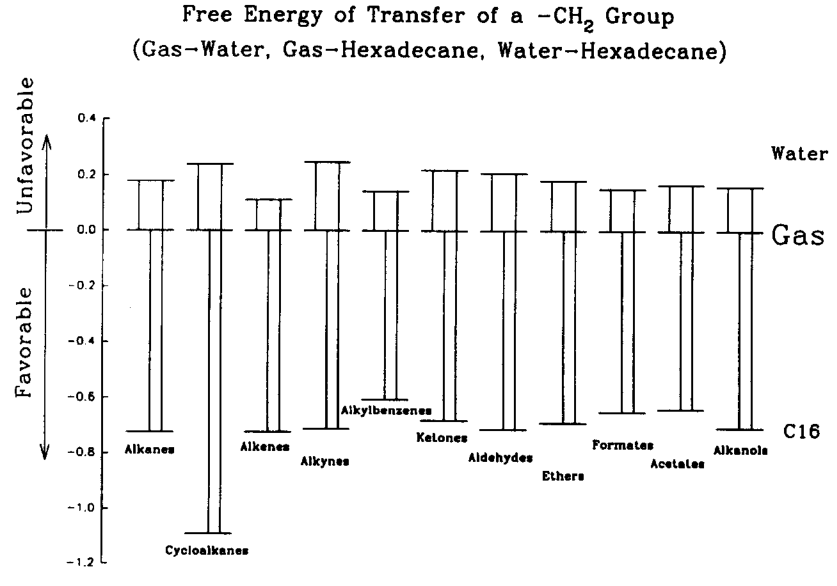

Fig. 9. Energy level diagram for methylene group derived from several homologous series of solutes. Values in kcal/mol (1 cal = 4.184 J).

the higher the water content of the mobile phase, the greater is the methylene group selectivity [25]. The same is also true of the effect of mobile phase on the selectivity of non-polar isomers [26]. Here again, to understand the data of Figs. 1–4 and their implications for RPLC, we feel that *the classical paradigm that processes in the stationary phase in RPLC are of secondary importance must be discarded.*

To understand the above in somewhat more detail we first examine the effect of a methylene group on the free energy of transfer from a series of liquids to the gas phase. Relevant data for *n*-alkanes and *n*-alcohols obtained in our laboratory [15,16] are shown in Figs. 10 and 11. Because the free energies differ so greatly in order to put the data on a common scale we have had to choose an arbitrary scale adjustment. For the hydrocarbons we use *n*-pentane as a zero point (see Fig. 11) because this is the lowest-molecular-mass alkane for which we have measured a large body of data. In the case of the alcohols we use methanol as the zero point of the scale (see Fig. 10). In either case, the important point is that the free energy of gas to liquid transfer becomes more favorable upon adding a methylene group to either an alkane or an alkanol for all organic solvents. However, this process becomes less favorable when water [or deuterium oxide which gives results that are indistinguishable from water (see superposition of data as shown in Fig. 10)] is the solvent. Clearly methylene groups are expelled from water into the gas phase whereas they are warmly welcomed by the other solvents. The data as displayed in Figs. 10 and 11 clearly show than the magnitudes of the changes in partition coefficients upon adding a methylene group are larger for any of these common organic solvents than for water. Thus the organic solvents are better able to discriminate between two solutes that differ by a methylene group than is water. Also note that the transfer of successively larger solutes from the gas phase to water becomes less favorable whereas as the transfer to an organic solvent becomes more favorable.

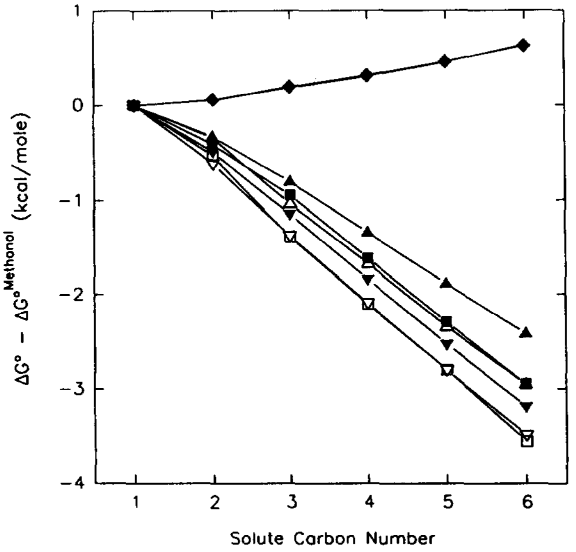

Fig. 10. Plot of log $K_{n\text{-alcohol}}/K_{\text{methanol}}$ *vs.* solute carbon number for various solvents. Data for all solvents were taken from ref. 15. ∇ = Hexadecane; ▼ = octanol; □ = tetrahydrofuran; ■ = isopropanol; △ = methanol; ▲ = acetonitrile; ◇ = water; ◆ = deuterium oxide.

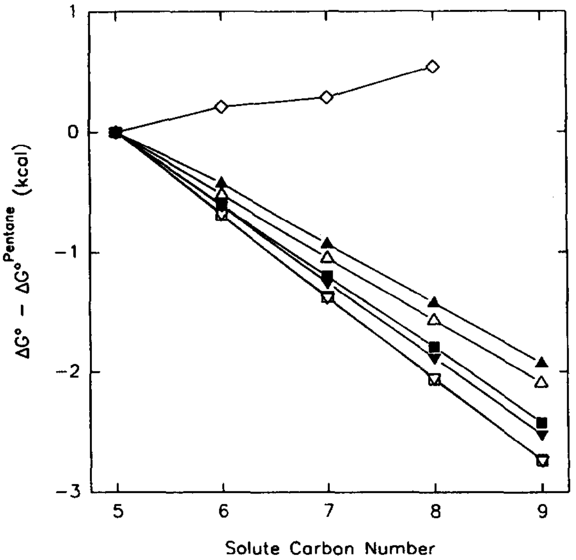

Fig. 11. Plot of log $K_{n\text{-alkane}}/K_{n\text{-pentane}}$ *vs.* solute carbon number for various solvents. Data for all solvents were taken from ref. 16. ∇ = Hexadecane; ▼ = octanol; □ = tetrahydrofuran; ■ = isopropanol; △ = methanol; ▲ = acetonitrile; ◇ = water.

Thus, the reason why a purely aqueous mobile phase is a better discriminator of methylene groups in RPLC than is a pure organic mobile phase is that the transfer of a methylene group out of water (even to the gas phase) is a favorable process, and thus this process augments the favorable increment to the gas to hexadecane transfer free energy. In contrast *with all other solvents, transfer of a methylene group to the gas phase is unfavorable* and thus diminishes the overall effect of addition of a methylene unit to the free energy of transfer from the mobile to the stationary phase in RPLC. This same result is shown much more explicitly in the series of "energy level" diagrams as a function of mobile phase composition shown in Fig. 12. These were derived from the data given in Fig. 1 and the partition coefficients from the gas phase into pure hexadecane.

Results with other types of solutes

So far, we have restricted our results to the interpretation of transfer free energies of methylene units. We can examine a great number of real species in terms of "energy level" diagrams, but we will restrict ourselves to benzene the prototypical, non-polar, test solute of RPLC. As shown in Fig. 13, the transfer of benzene from the gas phase to water, unlike transfer of a methylene group, is favorable; however, the magnitude of the free energy of transfer into water is smaller than that of the free energy of transfer into hexadecane. Consequently, although the transfers of benzene from gas to water, and from gas to hexadecane, are both favorable, the overall RPLC retention process is still favorable. This is the case because the net process of transferring benzene into hexadecane is so favorable that it more than counter-balances the net favorable process of transfer into water.

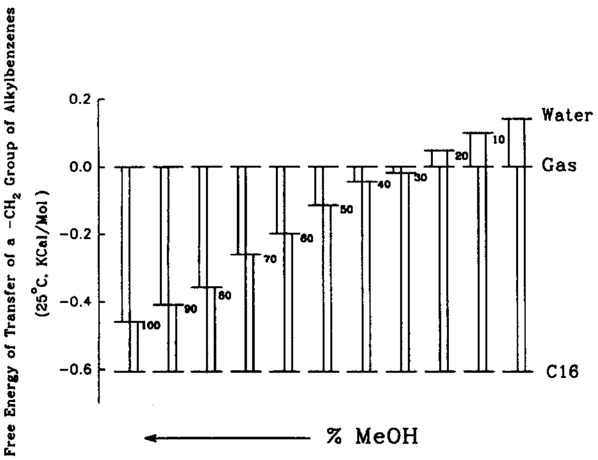

Fig. 12. Energy level diagram showing effect of volume fraction of methanol on transfer of methylene group in alkylbenzenes.

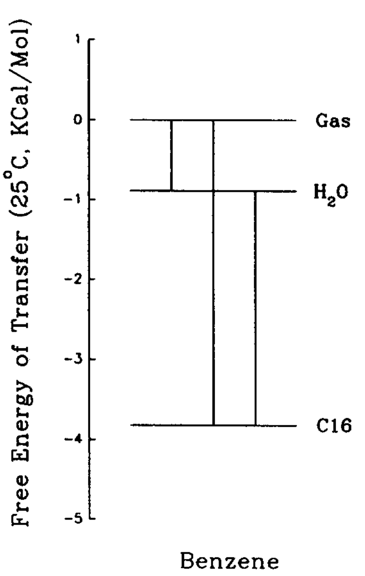

Fig. 13. Energy level diagram for transfer of benzene.

Clearly, the strong interaction of non-polar molecules with a non-polar phase is implicated in both the overall RPLC retention process and in methylene group selectivity. It is also manifested in more subtle factors such as that of molecular shape on the overall free energy of transfer. Energy level diagrams comparing *n*-propyl benzene and isopropyl benzene, and *o*-, *m*- and *p*-xylenes are shown in Figs. 14 and 15. Close inspection will show that variations in the interactions of these species with the non-polar phase contribute more to $\Delta G^0_{\mathrm{retn}}$ than do variations in the water phase.

The solvophobic effect

Given the above data and our interpretation, it seems that we are in disagreement with many groups [3,7] as well as our own prior conclusions [13]. It may also appear that we see nothing

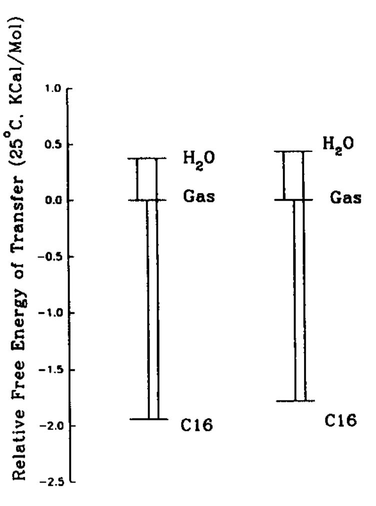

Fig. 14. Energy level diagram comparing branching effects. The data shown are differences in the free energies with respect to the same free energy for benzene.

unique about water as a solvent; that is, that we are denying the existence of a hydrophobic [14,27] or solvophobic effect. We do not feel that this is true. Data such as those displayed in Figs. 10 and 11 proclaim that water is a unique solvent. In comparison to all of the organic solvent shown here, even those exhibiting strong polar and hydrogen bonding interactions (*e.g.*, methanol and dimethyl sulfoxide), water is strongly disliked by methylene groups. It must also be borne in mind that water is also a most peculiar solvent because the unfavorable solubilities of non-polar substances in it, that is their free energies, are driven by a favorable enthalpy of solution, a very unfavorable entropy of solution and a very large change in the transfer heat capacity [14].

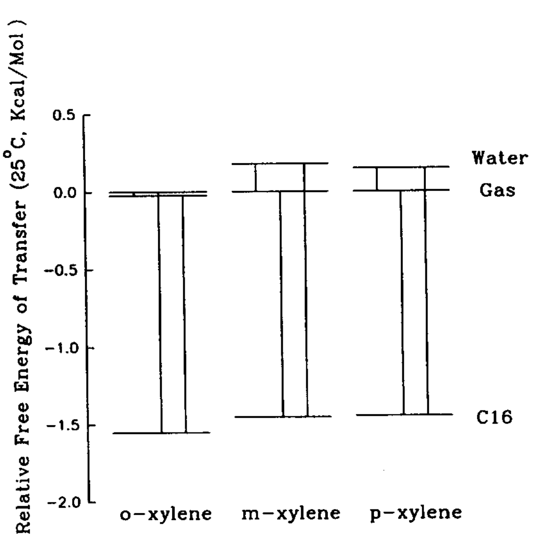

Fig. 15. Energy level diagram comparing isomerization effects. The data shown are differences in the free energies with respect to the same free energy for benzene.

The data of Figs. 10 and 11 clearly show that transfer of a methylene group from the gas phase into water is, based on free energy, an unfavorable process. This means that the water–water interactions that are disrupted or perturbed upon introduction of a methylene group are not completely compensated by the formation of methylene–water interactions. This is the essence of hydrophobicity. In contrast the transfer of a methylene group from the gas phase into any of the other solvents shown in Figs. 10 and 11 is, based on free energy, a favorable process. This means that the solvent–solvent interactions that are disrupted upon introduction of a methylene group are more than compensated by the strong interactions that take place between the methylene group and the solvent.

Nonetheless we do disagree with many others as to the dominance of water as the controlling factor in the net free energy of transfer of a methylene group from water to a non-polar environment. This idea seems to have originated in the biophysics community and is still widely held [28,29] although it is hotly debated [30]. One of the clearest arguments used to support the idea that water is the dominant element is related to the free energy of formation of a liquid–liquid interface from a liquid–air interface as described by Tanford [28]. Tanford argued that the free energy of forming a water–water interface from a water–air interface is very favorable (-144 erg/cm^2; 1 erg $= 10^{-7}$ J). In contrast, the free energies of forming water–hydrocarbon (*ca.* -40 erg/cm^2) and hydrocarbon–hydrocarbon (*ca.* -40 erg/cm^2) although favorable are much smaller and therefore the favorable transfer of a hydrocarbon from water to a non-polar environment is dominated by the free energy liberated by the formation of water–

water contacts upon removal of the hydrocarbon solute. This argument is evidently based on macroscopic properties (surface tensions). Even so, there have been many attempts to extend the use of the surface tension to a microscopic level [29,31]. Horváth *et al.*'s [6] formulation of the solvophobic model of RPLC is based on such arguments. Our view is based on measured microscopic properties, that is, the various phase transfer equilibrium constants and the 1 *M* gas reference state.

Our present interpretation of the differences in behavior of water is as follows: in almost all organic solvents the free energy of transfer of a methylene group from the gas phase to that solvent is favorable because of the favorable, largely dispersive (London) energy of a methylene group–solvent interaction overcomes the unfavorable cavity formation or solvent–solvent disruption process that accompanies insertion of the solute in the solvent. In water the favorable methylene group–water interaction process, which is also largely dispersive but must also involve some dipole–induced dipole (Debye) interactions, cannot overcome the unfavorable cavity formation process. Keep in mind that we are talking of free energies and thus both enthalpies and entropies must be taken into account. However, organic mixtures much more closely obey the behavior of regular solutions and consequently entropy effects are much smaller than mixtures containing water. *Thus transfer of a methylene group from a gas to all of the above organic solvents is solvophilic but its transfer from gas to water is solvophobic.* As organic solvent is added to water the mixture becomes less and less solvophobic as described above.

Second, we feel that our previous conclusion [13] is still partly correct. It is correct, but only in so far as it relates to the changes in retention of non-polar solutes upon changes in the mobile phase composition. Previously we showed that the variations in log k' of *non-polar solutes* as the mobile phase composition was varied very closely followed the changes in the solute's mobile phase activity coefficient. To a fair first approximation interactions of non-polar solutes with the stationary phase are unaffected by addition of organic modifier to the mobile phase. Thus the changes in retention (and activity coefficient) of such species are controlled by changes in the solute–mobile phase processes. The effect of addition of modifier to the mobile phase is to convert the solvophobic methylene group–solvent interaction in pure water to a solvophilic interaction with the mixed mobile phase. The points at which interactions in the mobile phase make no contribution to retention, either positive or negative, are the crossing points shown in Figs. 1–4 and summarized in Table I. In mobile phases that are richer in organic modifier than those indicated in Table I the net methylene group–mobile phase interaction, relative to the gas phase, is solvophilic. In this range of mobile phase compositions the endoergic (unfavorable) cavity formation effects are much less important than in pure water. Further the exoergic (favorable) solute–mobile phase interactions may be larger because a less polarizable solvent (water) has been replaced with a more polarizable organic modifier. Thus dispersive interactions may be stronger.

However, our previous statement quoted above is incorrect. It was based in large part on a comparison of the magnitudes of activity coefficients in the mobile and stationary phase. To quote ourselves again [13] "... it is unreasonable to expect that non-polar solutes will be 'pulled' into the stationary phase by virtue of a very small stationary phase activity coefficient". We now find this argument unpersuasive: while it is correct thermodynamically, it gives a highly biased view of the intermolecular process involved in retention.

It must be understood that the activity coefficients cited above are based on Raoult's law, and thus the pure solute liquid was used as the reference state. When solutes such as benzene and other non-polar solutes are used, the intermolecular interactions in the reference state are similar to those in the non-polar stationary phase, and thus there is very little difference in the energy of transfer from the reference state (pure solute) to the stationary phase state. The measured activity coefficients of benzene through *n*-butyl benzene in hexadecane at 25°C range from 1.07 to 1.30 [13]. If the pure liquid substance is chosen as the reference state and free energies of transfer are computed from the measured activity coefficients, then we obtain the data shown in Fig. 16. Based on this choice of reference state, the previous statements are completely justified. It is clear that *if we choose the pure substance as the reference state, then the dominant source of the overall free energy change is transfer from water to the new reference state.* This, however, is fundamentally misleading in that there are obviously very strong, dominantly dispersive, intermolecular interactions in the pure solute state and these are hidden from view when one makes comparisons on this basis.

Justification based on regular solution theory

It may help clarify our perspective to put it in terms of a model. The regular solution theory, although not at all appropriate to aqueous solutions (see below), is heuristically simple and has been very widely used in many chromatographic studies [32,33] even for RPLC [34,35].

According to the theory of regular solutions, the activity coefficient, γ_2^∞, of some solute (denoted 2) at infinite dilution in a solvent (denoted 1) can be written as [36]:

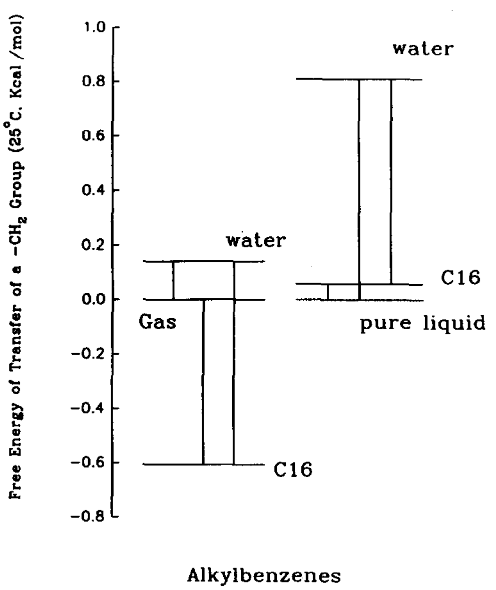

Fig. 16. Energy level diagram based on the use of the pure solute reference state.

$$g^{\mathrm{E}} = RT \ln \gamma_2^\infty = V_2(\delta_1 - \delta_2)^2 \tag{9}$$

where g^{E} is the partial molar excess free energy of transfer of species 2. This corresponds to the free energy liberated when 1 mol of solute is transferred from the pure liquid solute to a very large volume of solvent. δ_1 and δ_2 are termed solubility parameters, and V_2 is the solute molar volume. Karger *et al.* [32] have presented a molecular interpretation of this equation. It can be understood by writing out the squared term in detail.

$$g^{\mathrm{E}} = V_2(\delta_1^2 - 2\delta_1\delta_2 + \delta_2^2) \tag{10}$$

As pointed out by Karger *et al.* [32], the last term on the right ($V_2\delta_2^2$) corresponds to the energy of disrupting solute–solute interactions (denoted $E_{2:2}$ in Table III because the solute is designated as species 2). This energy corresponds to that of transferring a solute from its pure liquid state to the ideal gas phase. This is the quantity which is suppressed when one uses activity coefficients (or excess free energies) to quantify solute–solvent interactions. According to the calculations given in Table III, this term is really rather large, especially in comparison to the solute's interaction energies with less polar liquids.

The central term in eqn. 10 ($V_2\delta_1\delta_2$) corresponds to the energy of interaction of the solute with solvent (denoted $E_{1:2}$ in Table III). The first term ($V_2\delta_1^2$) corresponds to solvent–solvent interactions that are disrupted when the solute is placed in the solvent (denoted $E_{1:1}$ in Table III). This is the classical "cavity" formation term.

The magnitudes of these three terms for benzene and toluene are shown in Table III. It is quite clear that the energy corresponding to solute–solute interactions (denoted $E_{2:2}$ in Table III) is not small compared to the solvent–solvent interaction energy (see column headed $E_{1:1}$), or to the solvent–solute interaction energy (see column headed $E_{1:2}$). Further, note that the change in the solute–solute interaction contribution to the excess free energy of transfer upon changing the solute from benzene to toluene is quite large. This supports our view that the use of the pure solute liquid as a reference point is very misleading. Clearly, the above dissection of

TABLE III

DISSECTION OF THE ACTIVITY COEFFICIENT OF ALKYLBENZENES INTO COMPONENT PART BASED ON REGULAR SOLUTION THEORY

Solvent	Solute: benzene				
	$E_{1:1}$ [a]	$E_{2:2}$ [b]	$E_{1:2}$ [c]	$\ln \gamma_{-\text{theory}}$ [d]	$\ln \gamma_{-\text{exp}}$ [e]
Water	49.5	7.5	38.5	31.24	7.84
Methanol	18.9	7.5	23.7	4.36	1.95
Hexadecane	6.1	7.5	13.5	0.12	0.06
	Solute: toluene				
Water	59.0	8.6	45.0	38.36	9.12
Methanol	22.5	8.6	27.8	5.60	2.31
Hexadecane	7.3	8.6	15.8	0.08	0.00

[a] $V_2\delta_1^2$ (see eqn. 12), energy is in kcal/mol.
[b] $V_2\delta_2^2$ (see eqn. 12), energy is in kcal/mol.
[c] $V_2\delta_1\delta_2$ (see eqn. 12), energy is in kcal/mol.
[d] Infinite dilution activity coefficient in the indicated solvent at 25°C computed from regular solution theory.
[e] Experimental infinite dilution activity coefficient in the indicated solvent 25°C.

the activity coefficient into its component parts via regular solution theory justifies our perspective on the use of the gas phase *vs.* the pure solute as the reference state for comparing the strength of intermolecular interactions.

We turn now to the related issue of what regular solution theory can tell us about gas–liquid partition coefficients. Fortunately, Karger *et al.* [32] have derived an equation based on regular solution theory for the free energy of transfer from an ideal gas to a liquid phase. Their result is:

$$\Delta G^0 = V_2\,(\delta_1^2 - 2\delta_1\delta_2) \tag{11}$$

In this equation, the solute is at infinite dilution and the mol fraction scale is used to represent solute concentration in both phases. Using the mol per volume concentration scale, the free energy for the process described in eqn. 11, denoted ΔG^0_{conc}, is given by:

$$\Delta G^0_{\text{conc}} = RT \ln (V_1/RT) + V_2\,(\delta_1^2 - 2\delta_1\delta_2) \tag{12}$$

The subscript 1 denotes the solvent phase and 2 denotes the solute. Clearly, the difference in the two reference states used here is due to the absence of the $V_2\delta_2^2$ term in eqns. 11 and 12. This corresponds to the disruption of solute–solute interactions which are generally large. Not only are these interactions large, but as shown by the computations given in Table III, they vary greatly from solute to solute. It is vital to note that the cavity effect term ($V_2\delta_1^2$) is the same regardless of reference state. Methylene group increments inherently involve the comparison of free energies of one solute relative to another. It follows that when free energies of transfer between the same pairs of phases are compared for *different solutes*, we must not use *excess* free energies of transfer, as these are based on Raoult law type activity coefficients, and consequently, the inherent reference state sequesters important effects. On the other hand, when considering the effect of changing the solvent (species 1), as we do when examining the influence of mobile phase on the retention of a fixed solute [13,26], it does not matter at all whether we use the gas phase or the pure liquid solute as the reference state since interaction energies in both of these reference states are independent of changes in the mobile phase.

In Table IV, we used the regular solution theory to dissect the transfer from water to hexadecane into the two distinct gas to liquid

TABLE IV

FREE ENERGY OF TRANSFER OF BENZENE AND TOLUENE

All free energies (kcal/mol) are based on the mol per liter concentration scale at 25°C. They are computed based on regular solution theory using the following parameters: $\delta_{H_2O} = 23.53$, $\delta_{C_{16}} = 8.262$, $\delta_{benzene} = 9.142$ and $\delta_{toluene} = 8.946$, all in $(cal/ml)^{1/2}$. $V_{H_2O} = 18.07$, $V_{C_{16}} = 293.95$, $V_{benzene} = 89.42$ and $V_{toluene} = 106.87$, all in ml/mol.

Solvent	Free energy			
	Water[a]	Hexadecane[b]	Water to hexadecane[c]	Water to hexadecane_{exp}[d]
Benzene	8.97	−7.82	−16.79	−2.96
Toluene	12.11	−8.92	−21.03	−3.75

[a] For gas to water computed from regular solution theory.
[b] For gas to hexadecane computed from regular solution theory.
[c] For water to hexadecane computed from regular solution theory.
[d] Experimental data.

transfer processes described in Fig. 8. We see that based on regular solution theory, the solute interactions in water, while important, do not dominate the overall process.

Despite the fact that regular solution theory justifies our view as to the proper choice of reference state, we must reiterate the findings of Karger *et al.* [32] who showed many years ago that this approach is quantitatively very inaccurate when aqueous systems are considered. The above equations can only apply to those systems in which the excess entropy and volume of mixing are zero, and the geometric mean combining rule for unlike (1–2) pair interactions is valid. It does not apply to water as a solvent in view of the very large excess entropies of mixing non-polar solutes with water and the very unsymmetrical, highly direction nature of interactions in water. Thus, quantitative comparisons between measured data for non-polar solutes in water and the regular solution model cannot be taken very seriously. To emphasize the fact that regular solution theory should not be used quantitatively, we compare the estimated and measured activity coefficients in water, methanol and hexadecane. Clearly, the values in water and methanol are orders of magnitude too high, whereas in hexadecane agreement between theory and experiment is much better (see Table III). Simple applications of regular solution theory to RPLC should not be considered quantitatively.

Practical consequences

The above discussion rationalizes the fact that selectivity towards a methylene group in RPLC sometimes exceeds that observed in gas chromatographic separations on non-polar phases. This results from the primary effect of dispersive interactions between the solute's methylene group and the non-polar phase being augmented by the unfavorable interaction of a methylene group with water. Obviously, if the RPLC separation is carried out at a high volume fraction of organic modifier, this may not be so. Similarly, if the gas chromatographic separation is carried out at a low temperature, it might well be more selective than RPLC.

The primary consequence of our present view of the origin of the driving forces involved in RPLC is that it greatly complicates modeling the factors that control changes in retention as solute structure is altered. If retention were dominated by the interactions in either the mobile phase or the stationary phase we would have a far easier task of choosing appropriate parameters to model such factors as the solute size, shape and polarity [5–7,32–42].

It is not our intention that the above view encompass the details of what takes place in bonded phase RPLC. It offers little insight into the effect of variations in retention with the length [43], or shape [44], of the bonded phase chain. It certainly does not afford any detail as to the significant effects of the surface density of the bonded chain [4]. However, it does suggest that alternations in the non-polar interactions in the stationary phase by varying the nature, type and surface density of the bonded ligand should have significant effects on the grosser aspects of retention in bonded phase liquid chromatography even though changes in chromatographic selectivity are most often relatively minor [45]. The magnitude of the effect of such variations would be very difficult to rationalize if mobile phase interactions were to be the overwhelmingly dominant factor in establishing retention and selectivity in RPLC.

We further caution that we are not advocating a pure partition model of RPLC. As stated previously, we believe that interfacial processes are very important in bonded phase RPLC [13]. However, given the strength of the intermolecular interactions in the stationary phase, we feel that it is reasonable to expect that non-polar solutes will be in close contact with the bonded ligands, and very likely highly embedded in the stationary phase chains [2,4] (see below).

Effect of volume entropy

Thus far, we have used Ben Naim's [19,20] convention for comparing solvation thermodynamics. That is, we have adopted molar or number density concentration units, and not mol fractions and pressure units, in examining solute and solvent effects on the free energies of transfer. Based on Ben Naim's convention, we were then led to the conclusion that for non-polar species, solute interactions in the stationary phase involved greater free energies than do solute interactions in the mobile phase.

Recently Honig and co-workers [21,46] indicated that the chemical interpretations of free energies of solvation, such as those involved in hydrophobic binding, are best understood when the contribution of "volume entropy" is removed from the free energy contribution. Because such corrections can be very significant when solvents and solutes of very different sizes are considered, as is the case in this study, we felt that we should examine the "volume entropy" corrected free energy.

For the case of a gas to liquid transfer process, assuming an ideal gas phase, the "corrected" free energy free of volume entropy effects is, according to Honig and co-workers, given by the equation:

$$\Delta G^0_{\text{Honig}} = -RT \ln K - RT(1 - V_2/V_1) \qquad (13)$$

where V_1 and V_2 are the molar volumes of the pure solvent and solute. Clearly, when the solute is the same size as the solvent, the "volume entropy correction" is zero. The effects of such a correction for transferring a series of alkanes from the gas phase to a number of different solvents, including water and hexadecane, are given in Fig. 17. Comparison of these data to those shown in Fig. 11 indicates that the interactions of methylene groups with water relative to those with hexadecane are somewhat larger when viewed in terms of the "volume entropy corrected" free energy. However, it is still evident that the interactions of the methylene groups with hexadecane are still very significant compared to those in water.

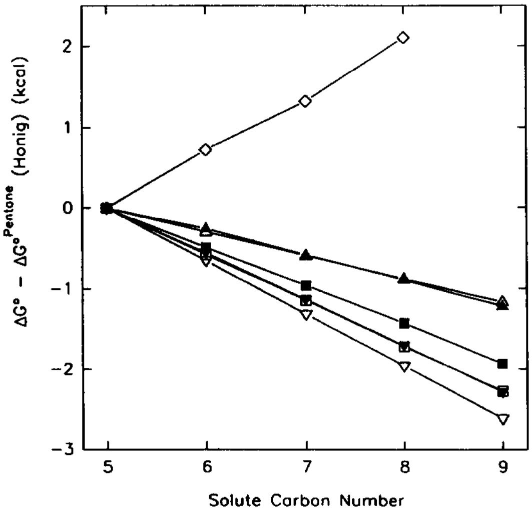

Fig. 17. Volume entropy corrected free energy of transfer for *n*-alkanes *vs.* solute homologue number. Symbols as in Fig. 11.

Comparison of bonded phase RPLC to the hexadecane model

In order to see whether the use of bulk hexadecane is at all reasonable as a model for bonded phase RPLC, we now compare the free energy of transfer of a methylene group from the mobile phase to a bulk hexadecane phase to the free energy of transfer from the mobile phase to a bonded RPLC support. To compute the free energy of transfer from the mobile phase to a bonded phase, one needs to know the column dead volume which is extremely difficult to measure [9,47]. In addition, as indicated by eqn. 7, a value for the phase ratio is needed. As shown by eqn. 8, Gibbs free energies of transfer of a particular functional group do not require knowledge of the phase ratio since this cancels out when one takes the difference of two capacity factors. However, some idea of the system dead volume is still required.

We computed the free energy of transfer of a methylene group to the bonded phase as a function of mobile phase composition from previously published data sets. In this work, we chose to use RPLC data that we had used in one of our previous studies [13]. Several different stationary phases were used and some of the data were taken from other workers [48,49].

As a preliminary test, we regressed the free energy of retention of a methylene group in RPLC against its free energy of transfer from the same mobile phase to bulk hexadecane using alkylbenzenes as the test solutes. The mobile phase was varied from 40% (v/v methanol) to 0%. The resulting regression equation is:

$$\Delta G_{\mathrm{CH_2,transfer}} = -0.04 + 0.976\Delta G_{\mathrm{CH_2,retn}} \qquad (14)$$

S.D. = 0.009, $r = 0.998$

In view of the fact that the intercept is close to zero and the slope is close to unity, the two free energies are almost identical.

Additional related results are shown in Fig. 18. If bulk hexadecane were a good model for a bonded phase, then the ratio shown on the y-axis in this figure should be close to unity and independent of the mobile phase composition. For methanol, the ratio is strikingly close to unity (see eqn. 14), given the complexity of the experiment (see below), and is more or less independent of composition. For the other types of mobile phases, the ratio is larger than unity and shows a greater composition dependence. It should be noted that the data in methanol and acetonitrile were obtained on the same type of column, while the data in isopropanol and tetrahydrofuran were obtained on a single but different column.

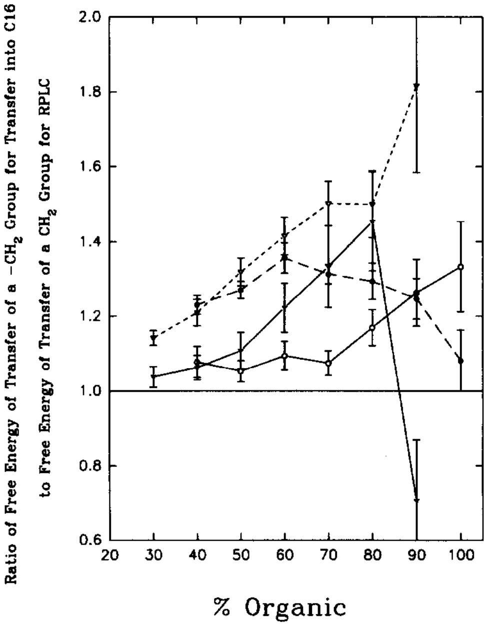

Fig. 18. Free energy of transfer of a methylene group for transfer into bulk hexadecane relative to the free energy of transfer of a methylene group in RPLC *vs.* mobile phase composition. ○ = Methanol; ● = acetonitrile; ▽ = isopropanol; ▼ = tetrahydrofuran.

Given the huge range in k' values covered by the data shown in Fig. 18, we feel that the ratios, at least in methanol, are sufficiently close to unity to justify our use of hexadecane as a model for the bonded phase. This was our chief objective in making the comparison shown in Fig. 18.

We must make several additional comments and qualifications about this result. First, we are using hexadecane to model a bonded octadecyl group. Use of hexadecane is an experimental necessity because octadecane is a solid at 25°C and gas–liquid partition coefficients on octadecane at this temperature cannot be obtained.

In addition, the high-quality RPLC data we took from the literature were obtained at 25°C. Elsewhere, we will carry out a similar analysis for different types of bonded phases and compare a variety of octadecyl bonded phases on different types of silica gel [50]. We point out here that in some cases the ratio plotted in Fig. 18 was less than unity, but in most cases, particularly when a short alkyl chain bonded phase was used, the ratio is considerably larger than unity and increases with the amount of modifier in the mobile phase. Second, because the mobile phase to hexadecane phase partition coefficients used in Fig. 18 span a very wide range, we used several different methods to obtain the data. Third, elsewhere we will consider the effect of the dissolved modifier on the properties of the bulk hexadecane phase [15]. For present purposes, we will state that from 0 to about 70% modifier, there is very little influence of dissolved modifier on the thermodynamic properties of the hexadecane phase towards a methylene group. However, towards polar species there is likely a significant effect of sorbed modifier on retention in RPLC (see below). In the limit when the mobile phase is a pure organic we must expect very substantial effects of the sorbed modifier. Above 70% modifier, in the case of tetrahydrofuran and isopropanol, there is really a very significant effect. Furthermore, at about tetrahydrofuran–water (90:10), hexadecane becomes very soluble in the mobile phase. Finally, we must point out that the effective bulk density of the bonded phase, even when a very high surface density is assumed (4.5 μmol/m^2), is considerably less than that of bulk hexadecane. That is, the bonded phase chains are not packed as compactly as the bulk fluid analogue. We feel that this should have some effect on the relative strength of the dispersive (London) interactions of the methylene groups of the probe solute and the stationary phase because dispersive interactions are short ranged.

"Partition" vs. "adsorption" mechanism of RPLC

Despite all of the above complications and our initial reluctance to enter into the discussion concerning the detailed mechanism of bonded phase chromatography, namely "adsorption" *vs.* "partition", our results are much more consistent with a "partition-like" process than an "adsorption-like" process. Intuitively, the free energy of an "adsorption-like" process is necessarily considerably less dependent on the solute chain length than is that for a "partition-like" process. According to Dorsey and Dill's lattice model [2,10], the ratio of the two free energies shown in Fig. 18 should be equal to the lattice coordination number, which for a cubic lattice is about 6. Thus, the free energy of transfer per methylene group for an adsorption-like process should be considerable smaller, *ca.* five-fold, than for a partition-like process. Given that the ratio shown in Fig. 18 is slightly greater than unity, which is the expected direction if we do not have full embedding of the solute, this indicates a very strong propensity for the nonpolar methylene group to be embedded in the bonded phase chain, and not simply to lay on top of the bonded phase chain. Thus, in accord with the definition of Dorsey and Dill [2], the alkyl chain of the test solute primarily "partitions" into the bonded phase chain. The preceding discussion does not provide any support for any specific lattice model of RPLC. In a recent theoretical study of a self-consistent field theory of RPLC Bohmer *et al.* [51] developed a model which allowed the prediction of the methylene group increment. Their work showed that the ratio of the free energy of transfer in a pure partition system to that in RPLC with a long bonded alkyl chain should vary from about 1.06 in a highly water-like mobile phase to 1.30 in a very organic-like mobile phase. These results are also in accord with the general upward trend seen in the data of Fig. 18. Finally we point out that it is difficult to reconcile the near equality of the free energies of the bulk partition process and RPLC if displacement of sorbed mobile phase were involved in the chromatographic retention process of nonpolar solutes.

Several caveats must be borne in mind. First, our results with isopropanol and tetrahydrofuran as modifier are clearly different from the results with methanol. This likely results from the very large amount of the isopropanol and tetrahydrofuran that sorb into the bonded phase. Second, results not shown here with other types of bonded phases and silica gels are substantially different. Third, all of the solutes considered in Fig. 18 were non-polar alkylbenzenes.

Polar solutes

We believe that the use of bulk hexadecane as a model for the bonded phase is justified for only non-polar solutes. We anticipate that the model will fail when polar solutes such as alcohols or amides are used as test solutes. This idea is supported by a plot of log k' *vs.* log P (Fig. 19) taken from the work of Tsukahara *et al.* [52]. In this figure k' is the capacity factor on an ODS column and P is the partition coefficient for the dodecane/(methanol–water) system at the same mobile phase compositions. Both polar and non-polar solutes were included in the data set. The data were taken at mobile phase compositions of 0.40, 0.60, 0.80 and 1.00 mole fraction methanol. The straight line in this figure is drawn on the assumption that P equals k'/ϕ, where ϕ is the column phase ratio. If bulk dodecane were to serve as a good model for the bonded phase, the distribution of the solutes between these two systems would only differ by the phase ratio, and thus all the data points would fall on the straight line. However, in many instances, the data points deviate positively from this line; the smaller is the log P value, the more conspicuous is this deviation. This implies that weakly hydrophobic compounds, *i.e.* polar solutes, prefer ODS to dodecane when being transferred from a methanol–water mobile phase. We point out that the interpretation of Fig. 19 in the region of low k' is complicated by the notoriously poor precision and accuracy of k' data when k' values are less than unity. Nonetheless, these results strongly suggest that bulk alkane will not be a good model for the bonded phase for polar solutes.

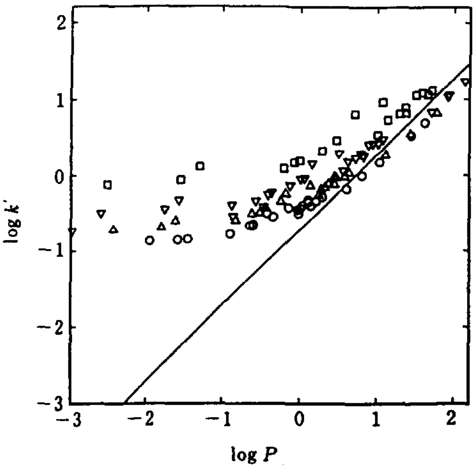

Fig. 19. Plot of the logarithm of chromatographic capacity factor (log k') in the ODS/(methanol–water) system *vs.* logarithm of the liquid–liquid partition coefficient (log P) in the dodecane/(methanol–water) system. Data from ref. 52 by permission of The Japan Society for Analytical Chemistry. Mole fraction of methanol in the methanol–water mixture, X: ○ = 1.00; △ = 0.80; ▽ = 0.60; □ = 0.40. The line represents the relation: log k' = log P + log ϕ.

We next attempted to determine which type of interaction between the polar solute and the stationary phase makes the alkyl bonded phase chemically different from the bulk-alkane phase. That is, we attempted to discern whether the deviations are due to dipole–dipole interactions, or to hydrogen bonding interactions. In order to understand this, we compare the linear solvation energy relationship (LSER) for $K_{\text{water,octanol}}$ and $K_{\text{water,hexadecane}}$ shown in eqns. 15 and 16, respectively. Here, $K_{\text{water,octanol}}$ and $K_{\text{water,hexadecane}}$ refer to the transfer of solute from bulk water to bulk octanol and from bulk water to bulk hexadecane, respectively. The symbols V_x denotes the volume; $\Sigma\pi_2^*$ the dipolarity/polarizability; R_2 the excess molar refraction; $\Sigma\alpha_2$ the hydrogen bonding acidity and $\Sigma\beta_2$ the hydrogen bonding basicity of solute. The coefficient for each solute property represents the net corresponding property between the two bulk phases. For example, the coefficient of the hydrogen bond acidity represents the net hydrogen bond basicity between the two bulk phases. The results given in these two equations were kindly provided by Abraham and Taft [53].

$$K_{\text{water,octanol}} = 0.07 + 3.80V_x/100 - 1.04\Sigma\pi_2^* + 0.56R_2 + 0.01\Sigma\alpha_2 - 3.40\Sigma\beta_2 \tag{15}$$

$n = 591$, S.D. $= 0.14$, $r = 0.9969$

$$K_{\text{water,hexadecane}} = 0.13 + 4.39V_x/100 - 1.60\Sigma\pi_2^* + 0.65R_2 - 3.55\Sigma\alpha_2 - 4.91\Sigma\beta_2 \tag{16}$$

$n = 374$, S.D. $= 0.13$, $r = 0.9980$

$K_{\text{water,octanol}}$ has been repeatedly related to the retention factor in RPLC [54–57]. It has also been shown that an octadecyl bonded phase in contact with an aqueous–organic mobile phase has a polarity similar to that of octanol [58]. Therefore, it is reasonable to use $K_{\text{water,ocatanol}}$ as to model retention in RPLC, and compare it with $K_{\text{water,hexadecane}}$. The largest difference between eqns. 15 and 16 lies in the coefficient for hydrogen bond acidity ($\Sigma\alpha_2$). Both water and octanol are good hydrogen bond acceptors and their basicity cancels. As a result, the net basicity between the two phases, which is reflected by the coefficient for hydrogen bond acidity in eqn. 15, is almost zero. In contrast, hexadecane cannot hydrogen bond and the net basicity between hexadecane and water is large, *i.e.* −3.55 as shown in eqn. 16. The coefficients for dipolarity/polarizability ($\Sigma\pi_2^*$) and hydrogen bond basicity ($\Sigma\beta_2$) are also substantially different. Again, this is because hexadecane cannot hydrogen bond and is much less polar than is octanol. Thus, compounds with significant values of $\Sigma\alpha_2$, $\Sigma\pi_2^*$ and $\Sigma\beta_2$ will tend to partition more into the octanol phase as compared to the hexadecane phase, both equilibrated with water. On the other hand, non-polar solutes which do not hydrogen bond, such as alkanes, will not differentiate between the hexadecane and octanol phases. In conclusion, if hexadecane were to be used to model alkyl bonded phase in RPLC, it will only be useful for non-polar compounds but not for polar compounds. This is exactly what is demonstrated in Fig. 19. Compounds which fall on the line are primarily non-polar compounds with insignificant hydrogen bond acidity/basicity, while those which fall off the line are mainly strong hydrogen bond donors such as alcohols, and compounds with high $\Sigma\pi_2^*$ and $\Sigma\beta_2$ such as amides.

The data of Fig. 19 also indicate that the bonded phase in RPLC is a much more polar environment than is bulk alkane, as has been shown in the many solvatochromic and fluorescence studies [58–62]. This does not contradict our findings that bulk hexadecane is a good model for the bonded phase for non-polar solutes. We are aware that water and organic modifier(s) in the mobile phase become incorporated into the bonded phase [63–66]. Our model simply emphasize the fact that the retention of non-polar solutes in RPLC is primarily due to the bonded alkane chains. In contrast, polar solutes will inevitably interact with the sorbed polar co-solvents through dipole–dipole and hydrogen bond interactions.

CONCLUSIONS

In contrast to the conventional solvophobic models of RPLC, we conclude that the net free energy of interactions of a methylene group with the stationary phase is larger than the net free energy of interactions of a methylene group with the mobile phase. Similarly, stationary phase processes contribute more to establishing the methylene group selectivity than do processes in the mobile phase. However, variations in retention and non-polar selectivity upon changing the mobile phase composition are certainly dominated by alterations in the solute–mobile phase processes, and not by alterations in the stationary phase. Based on comparison of experimental and computed activity coefficients, we have shown that regular solution theory is grossly wrong and should not be used for any quantitative predictions involving aqueous systems. Finally, the free energy of transfer of a methylene group from methanol–water mobile phase to bulk hexadecane is sufficiently similar to that for transform to a bonded phase that we conclude that there must be very significant embedding of the methylene group into the bonded phase.

ACKNOWLEDGEMENT

This work was supported by a grant from the National Science Foundation. P.W.C. wishes to acknowledge many fruitful discussions of this work with D.E. Martire, L.R. Snyder and S.G. Weber.

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