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STUDY OF PARTITION MODELS IN REVERSED-PHASE LIQUID CHROMATOGRAPHY BASED ON MEASURED MOBILE PHASE SOLUTE ACTIVITY COEFFICIENTS

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SUMMARY

Lack of accurate values for solute activity coefficients in aqueous organic solvent mixtures has been an impediment to the development of a detailed model of reversed-phase liquid chromatography (RPLC). In this study we have employed a recently measured set of infinite dilution activity coefficients for the alkylbenzenes (benzene to *n*-butylbenzene) in mixtures of water with four of the more common organic cosolvents in order to explore the mechanism of the retention process in RPLC. The work indicates 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. Analysis of relative solute activity coefficients of two solutes in the bonded phase shows that the stationary phase environment is considerably more polar than that of a bulk long chain alkane. This supports the idea that sorbed organic modifier plays a substantial role in establishing the chemistry in the bonded phase domain. The fact that measurements of the activity coefficients of non-polar solutes in methanol-saturated hexadecane are insignificantly different from those in pure hexadecane strongly suggests that the vastly different surface area to volume ratio of bonded and bulk phases is vitally important in bonded-phase RPLC.

INTRODUCTION

The primary purpose of this study was to examine the effect of mobile phase composition on the retention of a series of non-polar solutes and to apply the results to the study of the retention mechanism of reversed-phase liquid chromatography (RPLC).

In this work we choose to study the retention of the alkylbenzenes for many reasons. First we felt that the solute–condensed phase interaction would be simplest with a non-polar solute thereby making data interpretation easier. Secondly the alkylbenzenes can be readily measured with common liquid chromatographic detectors. Finally and most importantly the use of non-polar solutes would circumvent the complexities introduced when solutes interact with underivatized silanol groups which are inevitably present in bonded phases[1,2].

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The partition model

The retention of a series of homologous alkylbenzenes was studied and the results were analyzed in terms of three partition-like models of bonded-phase chromatography. The retention mechanism in RPLC has been the subject of a great deal of attention and controversy. Many mechanisms[3–10] have been proposed, but none has been conclusively demonstrated. This is due to the complexity of RPLC with bonded phases and difficulties in obtaining the appropriate experimental data needed to test the proposed mechanisms. It should be noted that most, but certainly not all, studies of RPLC have been rather qualitative. In many studies ln k' was correlated with some property of the mobile phase (*e.g.* its surface tension[8], or empirical solvent strengh parameters (*e.g.* a solvatochromic property of the solvent[11,12]). In contrast, there have been few attempts to relate observed capacity factors to capacity factors computed from the amount of stationary and mobile phases and fundamental theories involving specific properties of the phases and the probe solutes. The work of Schantz *et al.*[13] is a notable exception to this generalization.

Fundamentally a detailed mechanism of the retention process requires the development of an equation which predicts the free energy of transfer of the solute from the mobile to stationary phase in terms of independently measurable properties. This can be done at many levels of sophistication. In this work we choose to rely upon measurements of as many properties as possible and to minimize the need for estimating unknown parameters.

In the simplest terms retention can be viewed as a distribution of a solute between a mobile and stationary phase. The free energy change for the transfer process can be related to the activity coefficients of the solute in both phases under a given set of conditions (mobile phase, stationary phase, temperature). Technical and fundamental difficulties arise from the fact that the stationary phase in bonded-phase RPLC is not a uniform bulk liquid. Several different definitions of what constitutes the effective stationary phase in RPLC have been proposed[3–6,14–18].

Some workers have paid particular attention to the interaction of the solute and mobile phase with the hydrophobic surface[6,19–21]. Such models are most appropriately termed adsorption mechanisms. The above cited papers employed Everett's specific definition of the surface activity coefficient[22,23]. In these approaches one deals with an effective surface phase activity coefficient that is represented as the product of two terms. The first of these terms takes into account the contribution of the interfacial tension of the system to the solute chemical potential in the bonded phase. The second term is a more conventional activity coefficient, that is, it is related to the transfer free energy as if the interfacial contribution to the free energy of transfer were zero.

This emphasis on surface–solute and surface–eluent interaction terms can be contrasted with Snyder's view of normal phase liquid chromatography[24]. Snyder assumed that the difference between the surface–eluent and surface–solute interaction is the major driving force for solute retention in normal-phase chromatography and that the mobile phase effects are small compared to stationary phase effects.

In RPLC most models minimize the importance of the solute–surface interaction[3–5,7–9]. In contrast, work which emphasizes the adsorption model considers the surface effect to be a major term[19–21]. Everett's definition of a surface activity coefficient was used and consequently the surface interaction effect was separated

from the other energy terms. Of the many studies of the retention mechanism of RPLC the work of Locke[6] and Lochmuller and Wilder[4] are closely related. Locke[6] based his estimate of a solute's activity coefficient on its solubility in water and was able to demonstrate a linear relationship between the logarithm of this activity coefficient in the mobile phase and the logarithm of the capacity factor.

The study of Schantz *et al.*[13], who examined the partition model in detail, is very relevant to the present study. These authors compared measured partition coefficients (K) for a series of alkylbenzenes between various methanol–water mixtures and bulk phase hexadecane to the net retention volume (V_N) of the same solutes on a bonded-phase RPLC column operated at the same mobile phase composition. The ratio of K to V_N is completely independent of the solute activity coefficient in the mobile phase. They found that this ratio was not independent of the solute. The difference in the ratio varied by 0.4 ln units from benzene to *n*-butylbenene. This is unambiguous evidence that the effective activity coefficient of the solute in the bonded phase is not the same as the activity coefficient in bulk hexadecane. If they were identical there could be no variation upon change in solute.

More importantly they observed that the ratio of retention in bulk phase hexadecane to bonded phase octadecane decreased, roughly linearly, by 0.9 ln units as the mobile phase composition varied from pure methanol to about 50% (v/v) methanol. This implies that there is a very substantial differential effect of the mobile phase on the solute activity coefficient in the bonded and bulk phases. This ratio depends upon the amount of both phases. It is evident from their data that the volume of stationary phase will decrease with an increase in the fraction of water in the mobile phase thereby decreasing the bonded phase activity coefficients as water is added to the mobile phase. Martire and his co-workers also showed by direct measurement that the amount of methanol in hexadecane when equilibrated against pure methanol is very small (approximately 0.003 mole of methanol per mole of hexadecane). This seems to be a trivial quantity and ought not alter the activity coefficient of a solute in bulk hexadecane.

THEORY

The thermodynamic equilibrium constant for a partition model can be written as follows:

$$K_{th} = \frac{X_1^s \gamma_1^s}{X_1^m \gamma_1^m} = 1 \tag{1}$$

where

$$X_1^s = n_1^s/n_2^s \tag{2}$$

$$X_1^m = n_1^m/n_2^m \tag{3}$$

The terms X and γ denote the mole fraction and the activity coefficient of the solute (subscript 1) in the mobile (m) and stationary (s) phases, respectively. In addition the

terms n_1 designate the number of moles of solute in each phase whereas n_2^s and n_2^m represent the total number of moles of stationary phase and mobile phase in the column.

Certainly in the case of liquid–liquid chromatography with two perfectly immiscible fluids the meaning of the number of moles of stationary and mobile phase is quite clear. It is far more ambiguous in bonded-phase chromatography. For example we can certainly disregard the number of moles of silica in the column for all solutes that do not interact with silica. However, it is not clear whether sorbed organic modifier[18] should be counted as part of the stationary phase. If it can be shown that the sorbed organic modifier alters the properties of a solute when it is in the stationary phase then it is reasonable to include it as being part of the total number of moles in the stationary phase. The number of moles of ligand chemically bonded to the support should constitute a minimum estimate of the amount of stationary phase in any simple partition model of bonded-phase RPLC. Thus at this point we feel that it is best to defer a precise definition of what we mean by n_2^s.

The capacity factor can be defined as follows:

$$k' = n_1^s/n_1^m \tag{4}$$

Combining eqn. 1–4 we obtain

$$k' = (\gamma_1^m/\gamma_1^s)\,(n_2^s/n_2^m) \tag{5}$$

$$\ln k' = \ln \gamma_1^m - \ln \gamma_1^s + \ln n_2^s - \ln n_2^m \tag{6}$$

We now examine the above equations in terms of those quantities that are measureable. The capacity factor and the solute activity coefficient[25] in the mobile phase can be measured. Assuming that the column void volume is a good measure of the volume of mobile phase in the column the number of moles of the mobile phase in the column can be computed without further approximation as follows:

$$n_2^m = V_m d_m\,[w_0/M_0 + (1 - w_0)/M_w] \tag{7}$$

where V_m is the void volume of the column, d_m the density of the mobile phase, M_0 the molecular weight of the organic modifier, M_w the molecular weight of water, w_o the weight fraction of organic modifier in the mobile phase, and w_w the weight fraction of water in the mobile phase. Eqn. 6 can be rewritten as follows:

$$A = \ln k' + \ln (n_2^m/\gamma_1^m) = \ln (n_2^s/\gamma_1^s) \tag{8}$$

In eqn. 8, all of the measureable mobile phase terms are placed on the left and the stationary phase terms on the right. A very important idea is that the parameter A can be measured and studied as a function of the volume fraction of organic modifier in the mobile phase (φ). When A is independent of φ then it must follow, based only on thermodynamics, that n_2^s/γ_1^s will be independent of φ.

In an effort to define what actually constitutes the number of moles of material comprising the stationary phase we will consider three distinct "partition" type models

of the stationary phase. In model I we assume that the stationary phase only consists of the bonded phase ligand. It follows in this case that the number of moles of stationary phase must be independent of φ. The number of moles of stationary phase would also appear to be independent of φ if, beyond some minimum value of φ, the stationary phase were saturated with organic modifier. Thus in model II the stationary phase is assumed to consist, over the range in φ studied, of the bonded ligands and a fixed amount of sorbed modifier. In both of the above models it follows that the solute activity coefficient will not depend on φ since the solute's environment is fixed. A final possibility (model III), which will provide a solute environment that is independent of φ, is based on the idea that active stationary phase, that is the region into which the solute partitions, is comprised of a sorbed multilayer of pure organic modifier. In this model the bonded phase ligands serve only as a substrate onto which the multilayer of modifier sorbs. In model III the amount of stationary phase might vary with φ due to an increase in the number of multilayers but since the solute's environment is fixed one expects, as a first approximation, that the activity coefficient in the multilayer will be independent of φ.

In all of the above models a solute molecule in the stationary phase experiences an environment that is independent of φ. Consequently the stationary phase solute activity coefficient will be independent of φ. For cases I and II we must conclude on purely thermodynamic grounds that when the stationary phase composition is independent of φ the term A will be independent of φ. This is not true in case III.

Obvious cases II and III cannot hold over the entire range of φ (0–1.0). There must come a point where φ becomes so small that the amount of modifier in the stationary phase (case II) or the number of multilayers (case III) decreases. We will show below that for a variety of mobile phase modifiers and for a series of alkylbenzenes the term A and therefore n_2^s/γ_1^s does depend on φ but the dependence is weak.

A purely arithmetic examination of eqn. 8 indicates that A can appear to be independent of φ in two different ways. This will be so when both the solute activity coefficient in the stationary phase and the number of moles of stationary phase are both independent of φ. A second is that both the solute activity coefficient in the stationary phase and the number of moles of stationary phase vary with φ in such a fashion that the measured ratio appears to be independent of φ (see the right-hand side of eqn. 8).

In attempting to differentiate between these two possibilities we will consider the ratio of capacity factors for two similar solutes. Eqn. 5 can be rewritten for solutes 1 and 2 as follows:

$$k'(1)\, n_2^m/\gamma_1^m(1) = n_2^s/\gamma_1^s(1) \tag{9}$$

$$k'(2)\, n_2^m/\gamma_1^m(2) = n_1^s/\gamma_1^s(2) \tag{10}$$

Dividing eqn. 10 by 9 we get

$$[k'(2)/k'(1)]\,[\gamma_1^m(1)/\gamma_1^m(2)] = \gamma_1^s(1)/\gamma_1^s(2) = B_{2/1} \tag{11}$$

In this approach the number of moles of stationary phase drops out. If the left-hand

side of eqn. 11 is independent of φ then the ratio ($B_{2/1}$) of the stationary phase activity coefficients of the two solutes must be independent of φ. We hypothesize that when the ratio of activity coefficients is independent of φ then the individual activity coefficients will also be independent of φ or else the two activity coefficients must vary so similarly with φ that the ratio appears to be constant. This point will be discussed in more detail later.

EXPERIMENTAL

Activity coefficients

The activity coefficients of the alkylbenzenes in aqueous solvents were measured by head space gas chromatography (HSGC)[25]. The background and experimental details can be found in a previous report[26] and other references[27,28].

HPLC

Retention data for the methanol–water system[29,30] and the acetonitrile–water system[31] were taken from the literature. The data for the isopropanol–water and tetrahydrofuran–water systems were measured in this laboratory. A Hypersil ODS column (100 × 4.6 mm I.D., 5 μm, Hewlett-Packard, Avondale, PA, U.S.A.) was used throughout this study. The column was placed in a water jacket and the temperature was controlled at 25 ± 0.2°C. An Altex pump with a pulse dampener (Model 110AQ, Altex Scientific, Berkeley, CA, U.S.A.) was used to deliver the mobile phase. Samples were injected via a home-made auto-injector by using a Valco air-actuated injector (Model AC6W, Valco Instruments, Houston, TX, U.S.A.) equipped with a 10-μl loop. A Hitachi variable-wavelength UV–VIS dectector (Model 100-10, NSI/Hitachi Scientific Instruments, Mountain View, CA, U.S.A.) was used to generate the solute elution profiles. All retention times were based on the peak maximum position. The eluent flow-rate was varied from 0.2 to 1.0 ml/min depending on the mobile phase composition. Water was used as the void volume marker[32].

The capacity factor data for the isopropanol–water and tetrahydrofuran–water systems measured in this study are given in Tables I and II. Some important column characteristics were obtained from the literature[33,34] and are summarized in Table III.

TABLE I

CAPACITY FACTORS OF ALKYLBENZENES IN ISOPROPANOL–WATER MIXTURES

Measured in a Hypersil ODS column (46 × 100 mm, 5 μm) at 25°C with a 10-μl sample loop.

Solute	*Volume fraction of isopropanol*							
	1.0	*0.9*	*0.8*	*0.7*	*0.6*	*0.5*	*0.4*	*0.3*
Benzene	0.220	0.258	0.374	0.579	0.948	1.666	3.052	7.50
Toluene	0.224	0.306	0.455	0.722	1.253	2.425	5.031	14.78
Ethylbenzene	0.227	0.347	0.513	0.878	1.579	3.250	7.633	27.37
Propylbenzene	0.233	0.372	0.612	1.051	2.015	4.512	12.0	55.06
Butylbenzene	0.242	0.401	0.739	1.269	2.596	6.213	18.34	107.9
Cumene	0.238	0.346	0.589	0.972	1.852	4.097	10.64	–
tert.-Butylbenzene	0.256	0.382	0.648	1.099	2.174	5.008	14.04	–

TABLE II

CAPACITY FACTORS OF ALKYLBENZENES IN TETRAHYDROFURAN–WATER MIXTURES

Measured in a Hypersil ODS column (46 × 100 mm, 5 μm) at 25°C with a 10-μl sample loop.

Solute	*Volume fraction of tetrahydrofuran*						
	0.9	*0.8*	*0.7*	*0.6*	*0.5*	*0.4*	*0.3*
Benzene	0.210	0.312	0.572	0.955	1.674	3.292	7.486
Toluene	0.217	0.354	0.673	1.181	2.302	4.980	13.87
Ethylbenzene	0.253	0.378	0.754	1.401	2.972	7.350	25.07
Propylbenzene	0.261	0.423	0.849	1.720	3.965	11.18	47.27
Butylbenzene	0.274	0.452	0.965	2.048	5.130	17.10	–
Cumene	0.261	0.406	0.834	1.637	3.804	10.32	–
tert.-Butylbenzene	0.273	0.426	0.897	1.852	4.580	13.47	–

RESULTS AND DISCUSSION

Plots of $\ln k'$ and $\ln \gamma_1^m$ *versus* φ are shown in Fig. 1. We believe that these results provide unequivocal thermodynamic evidence that the principal driving force for the *change* in retention in RPLC upon variation in φ are due to changes in the solute–solvent interactions in the mobile phase and are only secondarily due to solute–solvent interactions in the stationary phase. The variation in the stationary phase contributions to k' are represented by the parameter A. Comparison of Fig. 2 to Fig. 1 shows that the variations due to the mobile phase interactions are much greater than those due to the stationary phase interactions. Note that the scale of the ordinate in Fig. 1 is five-fold larger than the ordinate in Fig. 2.

TABLE III

COLUMN CHARACTERISTICS

V = Column volume (ml), calculated by using the column dimensions; d_s = packing density of stationary phase (g/ml); V_m = column void volume (ml); W_s = weight of stationary phase (g); A_{sp} = specific surface area of silica substrate.

Column characteristic	*LiChrosorb RP-C_{18}*[a]	*Zorbax ODS*[b]	*Develosil ODS-5*[c]	*Hypersil ODS*[d]
V	3.77	2.492	2.492	1.661
d_s	0.5	0.783	0.8	0.8
V_m	2.26[e]	1.414	1.495[e]	1.150
W_s	1.885	1.95	1.99	1.329
A_{sp}	343	150[f]	150[f]	150[f]

[a] Used in work of Schoenmakers *et al.*[29].
[b] Used in Barmam's work[30].
[c] Used in Hanai and Hubert's work[31].
[d] Used in this study.
[e] Calculated assuming the total porosity is 0.6.
[f] Best available estimate.

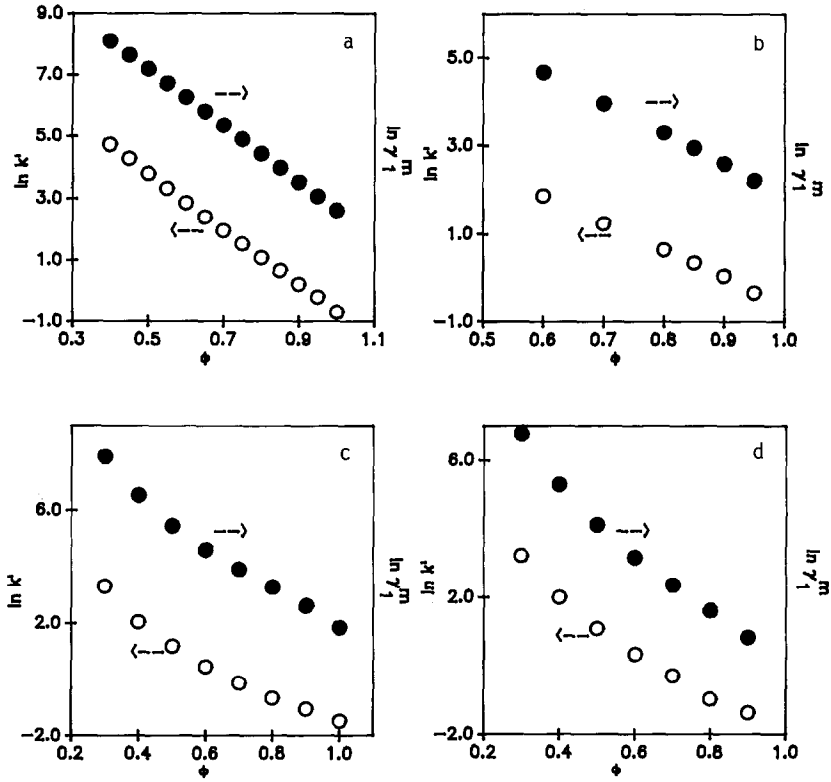


Fig. 1. The variation in capacity factor (○) and solute activity coefficients (●) in the mobile phase. The solute in all cases is ethylbenzene. Solvent system: (a) methanol–water; (b) acetonitrile–water; (c) isopropanol–water; (d) tetrahydrofuran–water.

It is evident from the data summarized in Fig. 1 that the variations in k' and γ_1^m with φ are both very large and strikingly similar. The data for ethylbenzene shown in Fig. 1 are quite typical. For all solutes examined plots of ln k' and ln γ_1^m *versus* φ were almost parallel. In contrast the variation in factors related to the stationary phase (see Fig. 2), summarized in term A (see eqn. 8), are much smaller compared to the variation in ln k' and ln γ_1^m. We note that for ethylbenzene in methanol a 270-fold greater change occurs in the mobile phase than in the stationary phase. For the other mobile phase modifiers the changes in the term A with φ are smaller (see Fig. 2) and in the case of isopropanol and tetrahydrofuran they are not monotonic. Because the variations in ln γ_1^m and ln k' with φ are quite large a 1% change in φ will cause a 10% change in k' or γ_1^m. Clearly small experimental errors in establishing the φ value for both k' and γ_1^m could easily account for some of the non-monotonic trends. It is unrealistic to believe that the A values are accurate to better than 0.1–0.2 ln units. On the whole it is clear that A does vary with φ in the methanol–water and tetrahydrofuran–water systems, possibly in the acetonitrile–water system and at most very slightly in the isopropanol–water system.

The data presented above do not prove that the major overall driving forces for retention in RPLC are the processes going on in the mobile phase. It is possible, but

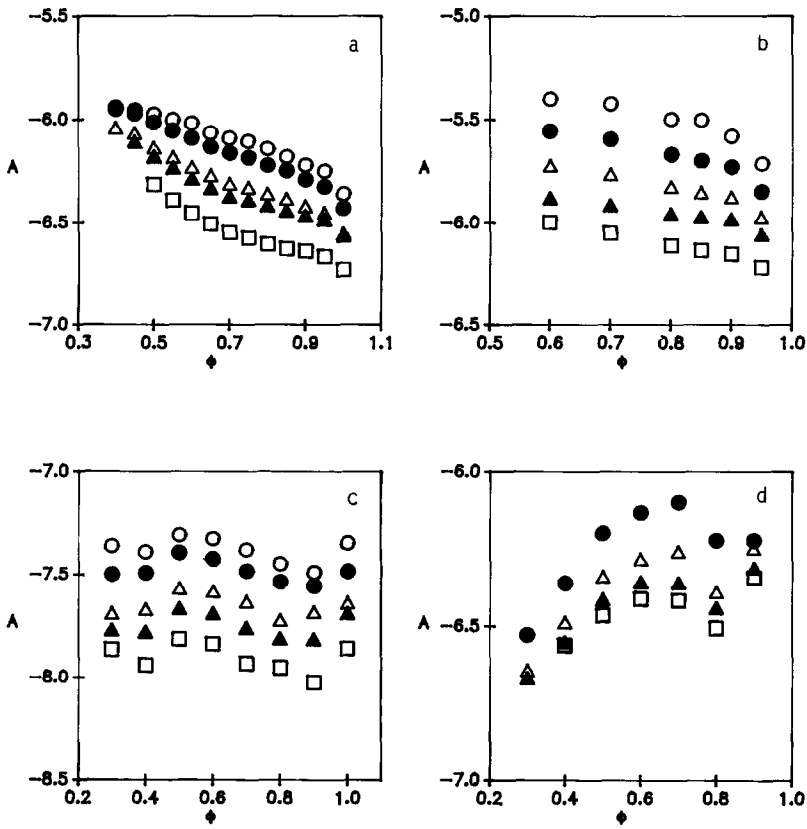


Fig. 2. The variation in A with mobile phase volume fraction. Solvent system: (a) methanol–water; (b) acetonitrile–water; (c) isopropanol–water; (d) tetrahydrofuran–water. Solutes: ○ = benzene; ● = toluene; △ = ethylbenzene; ▲ = *n*-propylbenzene; □ = *n*-butylbenzene.

very unlikely, that the large k' values are due to very low stationary phase activity coefficients. We addressed this issue by estimating the activity coefficients in the bonded phase by the use of bulk phase analogs. Two extreme points of view can be adopted in estimating the solute activity coefficients in the bonded phase. First one can assume, as in model I above, that a non-polar environment is established by the bonded alkyl chains. In this case solute stationary phase activity coefficients should be modeled by their values in, for example, *n*-hexadecane. In another study[35] we found that the activity coefficient of toluene in hexadecane was equal to 0.96. The values for the activity coefficients of a series of alkylbenzenes in hexadecane, as measured by Schantz and Martire[36], along with those computed by the UNIFAC method[37] for estimating limiting activity coefficients are given in Table IV. We see that all of the values are, in accord with intuition, similar and close to unity.

Values measured in this work by HSGC for benzene, toluene, ethyl benzene and propyl benzene are in good agreement with the literature. The value for *n*-butylbenzene disagrees with that of Schantz and Martire. The error in activity coefficients measured by HSGC for very low volatility solutes such as *n*-butylbenzene (b.p. 180°C) could be

TABLE IV

MEASURED AND COMPUTED SOLUTE ACTIVITY COEFFICIENTS IN HEXADECANE

Solute	*Activity coefficients*			
	Measured[a]	*Measured*[b]	*Measured*[c]	*Computed by UNIFAC*
Benzene	1.09	1.07	1.08	0.91
Toluene	1.03	1.05	1.07	1.03
Ethylbenzene	1.15	1.16	1.18	1.05
n-Propylbenzene	1.14	1.21	1.22	1.09
n-Butylbenzene	1.14	1.30	1.31	1.13

[a] From Schantz and Martire[36].
[b] In this work.
[c] In hexadecane saturated with methanol.

greater than 10% due to adsorption/condensation of the solute in the transfer lines especially when the solvent is also non-volatile.

In any instance it is evident that if bulk hexadecane were a good model for the stationary phase activity coefficients then the $B_{2/1}$ values would be close to unity and would not vary from solute to solute nearly as much as do the mobile phase values. Consequently based on model I we conclude that the principal driving force for retention in RPLC lies in the mobile phase.

A second perspective is that the stationary phase solute activity coefficient is completely controlled by the sorbed organic modifier as would be the case if model III were to prevail. Here the activity coefficients in the stationary phase should be modeled by their values in the pure bulk organic modifier. We measured these quantities as part of this study (see Table V). Relative to the huge activity coefficients in the

TABLE V

ACTIVITY COEFFICIENTS IN PURE ORGANIC LIQUIDS

Measured by head space gas chromatography in the indicated solvent at infinite dilution at 25°C. Based on the mole fraction concentration scale and Raoult's law reference state.

Solute	*Methanol*	*Acetonitrile*	*Isopropanol*	*Tetrahydrofuran*
Benzene	6.82	2.83	4.24	–
Toluene	9.67	4.03	5.08	0.84
Ethylbenzene	13.2	5.59	6.07	0.90
n-Propylbenzene	17.5	7.79	6.95	0.91
n-Butylbenzene	24.5	11.1	8.51	0.90
Reciprocal relative activity coefficients[a]				
Benzene	1.42	1.43	1.20	–
Ethylbenzene	0.73	0.72	0.83	0.93
n-Propylbenzene	0.55	0.52	0.74	0.93
n-Butylbenzene	0.39	0.37	0.60	0.94

[a] The activity coefficient of toluene divided by the activity coefficient of the solute of interest.

hydro-organic mobile phase these values are on the order of unity. Therefore regardless of the two extreme points of view outlined above it is unreasonable to expect that the non-polar solutes studied here will be "pulled" into the stationary phase by virtue of a very small stationary phase activity coefficient. In addition the differences in the solute activity coefficients in the pure organic modifiers are not nearly large enough to account for the solute to solute variations in k'.

Based on the above we believe that the basic concept that retention in RPLC is due to the solvophobic effect is validated. This is not to say that any specific solvophobic model, such as that of Horváth and co-workers[8,9], is correct in detail. Given that the mobile phase provides the major driving force for retention this does not mean that changes in the type of non-polar bonded ligand will be chromatographically unimportant. The results of Schantz *et al.*[13], as do our A values, indicate that solute activity coefficients in a bonded stationary phase are significantly altered by the mobile phase. One must expect that the type of the bonded phase will modify its propensity for sorption of the mobile phase. Based on the very large difference in the solubility of methanol in hexane and in hexadecane it would be surprising if there were no differences in the amount of organic modifier sorbed by bonded octyl and octadecyl groups. Further in view of the fact that a reasonably efficient column can easily sense differences in transfer free energies of two solutes of less than 100 cal/mol, subtle changes in the stationary phase can significantly alter chromatographic selectivity factors.

We next examine the relative retention of two solutes on the same column as a function of φ. The term $B_{2/1}$ is the relative stationary phase activity coefficient of two solutes. The data given in Table VI summarize our results using toluene as the reference solute. In view of the fact that the mobile phase activity coefficients are only precise to a few percent the relative stationary phase activity coefficients are, within experimental error, almost independent of φ over the range 0.3–1.0 for all four mobile phase modifiers (see Table V, and Figs. 3 and 4). The variation in $B_{2/1}$ with φ is much smaller, particularly for the methanol–water system, than the variation in A with φ. The sole possible exception being the tetrahydrofuran–water system where there does appear to be a significant increase in $B_{2/1}$ as the volume fraction of tetrahydrofuran approaches unity. These results may, however, be due to the experimental difficulties encountered in measuring the very low k' values in this system. This data should not be taken to mean that the stationary phase is not modified by the organic constituent of the mobile phase. We believe that it is (see below).

It is extremely interesting to note that despite the considerable variations of the solute activity coefficients in the pure polar organic solvents (see Table V) the differences in the relative stationary phase solute activity coefficients among the four types of organic modifiers are surprisingly small. In addition variations in the relative stationary phase activity coefficient from solute to solute are much greater than are those based on the use of bulk hexadecane as a model for the stationary phase. That is the variation in $B_{2/1}$ (see Table V) from benzene to *n*-butylbenzene is much greater than one would predict based on the activity coefficients in hexadecane given in Table IV.

Given the two-fold change in $B_{2/1}$ between benzene and *n*-butylbenzene we must conclude that the stationary phase environment is more polar than hexadecane. Thus case I, described above, is ruled out. This conclusion is in agreement with many fundamental studies of chromatography that indicate that a substantial amount of

TABLE VI

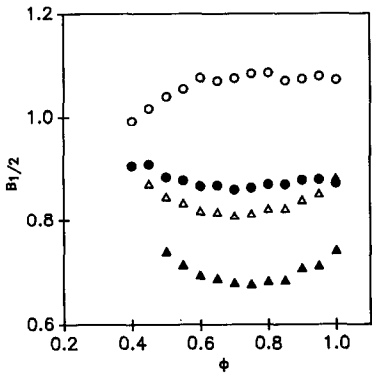
RELATIVE STATIONARY PHASE SOLUTE ACTIVITY COEFFICIENTS

Relative activity coefficients computed from eqn. 11. φ_{org} = Volume fraction of organic component in the mobile phase. $\gamma_1^{IPA}(1)/\gamma_1^{IPA}(2)$ = The ratio of the activity coefficient of toluene in bulk isopropanol to the activity coefficient of the indicated solute in isopropanol.

Solvent system	φ_{org}	*Benzene*	*Ethylbenzene*	*Propylbenzene*	*Butylbenzene*	*Cumene*	*tert.-Butylbenzene*
Methanol–water	1.00	1.07	0.872	0.881	0.742	–	–
	0.95	1.08	0.879	0.852	0.714	–	–
	0.90	1.07	0.878	0.839	0.709	–	–
	0.85	1.07	0.870	0.823	0.686	–	–
	0.80	1.08	0.870	0.822	0.684	–	–
	0.75	1.08	0.863	0.813	0.679	–	–
	0.70	1.07	0.860	0.809	0.681	–	–
	0.65	1.07	0.868	0.816	0.689	–	–
	0.60	1.08	0.867	0.819	0.696	–	–
	0.55	1.05	0.878	0.834	0.715	–	–
	0.50	1.04	0.884	0.846	0.741	–	–
	0.45	1.02	0.908	0.871	–	–	–
	0.40	0.99	0.905	–	–	–	–
Average		1.06	0.87	0.84	0.71	–	–
S.D.		0.03	0.01	0.02	0.03	–	–
Acetonitrile–water	0.95	1.15	0.882	0.811	0.693	–	–
	0.90	1.16	0.863	0.776	0.657	–	–
	0.85	1.21	0.857	0.761	0.649	–	–
	0.80	1.18	0.853	0.749	0.644	–	–
	0.70	1.19	0.841	0.726	0.636	–	–
	0.60	1.17	0.845	0.722	0.645	–	–
Average		1.18	0.85	0.76	0.66	–	–
S.D.		0.03	0.01	0.03	0.02	–	–

Isopropanol–water	1.0	1.15	0.857	0.814	0.689	0.828	0.841
	0.9	1.07	0.879	0.771	0.628	0.754	0.723
	0.8	1.09	0.828	0.758	0.661	0.789	0.721
	0.7	1.11	0.861	0.758	0.640	0.769	0.703
	0.6	1.10	0.852	0.766	0.662	0.776	0.719
	0.5	1.09	0.838	0.762	0.658	0.770	0.712
	0.4	1.11	0.836	0.749	0.640	0.759	0.697
	0.3	1.15	0.825	0.761	0.695	–	–
Average		1.11	0.84	0.77	0.66	0.78	0.73
S.D.		0.03	0.02	0.02	0.03	0.02	0.05
Tetrahydrofuran–water	0.9	–[a]	0.974	0.913	0.888	0.968	0.949
	0.8	–	0.845	0.802	0.754	0.825	0.780
	0.7	–	0.850	0.769	0.728	0.815	0.768
	0.6	–	0.859	0.797	0.757	0.824	0.790
	0.5	–	0.868	0.806	0.768	0.850	0.824
	0.4	–	0.879	0.825	0.818	0.854	0.828
	0.3	–	0.891	0.868	–	–	–
Average		–	0.88	0.83	0.79	0.86	0.82
S.D.		–	0.04	0.04	0.06	0.05	0.06
$\gamma_1^{IPA}(1)/\gamma_1^{IPA}(2)$[d]		1.20	0.83	0.74	0.60	0.77	0.69

[a] The mobile phase activity coefficient of benzene in tetrahydrofuran was not measurable by HSGC due to overlap with the solvent peak.



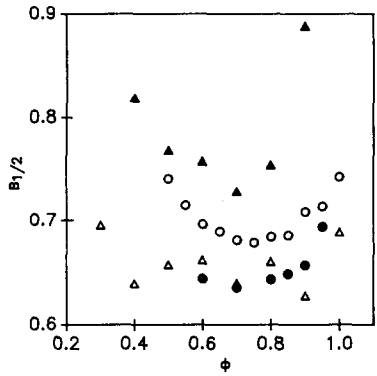


Fig. 3. Dependence of the relative stationary phase activity coefficient ($B_{2/1}$) on the solute and volume fraction of organic modifier in the mobile phase. All results are for the indicated solute relative to toluene in methanol–water mobile phases. Solutes: ○ = benzene; ● = ethylbenzene; △ = *n*-propylbenzene; ▲ = *n*-butylbenzene.

Fig. 4. Dependence of the relative stationary phase activity coefficient ($B_{2/1}$) on the type of mobile phase modifier and volume fraction of organic modifier in the mobile phase. All results are for the *n*-butylbenzene relative to toluene in the indicated mobile phases: methanol–water (○), acetonitrile–water (●), isopropanol–water (△), tetrahydrofuran–water (▲).

mobile phase is sorbed by the stationary phase[14,18]. More importantly it agrees with the solvatochromic studies of Stahlberg and Almgren[38], and Carr and Harris[39] in which the stationary phase polarity was directly probed with pyrene.

The $B_{2/1}$ values in all four organic modifiers are strikingly similar to those measured in bulk isopropanol (see the last line in Table V). This suggests the very simple idea that perhaps the absolute values of the stationary phase activity coefficients of all of the solutes in all of the mobile phases can be modeled by setting them equal to or proportional to their activity coefficient in pure bulk isopropanol (hereafter denoted as γ^{IPA}).

We will now investigate the consequences of making the following universal approximation:

$$\gamma_1^s = \alpha\, \gamma_1^{IPA} \tag{12}$$

Based on the approximate constancy of A and $B_{2/1}$ as φ is changed over the range 0.3–1.0 we believe that it is reasonable to make the rough approximation that the number of moles of stationary phase is constant. This is consistent with case II described above. We now combine eqn. 12 with eqns. 2 and 7 to give

$$k' = [\gamma_1^m/(\alpha\, \gamma_1^{IPA})]\ (n_2^s/\{V_m d_m\ [w_0/M_0 + (1 - w_0)/M_w]\}) \tag{13}$$

Since we have assumed that the number of moles of stationary phase is a constant the capacity factor can be computed as a function of φ to within an unspecified constant of proportionality. Taking the logarithm of both sides of eqn. 13 after combining the constants yields the following equation:

$$\ln k' = C + \ln \gamma_1^m - \ln \gamma_1^{IPA} - \ln \{V_m d_m [w_0/M_0 + (1 - w_0)/M_w]\} \quad (14)$$
$$= C + \ln k'_{calc}$$

We will refer to the sum of all the known terms on the right-hand side of eqn. 14 as ln k'_{calc}. In view of the above arguments the difference between ln k' and ln k'_{calc} is an unknown offset that should not affect the slope of a plot of ln k' *versus* ln k'_{calc}. The resulting plots for all four mobile phases for all solutes are shown in Fig. 5. Linear least squares analysis of this data was carried out and the results are summarized in Table VII. These are in all cases very good correlations, as are those based on the data shown in Figs. 6 and 7 (see below). The average least squares slopes for methanol, acetonitrile, isopropanol and tetrahydrofuran are: 1.089, 1.078, 1.007, and 0.941, respectively. For all but the isopropanol system there is a slight downward trend in the slope and a more distinct trend in the intercept as the solute size increases.

The slope for the isopropanol system is closest to unity and shows only random variations among the seven solutes investigated. The same is true of the intercepts, that

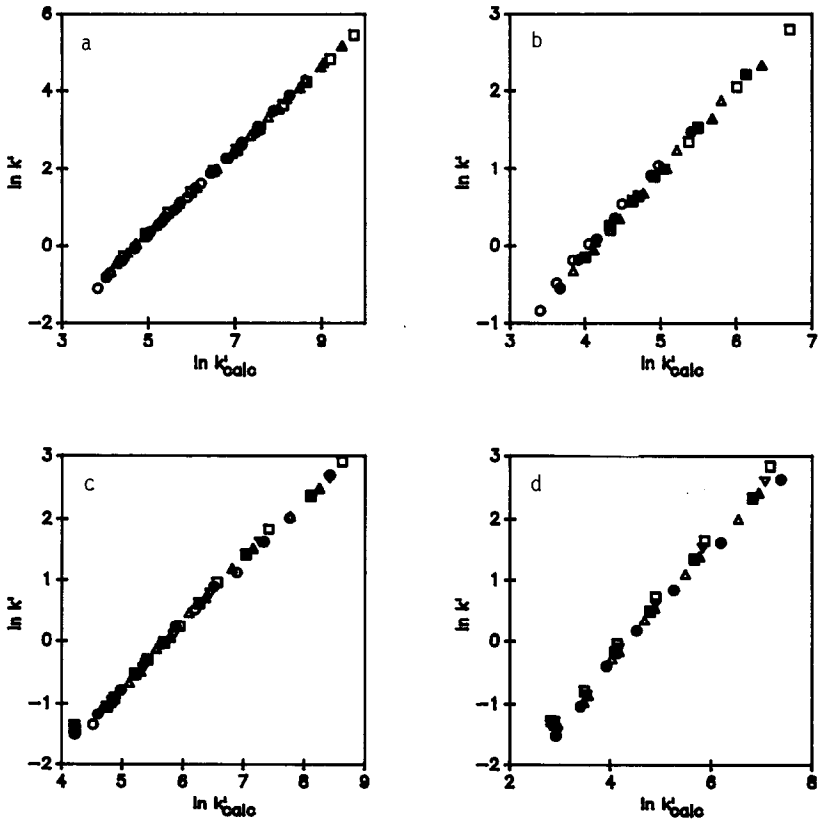


Fig. 5. Plot of measured ln k' *vs* ln k'_{calc} based on the isopropanol model for the stationary phase activity coefficient. The values of ln k'_{calc} were obtained from eqn. 14. Solvent system: (a) methanol–water; (b) acetonitrile–water; (c) isopropanol–water; (d) tetrahydrofuran–water. Solutes: ○ = benzene; ● = toluene; △ = ethylbenzene; ▲ = *n*-propylbenzene; □ = *n*-butylbenzene.

TABLE VII

REGRESSION ANALYSIS OF EFFECT OF φ ON SOLUTE RETENTION

The data below are the results of regressing the ln k' *vs.* the ln k'_{calc} as defined in eqn. 14. In all cases the solute activity coefficient in the stationary phase was assumed to be equal to that in bulk isopropanol. The results were obtained by regression of the individual solutes one at a time. The average result was obtained by regression of all of data for all of the solutes in the indicated solvent simultaneously.

Solute	*Intercept (S.D.)*	*Slope (S.D.)*	*r*	*n*
Methanol–water				
Benzene	−5.27(0.042)	1.103(0.007)	0.9999	13
Toluene	−5.23(0.026)	1.104(0.004)	0.9999	13
Ethylbenzene	−5.18(0.029)	1.095(0.004)	0.9999	13
Propylbenzene	−5.06(0.032)	1.076(0.005)	0.9999	12
Butylbenzene	−5.01(0.036)	1.068(0.005)	0.9999	11
Average	−5.15(0.02)	1.089(.003)	0.99976	62
Acetonitrile–water				
Benzene	−4.77(0.174)	1.177(0.043)	0.9974	6
Toluene	−4.73(0.111)	1.153(0.025)	0.9990	6
Ethylbenzene	−4.59(0.077)	1.116(0.016)	0.9995	6
Propylbenzene	−4.45(0.055)	1.070(0.011)	0.9997	6
Butylbenzene	−4.50(0.034)	1.087(0.006)	0.9999	6
Cumene	−4.54(0.063)	1.102(0.013)	0.9997	6
Average	−4.42(0.06)	1.078(0.011)	0.99797	36
Isopropanol–water				
Benzene	−5.870(0.110)	1.018(0.019)	0.9989	8
Toluene	−5.769(0.088)	1.010(0.015)	0.9993	8
Ethylbenzene	−5.709(0.084)	1.000(0.013)	0.9994	8
Propylbenzene	−5.724(0.086)	0.999(0.013)	0.9995	8
Butylbenzene	−5.750(0.092)	1.010(0.013)	0.9994	8
Cumene	−5.751(0.131)	1.007(0.022)	0.9988	7
tert.-Butylbenzene	−5.650(0.155)	0.989(0.025)	0.9983	7
Average	−5.76(0.04)	1.007(0.005)	0.99915	54
Tetrahydrofuran–water				
Toluene	−4.126(0.129)	0.927(0.026)	0.9980	7
Ethylbenzene	−4.088(0.096)	0.930(0.018)	0.9990	7
Propylbenzene	−4.124(0.071)	0.943(0.013)	0.9995	7
Butylbenzene	−4.063(0.085)	0.966(0.017)	0.9993	6
Cumene	−4.121(0.110)	0.953(0.021)	0.9990	6
tert.-Butylbenzene	−4.122(0.111)	0.958(0.020)	0.9989	6
Average	−4.090(0.056)	0.941(0.012)	0.99749	39

is, for the isopropanol system the intercepts for the various solutes do not differ beyond their individual standard deviations. In contrast the slope for the tetrahydrofuran system is the smallest, although it does not differ from unity as much as does the slope for methanol. We note that activity coefficients in bulk tetrahydrofuran are quite close to unity whereas the other bulk solvents induce much larger activity coefficients (see Table IV).

In three of the solvent systems the intercepts for the various solutes are so similar that the above approximation for γ_1^s leads to a universal curve for the retention of

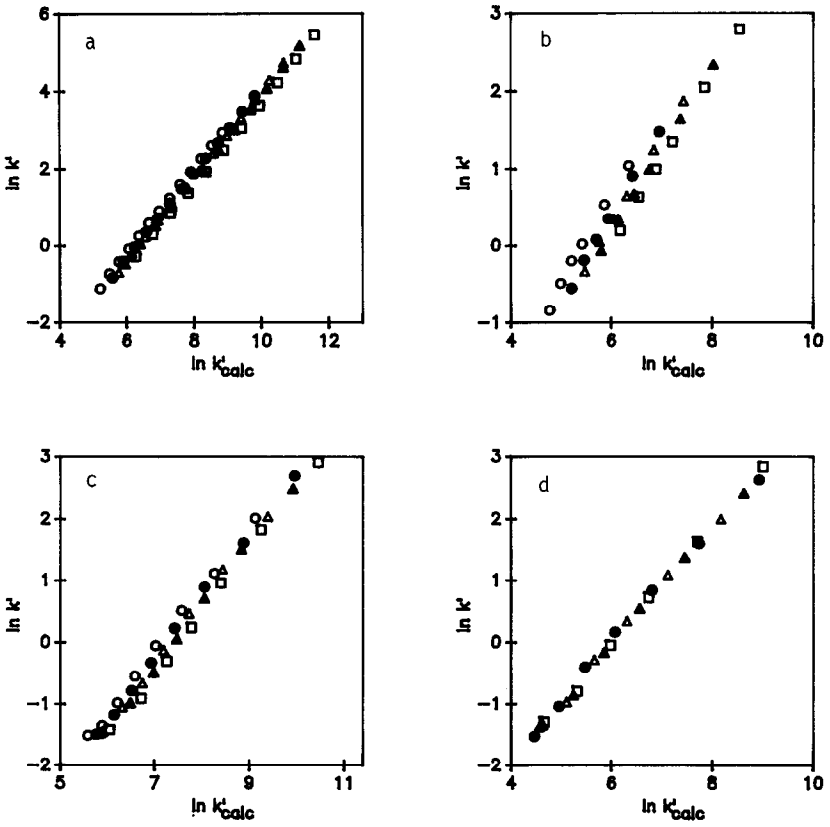


Fig. 6. Plot of measured ln k' *vs.* ln k'_{calc} based on the hexadecane model for the stationary phase activity coefficient. Solvent system: (a) methanol–water; (b) acetonitrile–water; (c) isopropanol–water; (d) tetrahydrofuran–water. Solutes: ○ = benzene; ● = toluene; △ = ethylbenzene; ▲ = *n*-propylbenzene; □ = *n*-butylbenzene.

non-polar solutes. In other words three of the data sets are very similar except for the presence of an offset in the plot; this amounts to a proportional difference in k' which corresponds to the term α in eqns. 12 and 13.

Other approaches to estimating the solute activity coefficient in the stationary phase were tested including the assumption that the activity coefficients are equal (or proportional) to those in bulk hexadecane (see Fig. 6), and assuming that they are equal (or proportional) to that in the pure bulk organic solvent used in the mobile phase (see Fig. 7). Systematic, that is solute dependent deviations, are evident in Figs. 6 and 7. It is clear that except for the tetrahydrofuran–water system the best single unifying factor is the isopropanol approximation (see eqn. 12).

We believe that this supports the idea that the effect of a change in stationary phase composition and the concomitant change in environment sensed by a solute immersed in the stationary phase are quite small relative to the enormous change in the solute environment in the mobile phase over the range in mobile phase compositions explored in this work. At this time we do not have an explanation for the different

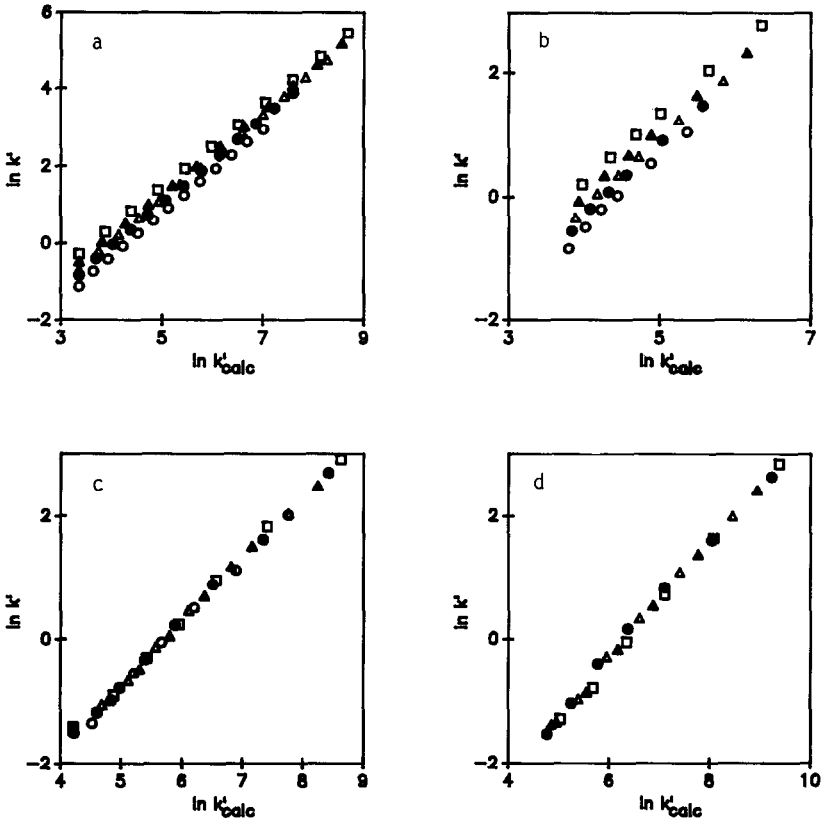


Fig. 7. Plot of measured ln k' *vs.* ln k'_{calc} based on the use of the solute activity coefficient in the bulk pure organic modifier used in the mobile phase as the stationary phase activity coefficient. Solvent system: (a) methanol–water; (b) acetonitrile–water; (c) isopropanol–water; (d) tetrahydrofuran–water. Solutes: ○ = benzene; ● = toluene; △ = ethylbenzene; ▲ = *n*-propylbenzene; □ = *n*-butylbenzene.

behavior of tetrahydrofuran other than the fact that it is the least polar of all of the modifiers based on factors such as its dielectric constant, Kamlet–Taft π^* dipolarity/polarizability[40] and the fact that the activity coefficients of non-polar solutes in tetrahydrofuran are close to unity. When the solute's activity coefficient in the stationary phase is set equal to its activity coefficient in bulk tetrahydrofuran the slope of the regression line of ln k' *versus* ln k'_{calc} is equal to 0.943 which is not different from that based on the isopropanol assumption.

An important point that should be addressed is why do the three modifiers act as if the stationary phase environment has a polarity similar to isopropanol. We have no solid answer to this question but believe that isopropanol may simply be the best model for the combined effect of the bonded phase ligands, sorbed mobile phase components, including water, and the polarity of the accessible silanol groups on the stationary phase chemical potential of the solute.

The intercepts of all of the plots shown in Fig. 5 are significantly different. Physically the intercepts correspond to the offset term in eqn. 14. If we assume that α in

TABLE VIII

COMPARISON OF MOLES OF BONDED PHASE FROM COLUMN CHARACTERISTICS AND VALUES CALCULATED FORM THE PARTITION MODEL

Data set	*Amount of stationary phase (mmoles)*	
	A[a]	*B*[b]
Methanol[c]	1.3	5.8
Acetonitrile[d]	0.6	12
Isopropanol[e]	0.4	3.2
Tetrahydrofuran[e]	0.4	17

[a] Computed from the column characteristics given in Table III and ligand surface coverage = 2 μmol/m^2.

[b] Computed from the intercept given in Table VII.

[c] This is the Zorbax column used by Barman[30].

[d] This is the Develosil column used by Hanai and Hubert[31].

[e] This is the Hypersil column used in this work.

eqn. 12 is unity, that is, the activity coefficient of the solute in the stationary phase is equal to the value in pure bulk isopropanol, then we can compute the number of moles of stationary phase. The results are summarized in Table VIII. It is evident that the number of moles of stationary phase is, in all cases, much larger than that computed based on estimates of the amount of bonded phase by using the column characteristics given in Table III. For the Hypersil column used in this work we find that the amount of stationary phase is much larger for the tetrahydrofuran mobile phase than for the isopropanol mobile phase despite the fact that the same column was used. In addition the number of moles of stationary phase is impossibly large. For example the data for the tetrahydrofuran system corresponds to a volume of 1.4 cm^3. This is larger than the void volume of the column (1.15 cm^3). The above computations are contingent upon the assumed value of the constant of proportionality (α) between the bulk phase activity coefficient and the activity coefficient in the stationary phase being unity. It is much more likely that the actual stationary phase activity coefficient is intermediate between the value in hexadecane and that in the pure organic liquid. A decrease in α will decrease our estimate of the number of moles of stationary phase to more reasonable values. However, activity coefficients in tetrahydrofuran are very close to those in hexadecane and the extraordinarily large amount of sorbed modifier computed based on a partition model cannot be rationalized. This suggests that the data analysis is not consistent with a "pure" partition model[41].

It seemed to us that, no matter how small, within reason, one chooses to make the activity coefficients in the stationary phase, one cannot deny that a considerable amount of the organic modifier is sorbed into the bonded phase thereby altering its properties. However, given the very small amount of methanol needed to saturate bulk hexadecane (0.3 mole %) we felt that it would be impossible for this minute amount of methanol to exert a substantial effect on the activity coefficient of a non-polar solute. This was verified by measuring the activity coefficient of a series of alkylbenzenes in bulk methanol-saturated hexadecane (see Table IV). Our assumption is clearly validated; saturation of bulk hexadecane with methanol does not change the environment experienced by a non-polar solute.

Based on this we are convinced that the very high surface to volume ratio of a bonded phase column, relative to that of a bulk liquid, must allow for the sorption of a much larger amount of organic modifier into the bonded phase than occurs in experiments with bulk liquid phases such as our HSGC experiment or the "sit flask" experiment of Schantz and Martire[36]. Using literature data on sorption of organic modifiers[7,14,18,34], we infer that at least 1–2 moles of the organic modifier per mole of bonded ligand can be adsorbed into the bonded phase. In essence then we feel that bonded-phase RPLC cannot be accurately described as a pure partition process even for non-polar solutes.

We turn now to reconciling our observations to those of Schantz *et al.*[13]. If one assumes that the activity coefficient of the solute in bulk hexadecane is independent of the amount of methanol that can partition into the phase then the change in our A term with φ should be directly comparable to the change in the natural logarithm of the ratio of the hexadecane partition coefficient to net retention volume studied by Schantz *et al.*[13]. For the methanol–water system A varied by about 0.5 ln units in the same direction and over the same range in φ that the parameter of Schantz *et al.*[13] varied by 0.9 ln units. Given the vastly different methodologies and the different experimental difficulties we believe that these results are only marginally inconsistent. Nonetheless if the difference is real it can be reconciled. Based on our preceeding results that the bonded phase acts as if it is more polar than an alkane we hypothesize that one must include some number of moles of organic modifier in the number of moles of stationary phase (see eqn. 8). Consequently the term A must vary less than the ratio of the sit flask partition coefficient to the net retention volume because of a cancellation of effects. That is the amount of stationary phase increases by sorbing the organic modifier and very possibly some water into the bonded phase domain. This has the effect of simultaneously increasing the stationary phase solute activity coefficient and the number of moles of stationary phase. Since the term A is a ratio of these two factors it appears to vary less than either factor alone.

CONCLUSION

It is clear from the above results that the solute–mobile phase activity coefficient is tremendously important in RPLC. Consequently all models of RPLC, such as that of Geng and Regnier[42,43], which do not incorporate this term should be re-examined. Because mobile phase interactions are the dominant factor in establishing the dependence of k' on φ and the γ^m values are so large it is evident that the solvophobic mechanism of RPLC is correct in spirit if not in detail. This does not mean that the stationary phase is a non-participating spectator. The results clearly show the importance of the very high surface area in bonded-phase RPLC. Solute activity coefficients in the stationary phase cannot be modeled by treating the solute as being dissolved in a non-polar bulk phase such as hexadecane.

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