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HYDROPHOBIC EFFECTS IN REVERSED-PHASE LIQUID CHROMATOGRAPHY*

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SUMMARY

The role of hydrophobic effects in reversed-phase liquid chromatography is examined. The molecular weight or structural selectivity resulting from hydrophobic effects relative to dispersion forces is discussed, and it is shown that in general a selectivity up to twice as great is possible in the former case. Hydrophobic selectivity can be accounted for using a new topological index, the molecular connectivity, to represent solute surface area. Selectivity as a function of organic modifier type and composition is explored. By simultaneously varying both of the above parameters such that retention is normalized, it is shown that hydrophobic selectivity is approximately independent of organic modifiers. Some differences in separation as a function of organic solvent are, however, observed when mixtures containing solutes of different functional groups are employed. Finally, hydrophobic selectivity is roughly constant under time normalization conditions based on temperature–organic phase composition changes.

INTRODUCTION

Recently, modern reversed-phase liquid chromatography (LC) employing chemically bonded stationary phases (typically *n*-octadecyl groups bonded to a silica surface) has become quite popular[1]. While the mechanism of retention on the hydrophobic stationary phase is still open to question, the importance of solubility in the mobile phase (most often consisting of water mixed in some proportion with a polar organic solvent) on retention and selectivity is clear. Indeed, as Locke has suggested the relative retention for closely related solutes may in certain cases be determined solely by the difference in solubility of the two components in the mobile phase[2]. Moreover, in both column operation[3] and previously in open bed operation[4], it has been demonstrated that the solubility of the substance in water is an important contributor to retention and hence relative retention. Thus, an understanding of reversed-phase LC requires an appreciation of solution phenomena in water.

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When a solute is introduced into an aqueous environment, the solution process is quite complex. This is especially the case when the solute is non-polar or contains a non-polar segment such as an alkyl group. The solubility of normal alkanes in water is remarkably low. Less known is the fact that the positive free energy is a result of a large negative entropy of solution; in fact, the solution of hydrocarbons in water is energetically favorable, since a small negative solution enthalpy results[5].

Frank and Evans[6] were among the first to suggest that the anomalously large negative entropy is a result of structural ordering around the hydrocarbon molecule. While details are at present uncertain, we can qualitatively visualize the formation of a cavity of water molecules surrounding the hydrocarbon[7]. In order to overcome this loss in entropy, non-polar segments of molecules will favor removal from the aqueous medium and/or will tend to group together. The term frequently used for cavity formation and, where appropriate, non-polar clustering is hydrophobic effects.

From the unusual solution behavior involved in hydrophobic effects, we can expect that structural selectivity will differ in the solution of non-polar entities in a non-polar solvent relative to that in water. This difference in selectivity can be seen in Table I which presents hydrocarbon relative retention values in gas–liquid chromatography with squalane as stationary phase (resulting mainly from dispersion forces) and relative solubilities of the same substances in water. A much larger methylene group increment is found when hydrophobic effects are operative (≈4.4) than when simple dispersion forces exist. Moreover, branched *vs.* straight chain selectivities are greater with hydrophobic interactions. Of special note are the very high values of cyclic *vs.* straight chain selectivities and the inverse order to that re-

TABLE I

COMPARISON OF SELECTIVITIES RESULTING FROM DISPERSION FORCES *VS.* HYDROPHOBIC EFFECTS

Dispersion: GLC retention volume ratios on squalane. Temp., 77° or 80°. Calculated from data in refs. 41 and 42. Hydrophobic effect: Inverse ratio of mole fraction water solubilities at 25°; data taken from ref. 8.

Solute pair	*Dispersion*	*Hydrophobic effect*
n-Alkanes	2.2	4.4
$\left(\frac{n\text{-Hexane}}{2,2\text{-dimethylbutane}}\right)$	1.6	1.9
$\left(\frac{n\text{-Octane}}{2,2,4\text{-trimethylpentane}}\right)$	2.3	3.7
$\left(\frac{\text{Cyclohexane}}{n\text{-hexane}}\right)$	2.1	0.17
$\left(\frac{\text{Methylcyclohexane}}{\text{cyclohexane}}\right)$	1.7	4.6
$\left(\frac{n\text{-Hexanol}}{n\text{-pentanol}}\right)$	2.3	4.2*
$\left(\frac{\text{Cyclohexanol}}{n\text{-hexanol}}\right)$	1.3	0.16*

* Data from ref. 9.

sulting from dispersion forces. Finally, it can be seen that to a first approximation the effect in water remains roughly constant when a hydrogen bonding substituent is added to the molecule. The large hydrophobic selectivities shown in Table I are moderated somewhat in reversed-phase LC because of the use of mixed solvents. Nevertheless, it is possible in a number of cases to operate under conditions in which hydrophobic effects play an important role in producing high structural selectivity.

The purpose of the present paper is to explore the role of hydrophobic selectivity in reversed-phase LC. This selectivity will be examined as a function of a variety of factors such as organic modifier, temperature and solute structure. Using a simple topological index, it is possible to estimate retention in reversed-phase LC.

THEORETICAL

Consider a standard reversed-phase packing of *n*-octadecyl groups attached to a silica surface in which unreacted silanols do not contribute to the overall retention of solute molecules. In such a case, as pointed out by Locke[2], stationary phase interactions will tend to be weak and non-selective. Retention and relative retention, as we have already noted, will thus be a strong function of solution phenomena in the mobile phase. This will be especially so if we consider the relative retention of a series of solutes with a common functional group.

Consider next a mixed aqueous–organic mobile phase. Soczewinski *et al.* have found the following equation to be frequently obeyed in paper chromatography[10,11]:

$$\log k'_{\text{mix}} = \varphi_{\text{H}_2\text{O}} \log k'_{\text{H}_2\text{O}} + \varphi_{\text{org}} \log k'_{\text{org}} \tag{1}$$

where k' = capacity factor, φ = volume fraction; the subscripts mix, H_2O and org denote mixed solvent system, pure water and pure organic phases, respectively. Although this equation may not fully apply to the reversed-phase systems considered here, Sleight[12] and Schmidt *et al.*[13], for example, have shown linear behavior for similar groups of substances of $\log k'_{\text{mix}}$ *vs.* $\varphi_{\text{H}_2\text{O}}$. Moreover, within given classes of substances and a given $\varphi_{\text{H}_2\text{O}}$, $\log k'_{\text{mix}}$ is found to be proportional to $\log S_{\text{H}_2\text{O}}$ (S = solubility, proportional to $k'_{\text{H}_2\text{O}}$) in both open bed[14] and column[2] operation.

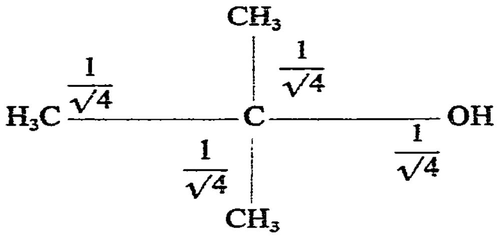
The group additivity approach of the Martin equation[15] has been shown to be a valid means of predicting retention in liquid chromatography on many occasions[16]. Moreover, a good appreciation now exists as to those cases in which a group contribution is not independent of the molecular environment (*e.g.* steric effects)[17]. Group additivity approaches have also been used to correlate water solubilities (*e.g.* refs. 9, 18–25). Since in reversed-phase LC, $\log k'_{\text{mix}}$ has been shown to be proportional to $\log S_{\text{H}_2\text{O}}$, it should therefore not be surprising that hydrophobic group constants can be obtained from $\log k'_{\text{mix}}$ (or inversely determine $\log k'_{\text{mix}}$). This approach has been used successfully in determining Hansch π constants[20,21], as reviewed by Tomlinson[14]. While most π constant determinations have been made with open bed chromatography, recent work has involved columns containing chemically bonded reversed-phase packings[26,27].

While π constants relate to the partition of a group between water and *n*-octanol, a different measure of hydrophobicity can be obtained from the size of the structural groups themselves. From the previous picture of cavity formation by water in the solution of non-polar entities, an interfacial solution model based on structural surface area has been successfully developed. Hermann[24] was able to relate the calculated surface area of the water cavity to the solubility of hydrocarbons in water. Hermann's method of calculating surface area has been employed by Amidon *et al.* in the successful prediction of the solubility of alcohols and other non-electrolytes in water[9,18].

While Hermann's approach could be used to obtain hydrophobic group constants for prediction of log k' in reversed-phase LC, a new topological index, called the molecular connectivity χ, has been introduced and shown to be proportional to the cavity surface area[28–30]. This index has the advantage of being quite simple to calculate and has successfully been related to the water solubility of non-electrolytes. The molecular connectivity is defined as

$$\chi = \sum_{k=1}^{k} \frac{1}{(\delta_i \delta_j)_k^{\frac{1}{2}}} \tag{2}$$

where $\delta = 1, 2, 3$ or 4, corresponding to the number of atoms attached to atoms i and j, respectively, and k = number of bonds in the group or molecule. In this calculation only the skeleton is important; the hydrogen atoms are neglected. As an example of the index calculation, consider *tert.*-butyl alcohol



$\chi = 2$

For saturated cyclic structures (*e.g.* cyclohexyl), the index involves a decrease of 0.5 to account for ring closure. Corrections for aromaticity and unsaturation can also be made[30]. As can be seen χ or degree of branching is quite simple to calculate and should find use in accounting for hydrophobic effects in reversed-phase LC. We shall examine this index in a later section.

EXPERIMENTAL

The liquid chromatograph was constructed in our laboratory and consisted of a Waters Assoc. (Milford, Mass., U.S.A.) M6000 pump, U6K injector and R401 differential refractometer detector. A Pye Unicam LCM2 moving wire detector and a Laboratory Data Control UV monitor (254 nm) were also used during part of this work. A commercial reversed-phase column μBondapak C$_{18}$ (Waters Assoc.) was employed for all separations. Column temperature was controlled with a circulatory constant temperature bath, Lauda K-4R (Brinkmann).

Methanol, acetonitrile and acetone were obtained from Burdick & Jackson Labs. The acetone was further purified by distillation from potassium permanganate.

Mobile phase mixtures were made up by volume using deionized, activated charcoal purified water. All solutes were reagent grade and used without further purification.

All samples were made up as solutions in either the organic modifier or the mobile phase and consisted of roughly 1–10 μg for each solute. Each data point was obtained from at least two injections. A careful reproducibility study involving seven injections revealed a relative standard deviation in retention of less than 2%. Agreement of retention between two columns was 5%. The column volume was measured using uracil (with the moving wire detector) or water–organic modifier solution, having a slightly different composition ratio from the mobile phase (with the differential refractometer).

RESULTS AND DISCUSSION

Molecular connectivity and retention

In order to illustrate the use of the above topological index in estimating non-polar group contributions to retention in reversed-phase LC, we have examined relative retention for mixtures of substances with a common functional group with the water–organic solvent composition maintained constant. In such cases, if log k' is proportional to log S_{H_2O}, we might expect log k' to be also proportional to χ.

We first tested this relationship in the prediction of R_M values for the open bed reversed-phase chromatographic retention of a series of para-substituted phenols[31,32]. The proportionality of R_M to χ_g (group contribution) was obtained from *p*-*n*-propyl, and this proportionality constant was assumed to be the same throughout the series of substances. The results are shown in Table II for 25% ethanol and 75% ethanol in water. The agreement between predicted and experimental retention is quite good, considering the approximations being made.

A second example of the quantitative estimate of hydrophobic selectivity in

TABLE II

PREDICTION OF R_M VALUES FOR VARIOUS SUBSTITUTED PHENOLS

χ_g = group connectivity index.

Reference	*Substituent*	χ_g	*25% Ethanol*		*37.5% Ethanol*		*70% Ethanol*	
			Pred.	*Exp.*	*Pred.*	*Exp.*	*Pred.*	*Exp.*
29, 30	*p*-*n*-butyl	1.71	0.55	0.56			−0.61	−0.62
	p-isopropyl	1.15	0.04	0.01			—	—
	p-*tert.*-butyl	1.50	0.34	0.17			—	—
	p-3-methylbutyl	2.06	0.84	1.00			—	—
	p-cyclohexyl	2.32	1.06	1.23			—	—
	p-cyclopentyl	1.82	0.63	0.72			—	—
	p-*tert.*-amyl	1.85	0.66	0.86			−0.59	−0.48
	p-*n*-pentyl	2.21	1.04	1.01			−0.48	−0.48
31	*p*-*n*-butyl	1.71	0.91	0.91	0.22	0.22		
	p-isopropyl	1.15	0.43	0.48	−0.12	−0.15		
	p-*sec.*-butyl	1.56	0.78	0.79	0.13	0.11		
	p-*tert.*-butyl	1.50	0.75	0.87	0.09	0.00		
	p-*sec.*-amyl	2.06	1.21	1.23	0.43	0.66		
	p-*n*-pentyl	2.21	1.34	1.20	0.52	0.55		

open bed chromatography comes from the work of Bark and Graham, who also examined various alkyl phenols in cellulose impregnated with ethyl oleate[33]. Using *p-n*-propyl again as the standard, the results at 25% ethanol and 37.5% ethanol are shown in Table II. Again, reasonably good fit is found, illustrating the usefulness of the molecular connectivity in prediction of retention in reversed-phase LC.

A third and final example is taken from our own work using a μBondapak reversed-phase column. Table III presents predicted and experimental log k' values for a series of acyclic and cyclic alcohols with a mobile phase composition of water–methanol (60:40). Three predictors of R_M are employed: χ; χ_g; and the weighted group connectivity, $\chi_{w,g}$. The latter is obtained from an empirical correlation of log S_{H_2O} for a series of 51 alcohols (eqn. A2 in ref. 29). For each topological index, the experimental log k' values of *n*-butanol and *n*-pentanol were used to obtain the slope and intercept of the linear equation of log k' *vs.* index. Hence, perfect agreement between predicted and experimental log k' results for these two alcohols.

TABLE III

LOG k' DATA FOR NORMAL, BRANCHED AND CYCLIC ALCOHOLS

Stationary phase, microparticle reversed-phase C_{18}; mobile phase, water–methanol (60:40). χ = total molecular connectivity; χ_g = group molecular connectivity; and $\chi_{w,g}$ = weighted group molecular connectivity (see text).

Alcohol	*Experimental*	*Predicted*		
		χ	χ_g	$\chi_{w,g}$
n-Butanol	−0.01	−0.01	−0.01	−0.01
3-Methyl-1-butanol	0.30	0.29	0.29	0.27
n-Pentanol	0.36	0.36	0.36	0.36
3,3-Dimethyl-1-butanol	0.54	0.47	0.47	0.44
2-Hexanol	0.62	0.63	0.72	0.47
3-Methyl-1-pentanol	0.61	0.67	0.67	0.64
2-Methyl-1-pentanol	0.65	0.67	0.67	0.64
4-Methyl-1-pentanol	0.66	0.63	0.63	0.61
n-Hexanol	0.71	0.73	0.73	0.72
Cyclopentanol	−0.04	0.13	0.22	−0.02
Cyclohexanol	0.28	0.35	0.44	0.19
Cyclooctanol	0.81	1.09	1.18	0.92

Examination of Table III reveals that the best agreement to the experimental data is obtained with the $\chi_{w,g}$, which is as expected. However, we cannot reasonably anticipate availability of such weighted group empirical indices for all classes of substances of interest.

If we examine closely the prediction of retention from the index χ_g in Table III, we find excellent agreement among primary alcohols, with the secondary alcohols uniformly high. This result is undoubtedly a reflection of the fact that the steric environment of the secondary alcohol is different from that of the primary alcohol and that primary alcohols were selected for standards. Thus, as with other group correlations[17], χ_g should only be used as a predictor of relative retention if the functional group is not altered. Finally, the total χ is seen to give a reasonable first order prediction of R_M for both the primary and secondary alcohols together. Thus, the difference in the two classes of alcohols is in part being accounted for in the connectivity of the C–OH bond.

The results in Tables II and III clearly show the validity of the χ as a means of predicting hydrophobic selectivity in reversed-phase LC. The topological index can be hand-calculated and thus has the advantage of simplicity over the cavity surface area approach of Hermann. Further studies of the role of this index in reversed-phase LC are warranted.

Influence of organic solvent and composition

The results of the previous section were obtained at a given water–organic solvent composition. It is worthwhile to explore the change in hydrophobic selectivity as a function of composition and organic solvents. We have done this by first measuring the retention of a series of *n*-alcohols on the μBondapak column using three solvent mixtures as mobile phases: water–methanol, water–acetonitrile, and water–acetone.

Log k' *vs.* carbon number plots were constructed for each set of *n*-alcohols eluted with a given mobile phase composition. Linear plots were obtained, with all data points on the lines with the exception of the lowest k' values in most cases. Alcohols of different chain-lengths were used for the various mobile phase compositions, with good overlap at two successive compositions. Complete overlap of all alcohols could not be achieved; with mobile phases containing *ca.* 20% (v/v) water, the lowest alcohols that could be eluted with measurable k' values were in the C_7–C_8 range; with *ca.* 80% (v/v) water, the highest alcohols that could be eluted in reasonable time (k' less than 30) were in the C_6–C_7 range.

Extrapolation to zero carbon number gave k'_0 values in the range of 0.02–0.05 for all mobile phase compositions. The small values of the intercepts suggest that unreacted residual SiOH groups on the silica surface do not contribute significantly to retention.

Log k' values for *n*-hexanol and *n*-octanol over the whole range of solvent composition were obtained from the log k' *vs.* carbon number plots and are shown in Fig. 1 for the acetonitrile–water and methanol–water systems, plotted *vs.* volume fraction of organic component. For the methanol–water system the plots are linear over the whole range of composition. Extrapolation of the line to 100% water gives log k' values of 3.05 and 1.85 for *n*-octanol and *n*-hexanol, and a corresponding methylene increment in k' of 4.0. This latter value is in good agreement with the methylene increment for the solubility in water of the *n*-alcohols, which ranges between 3.8 (ref. 34) and 4.2 (ref. 19). This extrapolated result for the 100% water mobile phase, combined with the linear relationship between log k' and volume fraction indicates agreement with eqn. 1 (see Fig. 2 as well).

The log k' *vs.* volume fraction plot for acetonitrile–water mixtures is curved indicating that eqn. 1 is not followed over the whole composition range in this case. Extrapolation of the straight line portion of the plot to 100% water gives log k' values of 2.90 and 1.70, again corresponding to a methylene increment of k' of 4.0. In the high water region the log of selectivity of acetonitrile–water mobile phases can then be linearly related to the log of selectivity in pure water. The behavior of log k' *vs.* percent composition for acetone is found to be intermediate of that for methanol and acetonitrile.

Fig. 2 shows plots of log α_{CH_2} (methylene group increments) *vs.* volume percent

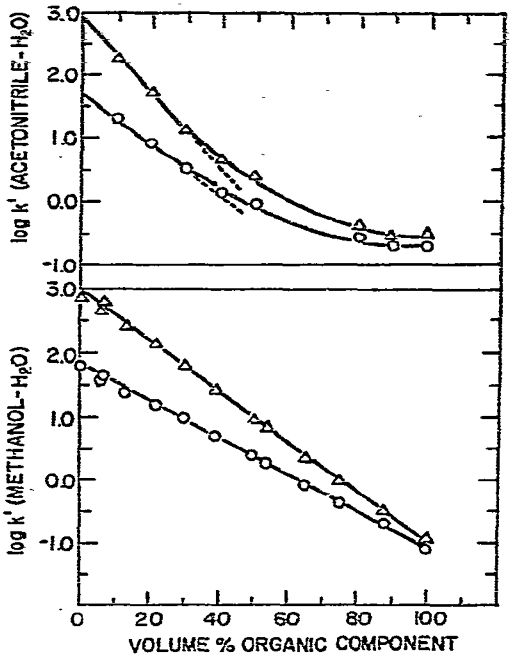


Fig. 1. Log *k'* vs. volume percent organic component for *n*-hexanol (○) and *n*-octanol (△) in water–acetonitrile and water–methanol mobile phases, on a chemically bonded octadecyl silane stationary phase at ambient temperature. *k'* values greater than *ca.* 20 and less than *ca.* 0.2 were obtained by extrapolation of log *k'* vs. carbon number plots for lower or higher alcohols.

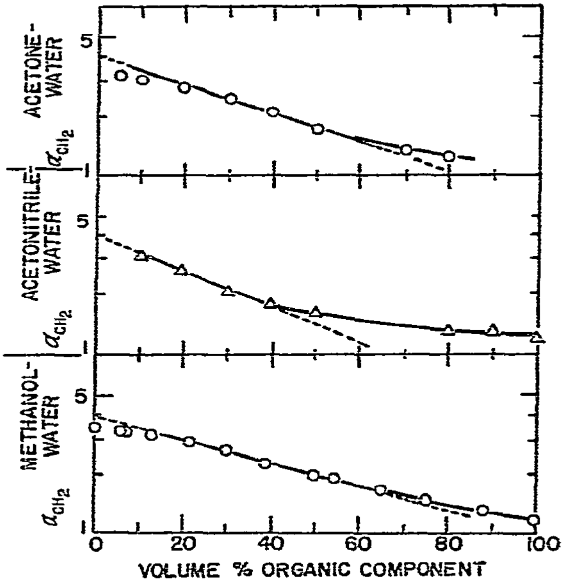


Fig. 2. Semilogarithmic plots of α_{CH_2} (obtained from log *k'* vs. carbon number plots for the *n*-alcohols) vs. volume percent organic component for water–acetone, water–acetonitrile and water–methanol mobile phases, on a chemically bonded octadecyl silane stationary phase at ambient temperature.

organic component for all three organic phases. All plots extrapolate to $\alpha = 4.0$ at 100% water, in agreement with Fig. 1. At the 100% organic solvent end, α_{CH_2} is much lower and approximately the same for all three modifiers: *ca.* 1.1. This shows the low non-polar structural selectivity to be expected in reversed-phase LC on chemically bonded stationary phases, when high concentrations of the organic component are used in the mobile phase.

For both the water–methanol and water–acetone plots, the points at organic component concentrations less than 10% lie below the extrapolated line, most probably because the mobile phase is not completely wetting the stationary phase in this concentration region. (Peak symmetry was much worse with low organic component concentrations in the mobile phase.) It is expected that water–acetonitrile compositions of less than 10% acetonitrile would show the same behavior. The net result of this effect is to complicate the retention mechanism in this region.

Leaving aside the region of $\varphi_{H_2O} > 0.90$, the shapes of the plots in Fig. 2 are most probably due to structural variations in the solvent systems as the concentration of the organic modifier is changed. Qualitatively, the addition of an organic modifier such as acetone, acetonitrile, or methanol to water should initially result in only minor perturbation of the water structure, since these small hydrophilic molecules should be relatively easily accommodated in the water structure. As the concentration of organic component in the water is increased a point must be reached when at least some of the structural aspects of the bulk water begin to disappear, and one or more intermediate structures occur[36]. The concentration (or range of concentration) at which this takes place will vary with the nature of the modifier. Finally, at very high organic component concentrations the liquid structure of the pure organic begins to predominate.

The fact that methanol–water mixtures follow eqn. 1 over almost the whole composition range is undoubtedly tied up with the fact that methanol can act as both a proton donor and acceptor[17,35]. Thus, methanol appears to provide simply a dilution medium for the water, at least until very large amounts of the alcohol are present.

Acetone–water and especially acetonitrile–water mixtures exhibit linear behavior of $\log \alpha_{CH_2}$ with mobile phase composition over a significantly lower organic modifier composition range than methanol. Moreover, the slope of $\log \alpha_{CH_2}$ *vs.* φ_{org} in the linear region is more negative for the former two solvents. Since both acetone and acetonitrile provide only proton accepting ability, we might expect that they might cause changes in the hydrogen bonded network of water molecules more readily than low molecular weight alcohols[35].

From the chromatographic point of view, the plots in Fig. 2 can be useful in predicting hydrophobic selectivity from one water–organic phase composition to another. Table III has already shown that from the molecular connectivity it is possible to obtain a reasonable prediction of hydrophobic selectivity for a given composition. Fig. 2 allows us to change from one composition to another. As an illustration of this approach consider acetonitrile–water as mobile phase. Over the linear portion of the plot in Fig. 2 we can write the following empirical relationship

$$\log \alpha_{CH_2,\,mix} = 0.95\,\varphi_{H_2O} - 0.35 \qquad (3)$$

The molecular connectivity for a methylene group is 0.5. We can therefore write that

$$\log \alpha_{CH_2,\,mix} = 0.5\beta \qquad (4)$$

where β is a proportionality constant relating χ_{CH_2} to log $\alpha_{CH_2,mix}$. Substitution of eqn. 3 into 2 results for acetonitrile–water mixtures in

$$\beta = 1.90\,\varphi_{H_2O} - 0.7 \tag{5}$$

At water–acetonitrile (80:20), $\beta = 0.82$, and this is the factor by which the hydrophobic selectivity is reduced from pure water. For example, the difference in molecular connectivity, $\Delta\chi$, between hexanol and 2-hexanol is 0.14, so that the predicted Δlog k' for these two substances is 0.14β or 0.12, whereas the experimental value is 0.16. As a second example, $\Delta\chi$ between butanol and 4-methyl-1-pentanol is 0.86. The predicted Δlog k' is then 0.71 *vs.* an experimental value of 0.75. Thus, a good prediction of relative retention can be made over the whole composition range in which log α_{CH_2} *vs.* φ_{org} in Fig. 2 is linear. Of course, knowledge of the retention of one substance at a particular mobile phase composition permits the prediction of all other substances within the class at that composition.

The constancy of non-polar increments, such as methylene groups, within different classes of substances has been illustrated many times in the past[17]. We may therefore expect the hydrophobic selectivities exhibited for the alcohols to be valid for other functional groups. In other work not reported here[37], we have found this non-polar selectivity behavior holds well for aliphatic ketones and diols, for example.

Fig. 2 provides one way in which hydrophobic selectivity as a function of organic modifier type and composition can be compared. A second approach, which is also chromatographically meaningful, is to compare the selectivities of the various mobile phases under normalized time conditions. In this method, both the organic modifier and its composition are simultaneously varied such that k' for a given solute is maintained constant. Table IV shows the results of the influence of organic modifier on α_{CH_2} for *n*-alcohols under normalized time conditions. We have included *n*-propanol along with the other three modifiers previously presented.

The value of α_{CH_2} under normalized time conditions is found to be approximately constant, even though there are large composition variations among the four organic modifiers. Hydrophobic selectivity within a class of substances (*i.e.* constant functional group) is expected to be roughly similar under normalized time conditions because of the opposing effects of the simultaneous change in two parameters. As seen from Figs. 1 and 2, at a given water–organic modifier composition acetonitrile leads to a lower k' and α_{CH_2} relative to methanol. Time normalization requires a larger water content for acetonitrile because of the lower retention (see Table IV). The greater water content compensates for the lower α_{CH_2} at a given composition, leading to roughly equal α_{CH_2} values for methanol and acetonitrile at constant time conditions. Similar arguments hold for acetone and *n*-propanol.

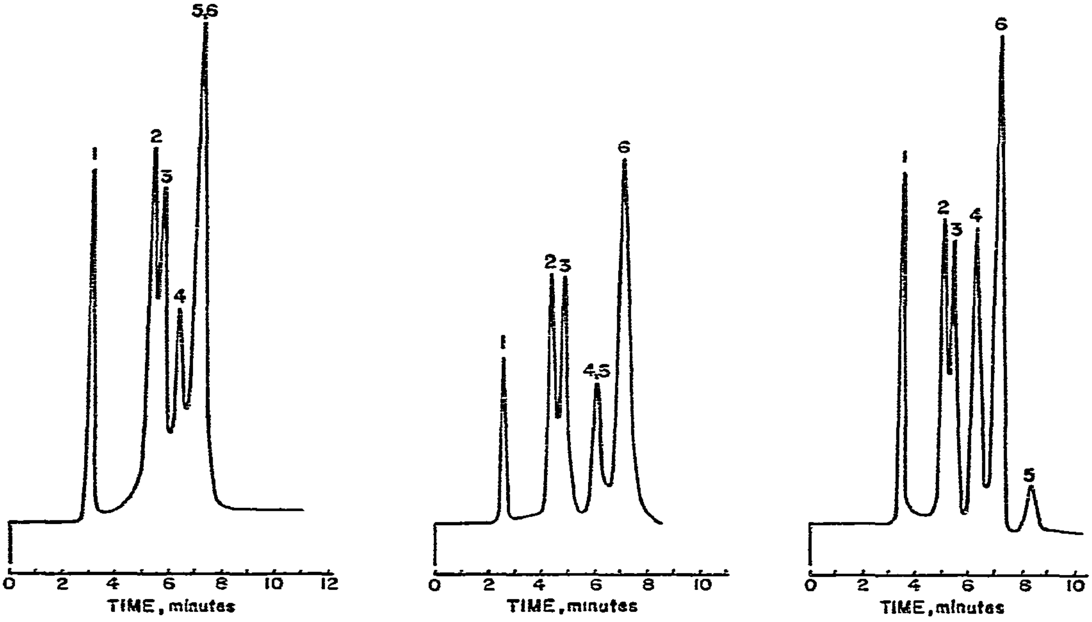
The next question is whether this similarity in selectivity under normalized time conditions holds when one considers mixtures of different classes of substances. Fig. 3 shows the separation of a series of six compounds with differing functional group (*i.e.* ketone, ester, primary and secondary alcohol), using three organic modifiers and normalizing to *n*-pentanol. In agreement with Table IV, the relative retention of 3-methyl-1-butanol to *n*-pentanol is constant in the three systems. On the other hand, large changes in retention are observed for cyclopentanone and methyl butyrate relative to *n*-pentanol. Most interesting in this regard is the reversal in elution order

TABLE IV

METHYLENE GROUP INCREMENT UNDER ORGANIC SOLVENT–VOLUME PERCENT NORMALIZATION

φ_{org} = volume fraction of organic solvent.

Normalization	*Organic solvent*	φ_{org}	α_{CH_2}
n-Pentanol (k' = 6.55)	methanol	0.20	3.03
	propanol	0.13	3.05
	acetonitrile	0.10	3.22
	acetone	0.07	3.10
n-Hexanol (k' = 3.00)	methanol	0.50	2.11
	propanol	0.25	2.13
	acetonitrile	0.31	2.17
	acetone	0.36	2.21
n-Hexanol (k' = 7.00)	methanol	0.37	2.48
	propanol	0.20	2.67
	acetonitrile	0.21	2.63
	acetone	0.23	2.64



(a) (b) (c)

Fig. 3. (a) Reversed-phase separation of selected C_5 compounds normalized to k' of *n*-pentanol. Column, 30 cm × 4.5 mm I.D. μBondapak C_{18}. Temperature, 25°. Mobile phase, methanol–water (30:70, v/v). Velocity, 0.33 cm/sec. 1 = cyclopentanone, 2 = 3-pentanol, 3 = 2-pentanol, 4 = 3-methyl-1-butanol, 5 = methyl butyrate, 6 = *n*-pentanol. Detection, differential refractometer at 8×. (b) Same conditions as in (a) except mobile phase: *n*-propanol–water (15:85, v/v). (c), Same conditions as above except mobile phase: acetonitrile–water (16:84, v/v).

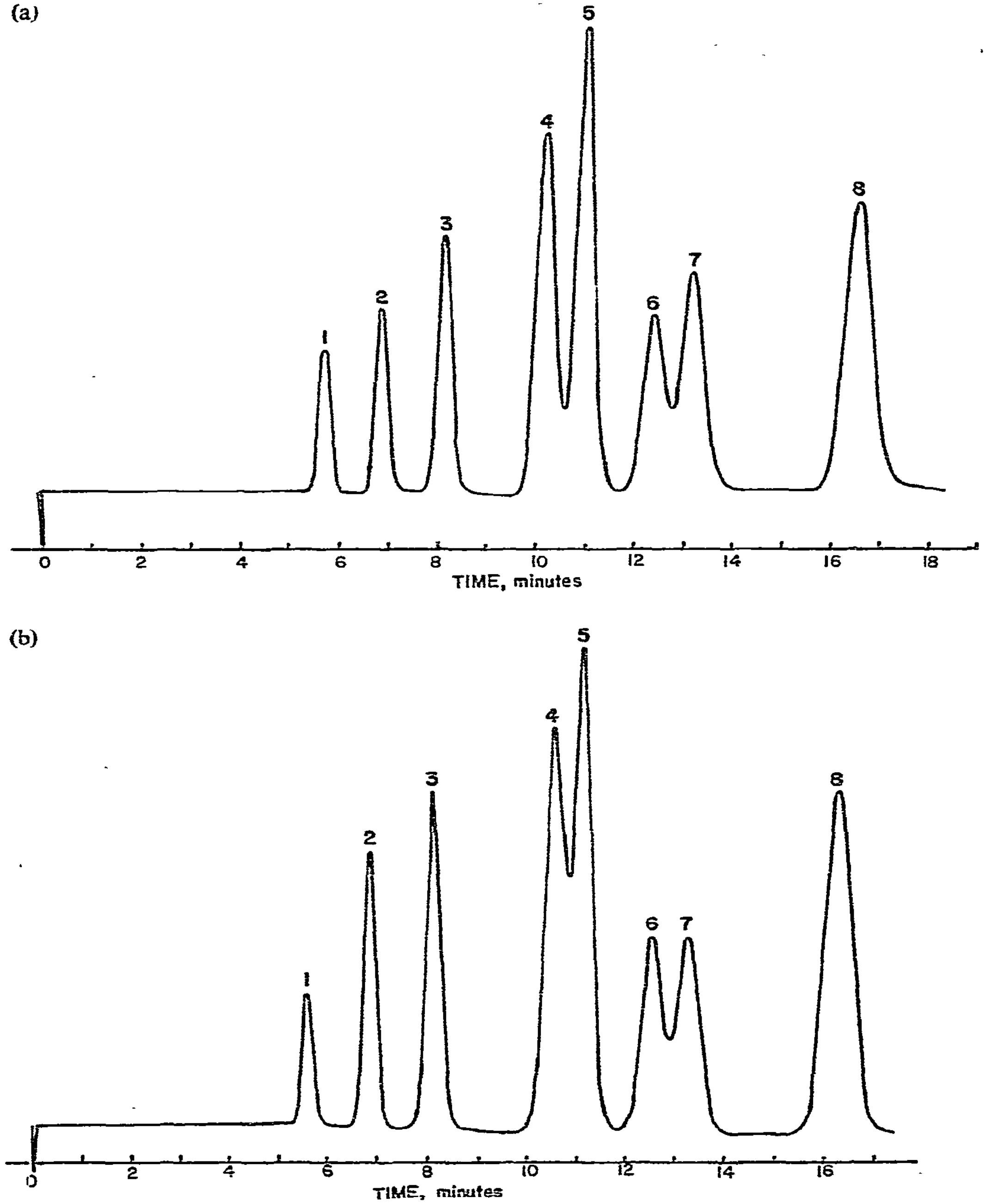


Fig. 4. (a) Reversed-phase separation of selected polynuclear aromatic hydrocarbons at normalized k' for pyrene. Column, same as Fig. 3. Temperature, 25°. Mobile phase, acetonitrile–water (60:40). Detector, UV at 254 nm. Attenuation, 0.16 a.u.f.s. Velocity, 0.33 cm/sec. 1 = benzene, 2 = toluene, 3 = naphthalene, 4 = biphenyl, 5 = acenaphthene, 6 = phenanthrene, 7 = anthracene, 8 = pyrene (b) Same conditions as in (a) except temperature, 60° and mobile phase, acetonitrile–water (53:47, v/v).

for methyl butyrate and n-pentanol in Fig. 3b (propanol) and 3c (acetonitrile) in which the water content is roughly the same. As expected, the changes in retention for the secondary alcohols relative to n-pentanol in the three systems is much smaller (*e.g.* the maximum change is 30%). The results in Fig. 3 suggest that functional group selectivity in reversed-phase LC is dependent to som eextent on the organic modifier of the mobile phase.

Effect of temperature

Temperatures above 25° have been employed in the past in reversed-phase LC for improved performance[13,38–40]. We decided to examine briefly the role of temperature on hydrophobic selectivity using chemically bonded reversed-phase packings. In agreement with other workers[13] we found a factor of roughly 2 decrease in absolute retention for alcohols with a 30° increase in temperature (mobile phase composition maintained constant). In addition, hydrophobic selectivity decreased to a small extent (10–15%) from 25° to 60°, independent of the organic modifier selected.

Hydrophobic selectivity was next compared under normalized time conditions, in which temperature and mobile phase composition were simultaneously varied. As previously, similar selectivities were obtained, in which the lower selectivity of the higher temperature was compensated by the use of a mobile phase of higher water composition. A particularly good example of this result is shown in Fig. 4 which illustrates the separation of a set of polynuclear aromatic hydrocarbons at 25° and 60° under normalized conditions. Almost identical chromatograms are obtained.

From our results it would appear that from a selectivity point of view, operation at 25° is equally as good as at 60°. Obviously, if the mass transfer is markedly improved at 60°, then the higher temperature ought to be used. However, if the changes in efficiency are not great, then room temperature might be preferred.

CONCLUSION

In this paper we have explored the role of hydrophobic effects in reversed-phase LC using chemically bonded C_{18} stationary phases. The use of a topological index to represent the surface area of a molecule (or a group) has been shown to be a useful method to estimate hydrophobic selectivity. The simplicity in calculating this index ought to prove valuable in predicting relative retention in reversed-phase LC.

Hydrophobic selectivity as a function of organic modifier types and composition has also been examined. Differences in the loss of selectivity with added modifier have been observed, depending on the type of organic solvent selected. However, when normalized time conditions are employed, roughly equivalent selectivities are obtained, as a result of the compensation caused by the simultaneous change in two variables. Such compensation is also observed in temperature–organic phase composition time normalization studies.

While most of our efforts have dealt with alkyl and cycloalkyl alcohols, it is expected that similar behavior would be observed for other classes of substances (*e.g.* acids, ketones, *etc.*). Moreover, the use of the topological index for aromatic groupings has already been demonstrated in predicting water solubility[30]. It is likely that the treatment can be extended to aromatic substances in reversed-phase LC.

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REFERENCES

1 A. Pryde, *J. Chromatogr. Sci.*, 12 (1974) 486.
2 D. C. Locke, *J. Chromatogr. Sci.*, 12 (1974) 433.
3 J. J. Kirkland, *Anal. Chem.*, 43 (1971) 36.
4 I. E. Bush, *The Chromatography of Steroids*, MacMillan, New York, 1961.
5 C. Tanford, *The Hydrophobic Effect: Formation of Micelles and Biological Membranes*, Wiley-Interscience, New York, 1973.
6 H. S. Frank and M. W. Evans, *J. Chem. Phys.*, 13 (1945) 507.
7 R. B. Hermann, *J. Phys. Chem.*, 75 (1971) 363.
8 C. McAuliffe, *J. Phys. Chem.*, 70 (1966) 1267.
9 G. L. Amidon, S. H. Yalkowsky and S. Leung, *J. Pharm. Sci.*, 63 (1974) 1858.
10 E. Soczewiński and C. A. Wachtmeister, *J. Chromatogr.*, 7 (1962) 311.
11 E. Soczewiński and G. Matysik, *J. Chromatogr.*, 32 (1968) 458.
12 R. B. Sleight, *J. Chromatogr.*, 83 (1973) 31.
13 J. A. Schmidt, R. A. Henry, R. C. Williams and J. F. Dieckman, *J. Chromatogr. Sci*,. 9 (1971) 654.
14 E. Tomlinson, *J. Chromatogr.*, 113 (1975) 1.
15 A. J. P. Martin, *Biochem. Soc. Symp.*, 3 (1949) 4.
16 J. Green and D. McHale, *Advan. Chromatogr.*, 2 (1965) 99.
17 I. E. Bush, *Methods Biochem. Anal.*, 13 (1965) 396.
18 G. L. Amidon, S. H. Yalkowsky, S. T. Anik and S. C. Vlavani, *J. Phys. Chem.*, 79 (1975) 2239.
19 G. J. Pierotti, C. H. Deal and E. L. Derr, *Ind. Eng. Chem.*, 51 (1959) 95.
20 T. Fujita, J. Iwasa and C. Hansch, *J. Amer. Chem. Soc.*. 86 (1964) 5175.
21 A. Leo, C. Hansch and D. Elkins, *Chem. Rev.*, 71 (1971) 533.
22 S. S. Davis, *Separ. Sci.*, 10 (1975) 1, and references therein.
23 M. J. Harris, T. Higuchi and J. H. Rytting, *J. Phys. Chem.*, 77 (1973) 2694.
24 R. B. Hermann, *J. Phys. Chem.*, 76 (1972) 2754.
25 J. A. Reynolds, D. B. Gilbert and C. Tanford, *Proc. Nat. Acad. Sci. U.S.*, 71 (1974) 2925.
26 W. J. Haggerty and E. A. Murrill, *Res. Develop.*, 25 (1974) 30.
27 R. M. Carlson, R. E. Carlson and H. L. Kopperman, *J. Chromatogr.*, 107 (1975) 219.
28 L. B. Kier, L. H. Hall, W. J. Murray and M. Randic, *J. Pharm. Sci.*, 64 (1975) 1971.
29 L. H. Hall, L. B. Kier and W. J. Murray, *J. Pharm. Sci.*, 64 (1975) 1974.
30 W. J. Murray, L. H. Hall and L. B. Kier, *J. Pharm. Sci.*, 64 (1975) 1978.
31 S. Marcinkiewicz, J. Green and D. McHale, *J. Chromatogr.*, 10 (1963) 42.
32 J. Green, S. Marcinkiewicz and D. McHale, *J. Chromatogr.*, 10 (1963) 158.
33 L. S. Bark and R. J. T. Graham, *J. Chromatogr.*, 23 (1966) 417.
34 G. H. Bell, *Chem. Phys. Lipids*, 10 (1973) 1.
35 F. Franks and D. J. G. Ives, *Quart. Rev., Chem. Soc.*, 20 (1966) 1.
36 F. Franks, in F. Franks (Editor), *Water, A Comprehensive Treatise*, Vol. 4, Plenum Press, 1975, Ch. 1.
37 J. R. Gant and B. L. Karger, unpublished results.
38 M. Novotny, S. L. Bektesh, K. B. Denson, K. Grohmann and W. Parr, *Anal. Chem.*, 45 (1973) 971.
39 J. H. Knox and G. Vasvari, *J. Chromatogr.*, 83 (1973) 181.
40 E. Grushka and E. J. Kikta, Jr., *Anal. Chem.*, 46 (1974) 1370.
41 W. O. McReynolds, *Gas Chromatographic Retention Data*, Preston Techn. Abstr. Co., Evanston, Ill., 1966.
42 O. E. Schupp, III and J. S. Lewis (Editors), *Compilation of Gas Chromatographic Retention Data*, ASTM Data Series, No. DS 25 A, Philadelphia, Pa., 1967.