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DESCRIPTION OF SOLUTE RETENTION OVER THE FULL RANGE OF MOBILE PHASE COMPOSITIONS IN REVERSED-PHASE LIQUID CHROMATOGRAPHY

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SUMMARY

In this paper we investigate the relationship between solute retention and mobile phase composition in reversed-phase liquid chromatography over the entire range of composition, with emphasis on mobile phases with a high water content.

It will be shown that a quadratic relationship between the logarithm of the capacity factor and the volume fraction of organic modifier is generally valid for mobile phases containing less than 90% water.

When more water is added to the mobile phase, a quadratic equation turns out to be insufficient. An experimental study of ten solutes and three organic modifiers is used to show that an extension of the quadratic equation by a term proportional to the square root of the volume fraction leads to a description of all experimental retention data within approximately 10%.

INTRODUCTION

In recent years we have been studying the relationship between solute retention in reversed-phase liquid chromatography (RPLC), and the composition of binary[1,2] and ternary[3] mobile phases. We concluded that a quadratic relationship is usually valid for relating the logarithm of the capacity factor (ln k) with the volume fraction(s), φ, of the organic modifier(s). Over limited ranges of binary composition, a linear relationship between ln k and φ can often be used as a good approximation[2].

However, a quadratic relationship between ln k and φ, although sufficient to describe the *experimental* data adequately, has never been completely satisfactory. The reason for this is that *extrapolated* data for the retention in pure water, which are usually too high to obtain experimentally, vary a great deal with the nature of the binary organic modifier–water system used for the extrapolation[1,2]. In other words, the quadratic relationship does not appear to be valid for mobile phases with a (very) high water content and low percentages of organic modifier. Recently, experimental studies[4,5] have underlined the shortcomings of the quadratic model in this range.

At the basis of the quadratic model lies the solubility parameter concept[1]. Recent studies on the use of this concept as a qualitative[6] or quantitative[7,8] model

for the description of retention in RPLC have emphasized its usefulness, as well as its limitations. The main limitation for the application of the solubility parameter concept in RPLC (and in chromatography in general) is that it describes *energy* effects upon mixing, while, fundamentally, retention is related to *Gibbs free energy* effects. For liquid mixtures, the energy effect described by the solubility parameter model approximates the enthalpy effect, *i.e.* the volume effect upon mixing can usually be neglected. In that case, the predominant difference between the solubility parameter model and chromatographic retention is the *entropy effect*.

In many physico-chemical systems a strong correlation between enthalpy and entropy effects is observed. Such an empirical relationship has also been observed in RPLC[1,9,10]. This so-called enthalpy–entropy compensation effect causes any model that describes retention in terms of enthalpy (or energy) to overestimate the effects induced by variations of the parameters involved. In the case of the solubility parameter model these parameters are the polarities of the mobile and stationary phase and of the solute. This is exactly what is observed when one tries to apply this model to the *quantitative* description and prediction of chromatographic behaviour[7,8,11].

On the other hand, a strong correlation between enthalpy and entropy effects suggests that a model describing either one will be qualitatively useful. Moreover, quantitative results may be obtained on a relative scale, indicating elution orders, eluotropic series, and so on. Hence, such a model can be used *semi-quantitatively*[6].

Another approximation often used when applying the solubility parameter concept in LC is the assumption that the stationary phase polarity (solubility parameter) is independent of the nature and the composition of the mobile phase[6]. However, unlike the above, this is not a fundamental limitation of the solubility parameter approach. This we already showed in previous work[1], when we first realized the problem of "stationary phase modification", *i.e.* a stationary phase that changes in character (composition; polarity) under the influence of a changing mobile phase. We then tried to account for this effect by incorporating a variation of the stationary phase polarity with mobile phase composition into the model. In the absence of experimental data at that time, we assumed a linear relationship between these two quantities[1].

Since then, experimental data have become available on the sorption of mobile phase components (organic modifiers) onto or into the stationary phase (eqns. 12–14), showing that the above assumption is incorrect. In fact, the sorption of organic modifiers appears to be most important at low concentrations.

In this study we will show how a variation of the stationary phase with mobile phase composition can easily be accounted for in the solubility parameter model. A very simple equation will be shown to describe experimental ln k *vs.* φ relationships over the full range of composition, including the capacity factor in pure water.

THEORETICAL

Retention in LC can be expressed in terms of solubility parameters as follows[3,6]:

$$\ln k_i = \frac{v_i}{RT}\left\{(\delta_m - \delta_i)^2 - (\delta_s - \delta_i)^2\right\} + \ln \frac{n_s}{n_m} \tag{1}$$

where: k_i is the solute (i) capacity factor, R is the gas constant (1.9865 cal K^{-1} mole^{-1}), T is the absolute temperature (*.K), v_i is the molar volume of the solute (cm^3 mole^{-1}), δ is the solubility parameter (cal$^{1/2}$ cm$^{-3/2}$) and n is the number of moles of mobile (m) or stationary phase (s) present in the column.

For the solubility parameter of a mixture, we can use the average value of the constituents, weighted by volume fractions. *E.g.*, for a binary mixture of water (w) and an organic modifier (o), the volume fraction of the latter given by φ, we can write[1,3,6]:

$$\delta_m = (1 - \varphi)\,\delta_w + \varphi\,\delta_o \tag{2}$$

Clearly, if we substitute eqn. 2 into eqn. 1 we get an equation that is *quadratic* in φ, hence

$$\ln k_i = A\varphi^2 + B\varphi + C \tag{3}$$

In eqn. 3 it is assumed that the phase ratio term $\ln n_s/n_m$ is either independent of the mobile phase composition (φ) or is varying in such a way that it can be approximated by a quadratic curve.

Eqn. 3 has been shown to give an accurate description of the variation of retention with composition in binary RPLC systems[1,2], and a similar second-order equation applies when ternary mobile phase mixtures are used[3]. However, mobile phases of high water content ($\varphi \leqslant 0.1$) were used only scarcely in these studies, owing to the very high retention in this region for most solutes.

Let us now assume that the polarity of the stationary phase is some function of the mobile-phase composition φ:

$$\delta_s = f(\varphi) \tag{4}$$

Substitution of both eqns. 2 and 4 into eqn. 1 yields an equation that is quadratic in φ as well as in $f(\varphi)$:

$$\ln k_i = A\varphi^2 + B\varphi + C + Df^2(\varphi) + Ef(\varphi) \tag{5}$$

Eqn. 5 gives a simple relationship between $\ln k_i$ and composition, provided that the function $f(\varphi)$, *i.e.* the way in which the stationary phase polarity varies with the mobile phase composition is known. Previously[1] we have assumed that $f(\varphi)$ is linear in φ. In that case, eqn. 5 conforms to eqn. 3.

Direct data on the variation of the stationary phase polarity with mobile phase composition are not available. As was made clear in the introduction section, it is not a sensible approach to try and calculate δ_s from eqn. 1 and experimental values for k_i[7,8], because eqn. 1 does not provide a quantitatively correct description of LC retention behaviour.

Instead, we turn back to eqn. 2, which states that *polarity* is linearly related to *composition*. Experimental data on stationary phase composition are available from the literature, *i.e.* data on the sorption of organic modifiers into the stationary

TABLE I

VARIATION OF STATIONARY-PHASE COMPOSITION ACCORDING TO EQN. 6

Experimental data taken from ref. 14.

Modifier	α	β	$r^{\star}$	$N^{\star\star}$
Methanol	0.37	3.47	0.85	4
Acetonitrile	0.56	4.90	0.993	5
THF	0.52	5.22	0.999	5

$\quad$ * Correlation coefficient.
$\quad$ ** Number of data points.

phase[12-14]. We can reasonably assume the stationary phase polarity to vary according to its composition, in order to obtain information on the type of function suitable for describing the function $f(\varphi)$.

To this end we replotted the data from McCormick and Karger[14] logarithmically. This yielded the results summarized in Table I, corresponding to the equation

$$\ln \varphi_s = \alpha \ln \varphi + \beta \tag{6}$$

where φ_s is the amount of organic modifier sorbed (mg/g).

From Table I we see that for acetonitrile and tetrahydrofuran (THF) an almost perfectly straight line is obtained. For the sorption of methanol only four data-points are available, which show a considerable experimental scatter. Therefore, eqn. 6 seems to be useful as an indication of how φ_s (and hence δ_s) varies with φ. It should be noted that the use of different units for φ_s (mg/g) and φ (dimensionless) will reflect on the intercept β, but not on the slope α.

Analogous to eqn. 2 for the mobile phase we can assume the polarity of the stationary phase to vary proportional to φ_s. Since eqn. 6 shows φ_s to be proportional to φ^α, we can write

$$\delta_s = \delta a + q \, \varphi^\alpha \tag{7}$$

where q is a constant for a given organic modifier, and δ is the solubility parameter of the "bare" stationary phase (no organic modifier sorbed), i.e. the polarity of the stationary phase in pure water.

Combination of eqns. 1, 2 and 7 yields a specific form of eqn. 5:

$$\ln k_i = A\varphi^2 + B\varphi + C + D\varphi^{2\alpha} + E\varphi^\alpha \tag{8}$$

From Table I we may conclude that typical values for the exponent α are around 0.5. The overall shape of the curve described by eqn. 8 is not critically influenced by the exact value of α, i.e. an accurate description of experimental retention data can be achieved even if the α-value used in eqn. 8 is not exactly correct. If we assume α to equal 0.5 in eqn. 8 an even simpler general equation for the relationship between retention and composition is obtained:

$$\ln k_i = A\varphi^2 + B\varphi + C + E\sqrt{\varphi} \tag{9}$$

The coefficient D has disappeared from eqn. 9, thus changing the value of the parameter B. The coefficients A, B, C and E can be expressed in terms of solubility parameters. The derivation is given in the Appendix. Comparison of eqns. 9 and A-2 yields:

$$A = (v_i/RT) (\delta_0 - \delta_w)^2 \tag{10}$$

$$B = - (v_i/RT) [(\delta_w - \delta_0) (\delta_w - \delta_i) + q^2] \tag{11}$$

$$C = (v_i/RT) [(\delta_w - \delta_i)^2 - (\delta_a - \delta_i)^2] + \ln (n_s/n_m) = \ln k_w \tag{12}$$

$$E = (2 v_i/RT) q (\delta_i - \delta_a) \tag{13}$$

We conclude that the coefficient A is always positive, and, given the extremely high polarity of water, the coefficient B will be large and negative. Since the difference between δ_w and δ_i will usually be much larger than that between δ_a and δ_i, $C = \ln k_w$, the logarithm of the solute capacity factor in pure water is usually positive.

Eqn. 13 predicts that the coefficient E will be small for a solute with a polarity comparable to that of the stationary phase in pure water (δ_a). The sign of E depends on the polarity of the solute and on the sign of the parameter q (eqn. 7). Intuitively, we expect the polarity of the stationary phase to *increase* upon the sorption of mobile phase molecules. In that case q is positive and for polar solutes E will also be positive. However, it is not evident that the alkylated stationary phase in contact with a purely aqueous mobile phase can be assigned an equally low value for its solubility parameter (δ_a) as is attributed to bulk alkanes. Apart from the chemical structure of the modified silica, sorption of water may be a factor that influences the value of δ_a. Consequently, a positive value for q may still lead to negative values for E for non-polar solutes.

Moreover, if the stationary phase polarity in pure water is not very low, it may even be possible that δ_s *decreases* upon sorption of mobile phase molecules, so that q will be negative. In this case, E-values would be positive for non-polar solutes, but negative for very polar ones.

Therefore, both positive and negative values for E seem to be entirely feasible.

The effects caused by the E term in eqn. 9 on the shape of the $\ln k$ vs. φ curve are illustrated in Fig. 1. In this figure three curves have been drawn, which have been designed to overlap in the range $0.5 \leqslant \varphi \leqslant 1$. However, the E-coefficients for each of the curves have been fixed at different arbitrary values. Clearly, and not surprisingly, the influence of the stationary phase modification term (E-term) can be discerned only in the region of low φ.

The coefficients corresponding to eqn. 9 for the curves drawn in Fig. 1 are given in Table II. From this table it is clear that, although the three curves coincide completely in the high-φ region, the A, B anc C values turn out to be very different as a consequence of the varying values for E. Hence, the inclusion of an E term is not necessary for describing retention for all but the very low φ values ($\varphi < 0.1$). On the other hand, coefficients obtained from quadratic fits to experimental data not including this low-φ region[1,2] cannot be assigned any fundamental value. Most importantly, this is true for the coefficient C, the extrapolated retention in pure water
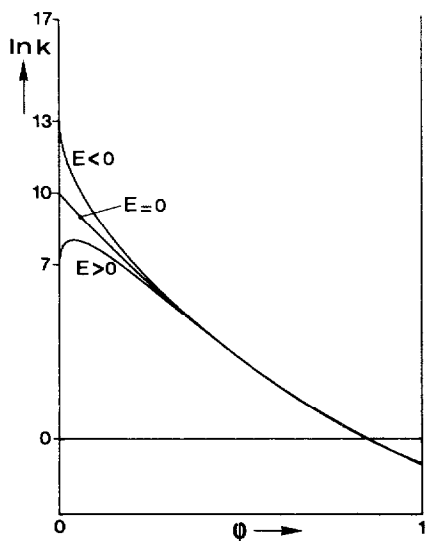
Fig. 1. Illustration of the relationship between solute retention and mobile-phase composition according to eqn. 9. Arbitrary curves designed to overlap for $\varphi \geq 0.5$. For coefficients see Table II.

TABLE II

COEFFICIENTS CORRESPONDING TO EQN. 9 FOR THE THREE CURVES DRAWN IN FIG.

A	B	C	E
5*	− 16*	10*	0
7.53**	− 25.60**	7.10**	10*
2.47**	− 6.40**	12.90**	− 10*

  * Arbitrarily selected value.
 ** Value designed to yield the best correspondence (least squares) to the quadratic curve ($E = 0$) in the range $0.5 \leq \varphi \leq 1$.

($C = \ln k_w$). Both from Table II and from Fig. 1 it will clear that extrapolation of retention data towards pure water will be seriously obstructed by the occurence of a significant E term.

     In this paper we will investigate the relationship between $\ln k_i$ and φ, with emphasis on the high-water region, where the influence of the coefficient E is significant and discernible from the other terms in eqn. 9.

     We will examine the validity of eqn. 9 as a convenient approximation for the description of retention in RPLC over the complete range of composition (100% water to 100% organic modifier).

EXPERIMENTAL

     Chromatographic experiments were performed with different instruments, assembled from modules from Waters (Model M6000A and M45 pumps, a Model 440 UV photometer and a U6K injector), Perkin-Elmer (LC1 pump and LC75 spectrophotometer), Rheodyne (Model 7120 injection valves) and Zeiss (PM2 DLC-spectrophotometer). Some of the measurements in mobile phases with low-volume frac-

tions of methanol in water were performed by Dr. C.E. Werkhoven-Goewie in the laboratory of the Free University of Amsterdam.

In order to obtain measurable retention volumes for ten solutes varying in size and polarity over the complete range of binary composition (0–100% of organic modifier) on ODS-silica, different columns of supplementary dimensions had to be used. A 150 × 4.6 mm analytical column and a 71 × 2.2 mm precolumn were obtained from Chrompack, and two 11 × 2 mm precolumns of special design[16,17] were made available for the present project by the Department of Analytical Chemistry of the Free University of Amsterdam. Each of these columns was packed once from the same batch (No. 6/872) of ODS-hypersil (5 μm) from Shandon.

A typical series of mobile phase compositions included mixtures of 0, 1, 2, 5, 10, 15, 20, 33, 50, 67, 80, 90 and 100% of organic modifier in water. This illustrates the emphasis we put on the high water range. Frequently, several retention volumes were measured successively under identical conditions. Such data were averaged and will be referred to as a single datapoint.

Given the divergent experimental conditions and columns used, care had to be taken to combine all individual datapoints into a consistent set. To this end, retention times obtained on columns of different dimensions and on different instruments, but with the same mobile phases were used to establish correlations in the form of linear regression curves. To correlate columns of different dimensions, 35–50 datapoints were used to establish straight lines with correlation coefficients well exceeding 0.99. To correlate the two different precolumns of the same size, a set of seventeen overlapping datapoints was found to be sufficient.

All data were recalculated to yield retention volumes on the analytical column and then corrected for the extra-column residence volume of 20 μl. For reasons discussed before[3,15], a uniform column hold-up volume independent of the mobile phase (1.50 ml for the analytical column) was used to calculate capacity factors.

Methanol, acetonitrile and THF of HPLC grade were obtained from Rathburn, Scotland. The water used was specially treated with ion-exchange resins and carbon filters after distillation. Solvents were formed from independently measured volumes of the individual components and thoroughly mixed and degassed by extensive ultrasonic vibration before use. Long mixing times were found to be special importance for mixtures of THF and water, especially in the range 10–33% THF.

RESULTS AND DISCUSSION

Retention data were measured by using three different organic modifiers for a set of ten solutes, chosen for their divergent polarities and to allow comparison with the results obtained by Gilpin and Squires[5] on other materials. These data were fitted to a three-dimensional version of eqn. 9:

$$\ln k_i = C + A_M \varphi_M^2 + B_M \varphi_M + E_M \sqrt{\varphi_M}$$
$$+ A_A \varphi_A^2 + B_A \varphi_A + E_A \sqrt{\varphi_A} \qquad (14)$$
$$+ A_T \varphi_T^2 + B_T \varphi_T + E_T \sqrt{\varphi_T}$$

TABLE III

COEFFICIENTS ACCORDING TO EQN. 14 OBTAINED FROM REGRESSION ANALYSIS OF EXPERIMENTAL RETENTION DATA

Solute	$\ln k_w$ (exp)	C	Methanol			Acetonitrile			THF			N*	Δ**
			A	B	E	A	B	E	A	B	E		
Phenol	3.12	2.99	2.46	−8.38	1.02	4.82	−10.05	0.49	2.53	−9.93	1.42	54	0.09
p-Cresol	4.32	4.26	2.84	−10.01	1.12	5.19	−11.00	−0.13	2.56	−9.54	0.30	50	0.08
p-Chlorophenol	4.62	4.47	1.50	−9.24	1.45	6.75	−15.16	2.34	9.41	−24.07	7.50	52	0.08
2,4-Dichlorophenol	6.00	5.93	1.24	−10.00	1.24	8.77	−18.71	2.77	10.77	−27.33	7.78	41	0.08
Nitrobenzene	4.49	4.29	1.73	−8.32	0.74	2.74	−8.87	0.21	0.42	−5.53	1.92	52	0.10
m-Dinitrobenzene	4.58	4.14	1.45	−7.02	−0.59	2.71	−8.95	−0.07	−1.62	−3.24	2.60	52	0.11
Benzene	3.94	3.96	0.38	−7.09	1.43	2.45	−9.07	1.37	1.20	−7.15	0.17	45	0.09
Chlorobenzene	5.40	5.25	−0.89	−6.58	0.88	4.39	−13.30	2.59	6.34	−17.25	3.64	41	0.11
Benzophenone	8.99	8.75	5.33	16.97	1.56	6.01	−11.85	−3.94	0.50	−1.19	10.93	37	0.11
Naphthalene	7.22	7.25	14.00	−36.75	14.88	5.69	−14.63	0.80	6.06	−15.64	0.02	37	0.15

* Number of independent datapoints.

** Average deviation of experimental datapoints from the regression curve $\Delta = \dfrac{1}{N} \cdot \sum_{i=1}^{N} (\ln k_{\text{eqn. 14}} - \ln k_{\text{exp}})$.

where the subscripts M, A and T denote methanol, acetonitrile and THF, respectively. Since only binary mixtures were used, only one of the three φ values in eqn. 14 will differ from zero for each datapoint. The difference between eqns. 9 and 14 is the fact that the latter acknowledges the C term as a constant, independent of the nature of the organic modifier. The results of the regression analysis are presented in Table III. The final two columns in this table list the number of independent data points used and their average deviation from the regression curves. For most solutes errors tend to be largest in the high-φ region, where experimental errors (and errors connected to the assumption of a uniforme V_0 value) will be most pronounced. Data obtained with mixtures of 20% THF in water showed a systematic deviation from the model, the experimental value for ln k always being higher than the calculated one. All other deviations appeared to be random and small, especially if we consider the use of four different columns for each solute.

In order to evaluate the improvement of eqn. 9 over eqn. 3 the data points were fitted to eqn. 14 with and without the inclusion of the E terms. With incorporation of the E terms the average deviation of the datapoints from the regression curves varies from 0.08 to 0.15 for different solutes (Table III). Similar deviations have been observed previously when a quadratic expression (eqn. 3) was fitted to datapoints ranging from about $\varphi = 0.1$ upward. However, when all datapoints over the full range from $\varphi = 0$ are fitted with $E = 0$, the average deviation increases to values as high as 0.34 (for naphthalene). This is due to the poor fit in the low-φ region. Indeed, with $E = 0$, the datapoints for $\varphi < 0.25$ deviate from the regression curves by average values ranging from 0.25 to 0.5 for different solutes. As a result, the C values found in this case differ from the experimental retention data in pure water (ln k_w) by values up to about 0.5, corresponding to an error of 65% in k_w.

In contrast, when the E terms are included in the regression analysis, the data points for $\varphi < 0.25$ behave similar to the datapoints in other regions and show deviations of about 0.1 in ln k. The C values now agree with the experimental values of ln k_w to within the random error of about 0.2 (Table III). The necessity of incorporating the E terms in eqn. 14 is thus amply demonstrated.

Following the experimental retention data for pure water (ln k_w) and the uniform C values, Table III gives the values of A, B and E for all ten solutes in the three modifier systems studied. Despite an occasional (small) negative A value and a low value for B in the case of benzophenone in THF (where E is exceptionally large and negative), the values for A and B generally agree with the expectations formulated in the theory section.

The values found for E are less easily interpreted. Obviously, positive as well as negative values are observed, and the magnitude of E varies from virtually zero to values well over 10. As has already been remarked in connection with Fig. 1 (Table II) extreme values for E will have a significant effect on the values found for A and B. Generally, the magnitude of the E coefficient tends to be higher in THF than in acetonitrile and in methanol. Indeed, in the methanol–water system all values of E appear to be fairly small, except for naphthalene, which shows a surprisingly large and positive E value. This result reflects the exceptionally high retention volumes observed for naphthalene in mobile phases containing only a few percent methanol in water.

It is difficult to explain why naphthalene, definitely one of the least polar

solutes in our set, yields such a extreme value for E. From eqn. 13 an increase in E with decreasing solute polarity (δ_i) can only be explained by assuming a negative value of q. This implies a decrease in the polarity of the stationary phase upon sorption of methanol. Although bulk liquid methanol is much more polar ($\delta \approx 16$ cal[12] cm^{-32}) than we envisage our base support material to be, such a situation could at least be explained in two ways. First, the sorption of methanol may accompany a desorption of water ($\delta \approx 25$) from the column. Secondly, residual silanols may be specifically shielded by methanol molecules, which are much smaller than the smallest silanizing agent (trimethylchlorosilane) and could, in theory, be chemisorbed on the surface through hydrogen bonding, thus yielding a stationary phase that appears to be less polar.

However, this theory is highly speculative, since it is based on only a single piece of evidence, the surprisingly high value for E in the case of naphthalene. Other non-polar solutes, such as benzene and, to a lesser extent chlorobenzene, show E values that are only slightly positive and very similar to those of the (polar) phenolic solutes.

When acetonitrile is used instead of methanol, we see significant, positive E values for the two chlorophenols and for chlorobenzene, as well as a large negative value for benzophenone. Again, the correlation with solute polarity cannot easily be understood. The chlorophenols are rather polar solutes, but they behave different from the two other (polar) phenolic solutes. On the other hand, chlorobenzene is only moderately polar and as such comparable to benzophenone, which in turn shows a large, negative E value.

It is more rewarding to compare the E values obtained with acetonitrile to those obtained with THF. With THF, the effects described above for acetonitrile appear to be enlarged. Indeed, there appears to be a correlation between the E values obtained with these two modifiers as is demonstrated in Fig. 2.
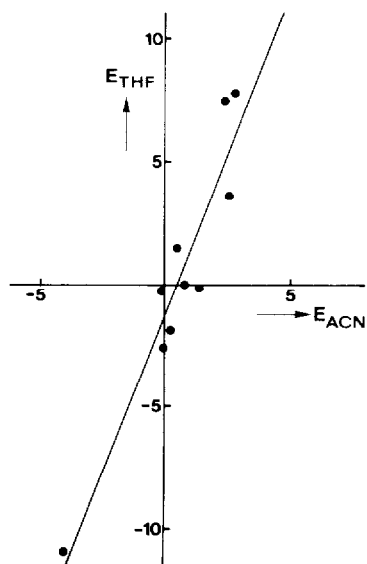

Fig. 2. Correlation between the E values observed for acetonitrile (ACN) and for THF. Data taken from Table III. Straight line follows $E_{THF} = 2.61 E_{acetonitrile} - 1.24$.

A least squares estimate for a regression line is shown in the figure. The observation of such a linear correlation, as well as the fact that the intercept is close to zero (-1.24) both support the suggestion in eqn. 13 that the type of modifier only contributes a constant factor (q) to the value of E for different solutes. The slope of the line is 2.61, indicating that the absolute value of q in eqn. 13 is about three times as high in the case of THF as it is for acetonitrile.

Apart from the E value for naphthalene, all values for E obtained with methanol are low, thus suggesting that

$$|q_{methanol}| < |q_{acetonitrile}| < |q_{THF}|$$

a sequence that agrees with the sorption isotherms measured for these three modifiers[14].

Fig. 3 gives some examples of ln k as a function of φ according to eqn. 9 using the coefficients given in Table III. Since the inclusion of all the individual datapoints would obscure the effects we want to demonstrate, only the most significant part of the curve for p-chlorophenol in THF is inserted as an example (Fig. 3, right). In this three-times-enlarged insert, some deviations are still observed between the calculated curve (eqn. 9) and the individual datapoints, but the necessity of including one or more stationary phase modification terms is underlined. For the particular, but not atypical, case of p-chlorophenol, the datapoints suggest that a value for α slightly lower than 0.5 in eqn. 8 might further increase the accuracy of the description in the low-φ region, at the cost of an increase in complexity. The present set of experimental data does not allow a sensible estimate of all six parameters (A–E and α) in eqn. 8 by means of regression analysis. In Fig. 3 (left), ln k vs. φ is plotted for p-chlorophenol, benzophenone and naphthalene by using methanol–water mixtures as the mobile phase. In Fig. 3 (right), THF is used instead of methanol. The different character of the two plots is clear, as well as the considerable influence of the E term.
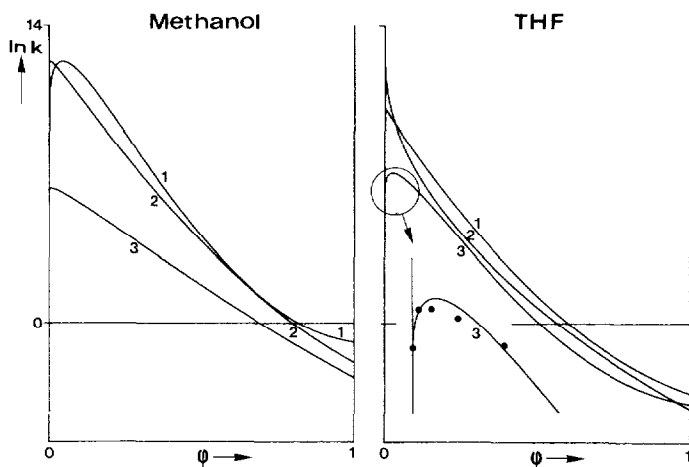

Fig. 3. Examples of experimentally observed ln k vs. φ relationships. Coefficients taken from Table III. Left: Methanol–water system; right: THF–water system. Solutes: 1 = naphthalene; 2 = benzophenone; 3 = p-chlorophenol.

The data presented here appear to confirm data reported by Gilpin and Squires[5], who earlier demonstrated the occurence of what we refer to as an E term or stationary-phase modification term, but interpreted their results differently, *i.e.* as a consequence of a conformational change in the stationary layer. Obviously, the occurrence of such a phenomenon, although not in conflict with the present study, is by no means a prerequisite for the explanation of the experimental data.

In the terminology of this paper, Gilpin and Squires observed an increase in E values in the order

$$|q_{methanol}| < |q_{acetonitrile}| < |q_{dioxane}|$$

in close agreement with the sequence given above, especially if we realize that dioxane and THF have comparable polarity[6] and belong to the same group in Snyder's triangular solvent classification scheme[19]. The influence of the solute upon the E values in the study of Gilpin and Squires can be summarized as

$$E_{p\text{-cresol}} < E_{phenol} < E_{chlorobenzene} \approx E_{p\text{-chlorophenol}} \approx E_{2,4\text{-dichlorophenol}}$$

a sequence that can roughly also be observed in Table III, but bears little relation to a steadily increasing or decreasing polarity.

Extrapolation of retention data towards pure water

Although actual retention data in the range $0 < \varphi < 0.1$ are not of great importance in current liquid chromatographic practice, the retention in pure water has the assigned practical significance that it can be related to the breakthrough volume on precolumns used for the preconcentration of samples from aqueous solutions[4].

In Table IV we have investigated what effect the stationary phase modification terms (E values), as listed in Table III, would have on the result obtained by extrapolating retention data for $\varphi \geq 0.25$ towards $\varphi = 0$. To this end we calculated the quadratic curves that gave the best approximation of the curves described by eqn. 9 and the coefficients of Table III over the range $0.25 \leq \varphi \leq 1$. In Table IV we compare the C values obtained from this simulated extrapolation with the experi-

TABLE IV

COMPARISON OF SOME EXPERIMENTAL DATA FOR ln k_w WITH EXTRAPOLATED DATA (EQN. 3 USING DATA FOR WHICH $\varphi \geq 0.25$)

Solute	Modifier*	E	ln k_w	C	C − ln k_w
Naphthalene	T	− 0.20	7.22	7.20	−0.02
p-Cresol	T	− 0.30	4.32	4.18	−0.14
Chlorobenzene	M	0.88	5.40	5.49	0.09
Benzene	A	1.37	3.94	4.33	0.39
Phenol	T	1.42	3.12	3.38	0.26
Nitrobenzene	T	− 1.92	4.49	3.77	−0.72
Benzophenone	A	− 3.94	8.99	7.67	− 1.32
2,4-Dichlorophenol	T	7.78	6.00	8.05	2.05
Naphthalene	M	14.88	7.22	11.31	4.09

* M = Methanol; A = acetonitrile; T = THF.

mental values for ln k_w for a series of examples listed in order of increasing absolute values of E.

From Table IV we see that E values smaller than 1 (first three examples) will not lead to large extrapolation errors. For $1 < E < 3$ (next three examples) the errors are found to increase up to a factor of 2 in terms of k_w, while for even larger values of E (bottom three lines in table IV) the results become quickly worse up to a factor of 60 for naphthalene in methanol–water.

Since all other solutes show E values less than about 1.5 for methanol, this modifier may be expected to yield the most reliable results, although exceptions do occur. Unfortunately, it is not yet possible to predict E values, so that such exceptions cannot be anticipated.

It is interesting to note that in an experimental study of the possibilities for extrapolating retention data towards $\varphi = 0$ (ref. 4), the experimental retention volume for naphthalene in pure water turned out to be overestimated by almost exactly a factor of 60 when quadratic extrapolation was used. For the seemingly similar solute biphenyl, however, almost exact agreement between the extrapolated and the experimental values was obtained, as was also the case for dimethyl phthalate.

CONCLUSIONS

For the description of retention as a function of composition in RPLC a quadratic equation is generally adequate. Only when the region of very high water content (10% or less organic modifier in water) has to be included at least one extra term is necessary to account for the effects caused by sorption of the organic modifier.

An adequate and yet convenient equation that describes ln k as a function of φ over the full range of composition $0 \leqslant \varphi \leqslant 1$ can be obtained by incorporating an extra term that is proportional to the square root of φ.

The coefficient E for this square root term can either be positive or negative and its absolute value has been found to vary from zero to almost fifteen for a set of ten solutes when three different organic modifiers were used. E values for a given solute tend to increase in order

$$|E_{\text{methanol}}| < |E_{\text{acetonitrile}}| < |E_{\text{THF}}|$$

However, one noticeable exception to this rule has already been observed, *i.e.* a very large positive E value for naphthalene in methanol–water mixtures.

The influence of solute polarity on the value of E is not straightforward.

The occurence of significant values for E forms a serious obstacle to the extrapolation of experimental retention data in order to estimate the retention in pure water. If such data are required, the methanol–water system does seem to yield good results in almost all cases. To be sure of a correct estimate for the retention in pure water, however, it is advisable to measure this quantity directly on a very short (pre-)column.

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APPENDIX

Derivation of the retention equation
Retention is described by eqn. 1:

$$\ln k_i = \frac{v_i}{RT} \cdot \left[(\delta_m - \delta_i)^2 - (\delta_s - \delta_i)^2 \right] + \ln \frac{n_s}{n_m} \tag{1}$$

The mobile phase polarity follows eqn. 2:

$$\delta_m = (1 - \varphi)\delta_w + \varphi\delta_0 \tag{2}$$

and the stationary phase polarity can be assumed to follow eqn. 7:

$$\delta_s = \delta_a + q\,\varphi^\alpha \tag{7}$$

Combination of these three equations results in

$$\ln k_i = \frac{v_i}{RT} \cdot \left\{ [(1 - \varphi)\,\delta_w + \varphi\delta_0 - \delta_i]^2 - (\delta_a + q\varphi^\alpha - \delta_i)^2 \right\} + \ln \frac{n_s}{n_m} =$$

$$= \frac{v_i}{RT} \cdot \left[\varphi^2 (\delta_0 - \delta_w)^2 - 2(\delta_w - \varphi_0)(\delta_w - \delta_i)\varphi + (\delta_w - \delta_i)^2 - (\delta_a - \delta_i)^2 \right.$$

$$\left. - 2q(\delta_a - \delta_i)\varphi\alpha - q^2\varphi^2\alpha \right] + \ln \frac{n_s}{n_m} \tag{A-1}$$

For $\alpha = 0.5$ this equations turns into

$$\ln k_i = \frac{v_i}{RT} \cdot \left\{ \varphi^2(\delta_0 - \delta_w)^2 - [2(\delta_w - \delta_0)(\delta_w - \delta_i) + q^2]\varphi \right.$$

$$\left. + (\delta_w - \delta_i)^2 - (\delta_a - \delta_i)^2 + 2q(\delta_i - \delta_a)\sqrt{\varphi} \right\} + \ln \frac{n_s}{n_m} \tag{A-2}$$

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