

## Review

# Retention in reversed-phase liquid chromatography as a function of mobile-phase composition

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### ABSTRACT

The dependence of solute retention ( $k'$ ) on mobile phase composition (%B for binary-solvent mixtures A–B) is reviewed and compared with various empirical and theoretical equations that have been proposed for this relationship. Because the functional dependence of  $k'$  on organic modifier composition varies from one system to another, it is not possible from these data to draw any overall conclusions as to the nature of the retention process in reversed-phase chromatography. Likewise, there is probably no one best equation for extrapolating all retention data to 0 %B for purposes of predicting  $\log P_{o,w}$  values from chromatographic data.

The relative change in  $k'$  with change in %B can be described in terms of the parameter  $S = -d(\log k')/d\phi$  ( $\phi = 0.01$  %B). Values of  $S$  as a function of solute structure, mobile-phase composition, column type and experimental conditions are of interest for several reasons: insight into the retention process or “mechanism”, mobile phase optimization, etc. Previous work relating to this question is reviewed here and some conclusions are presented.

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## 1. INTRODUCTION

The dependence of reversed-phase retention ( $k'$ ) on organic modifier composition (%B in binary solvent systems) is of interest for several reasons. First, the study of the relationship for a wide range of solutes and separation conditions might provide insight into the fundamental basis of sample retention in reversed-phase liquid chromatography (RP-LC); *i.e.*, the retention “mechanism”. Second, reversed-phase retention data are widely used to estimate octanol–water partition coefficients ( $\log P_{o/w}$ ) as discussed elsewhere in this volume [1]. These procedures typically require extrapolation of values of  $k'$  as a function to %B to pure water (0 %B); hence a knowledge of how  $k'$  depends on %B is relevant to this application. Third, changes in %B often result in significant changes in relative retention or selectivity [2]; a better understanding of the  $k'$ –%B relationship should lead to a more effective use of solvent strength (%B) as a means of optimizing HPLC separation. Finally, for ternary and higher-order solvent systems, the relative proportions of different organic modifiers (%B, %C, etc.) to each other and water offer additional selectivity for separation optimizations [3–6].

In the present paper we will review the dependence of  $k'$  on %B first in binary-solvent RP-LC systems and then in ternary and higher-order solvent systems. Previous workers have proposed both empirical [7] and theoretical [8–11] equations that describe  $k'$  as a function of %B for binary-solvent systems. For higher-order solvent

systems, extensions to these have also been proposed [6,12,13]. It is of interest to review and compare these different approaches in terms of experiments where solute structure and experimental conditions are systematically varied.

A large body of published data exists that describes the dependence of  $k'$  on %B for different solutes and varied experimental conditions; one study [11] lists 332 data sets (tabulations of  $k'$  vs. %B for different solutes and conditions) drawn from five different papers. Today it is possible to cite a much larger number of such examples, and a detailed analysis of this entire data base would be a formidable task. In the present paper we will make selective use of a small part of this substantial literature—mainly those papers in which retention data have been reduced to a form that is readily interpreted.

Our goals for the present study include the following. First, the *general* relationship that describes the dependence of  $k'$  on %B in binary systems will be examined. The extension to higher-order solvent systems will also be considered. Second, the rate of change of  $k'$  with %B will be expressed as a single parameter ( $S = -100 \, d[\log k'] / d[\%B]$ ) which will then be considered as a function of solute structure and experimental conditions. Third, some implications of our results for a better understanding of the retention process in reversed-phase HPLC will be examined. Finally, the significance of our findings for the prediction of  $\log P_{o/w}$  values and the optimization of HPLC separation will be explored.

## 2. BACKGROUND: $k'$ VS. %B AND DIFFERENT MODELS OF RETENTION

An increase in organic content (%B) of a mobile phase such as methanol–water usually leads to a decrease in solute  $k'$  values. Early workers [7] recognized that RP-LC retention could be approximated by the relationship

$$\log k' = \log k_w - S\varphi \quad (1)$$

Here  $k_w$  is the value of  $k'$  in pure water as mobile phase,  $S$  is a constant for a given solute and a given RP-LC system, and  $\varphi$  is the volume fraction of the organic solvent (equal %B/100). [Note in eqn. 1 and some others in the present paper, the base 10 logarithm is used; unless natural logarithms are explicitly stated (“ln”) in this paper, base 10 logarithms are understood]. While eqn. 1 usually provides a reasonable fit to experimental data over a limited range in  $\varphi$ , there is often a tendency toward concave plots of  $\log k'$  vs.  $\varphi$ . This is illustrated in Fig. 1a for the retention of various di-*n*-alkyl phthalates on a  $C_{18}$  column with acetonitrile–water mobile phases.

Curvature of plots of  $\log k'$  vs.  $\varphi$  can be especially severe for basic or cationic solutes when  $\varphi > 0.5$ . A similar behavior was first noted for various crown ethers as solute and attributed to silanol interactions [14]. That is, “basic” solutes interact with accessible silanols present in the packing via a normal-phase process; see ref. 15 for a recent review. An example of this curvature is seen in Fig. 1b, for four amino-substituted compounds and a column which has accessible, acidic silanols. The same separation on a different column with fewer acidic silanols (Fig. 1c), on the other hand, shows plots of  $\log k'$  vs.  $\varphi$  that are nearly linear. These examples illustrate the importance of the column in regard to the accuracy of eqn. 1. In many cases it is possible to select experimental conditions that minimize these silanol effects [15]; e.g., a less “acidic” column plus a low pH mobile phase having a buffer concentration  $>25$  mM and an amine modifier such as triethylamine. The present paper will ignore the effects of silanol interactions, but see ref. 16 (in this volume) for a good review and discussion.

### 2.1. Solubility parameter model

Solubility parameter theory has been used to derive a relationship between  $k'$  and  $\varphi$  [17]:

$$\ln k' = A\varphi^2 + B\varphi + C \quad (2)$$

Here  $A$ ,  $B$  and  $C$  are constants for a given solute and reversed-phase system. The model from which eqn. 2 is derived assumes that solvent–solute interactions in solution are of primary importance in determining retention, and this in turn implies that retention results from a partition rather than an adsorption process. Eqn. 2 provides a better fit to experimental data vs. eqn. 1 [17], as is expected for a fitting function with one additional term.

### 2.2. Solvatochromic models

An extension of the solubility parameter model above is the use of “solvatochromic” relationships for the prediction of  $k'$  as a function of  $\varphi$ . This approach assumes that (a) retention is controlled by solvent “polarity” and (b) the latter can be approximated by certain spectroscopic measurements. Dorsey and co-workers [11,18] have used so-called  $E_T(30)$  values as a measure of solvent polarity and have shown that plots of  $\log k'$  vs.  $E_T(30)$  are more nearly linear than are plots of  $\log k'$  vs.  $\varphi$  i.e.,

$$\log k' = a + bE_T(30) \quad (3)$$

The latter retention model can be criticized [19], in that it assumes that mobile phase polarity can be described by a single parameter. A more detailed application of solvatochromic theory has been used to successfully model retention as a function of  $\varphi$  for different organic solvents [20]. However a considerably larger number of experimental parameters are then required.

### 2.3. Stoichiometric displacement model

Geng and Regnier have suggested yet another model of retention for reversed-phase HPLC [9,10]. These workers assume that a sorbing solute molecule X displaces some number  $Z$  of previously retained organic solvent molecules S

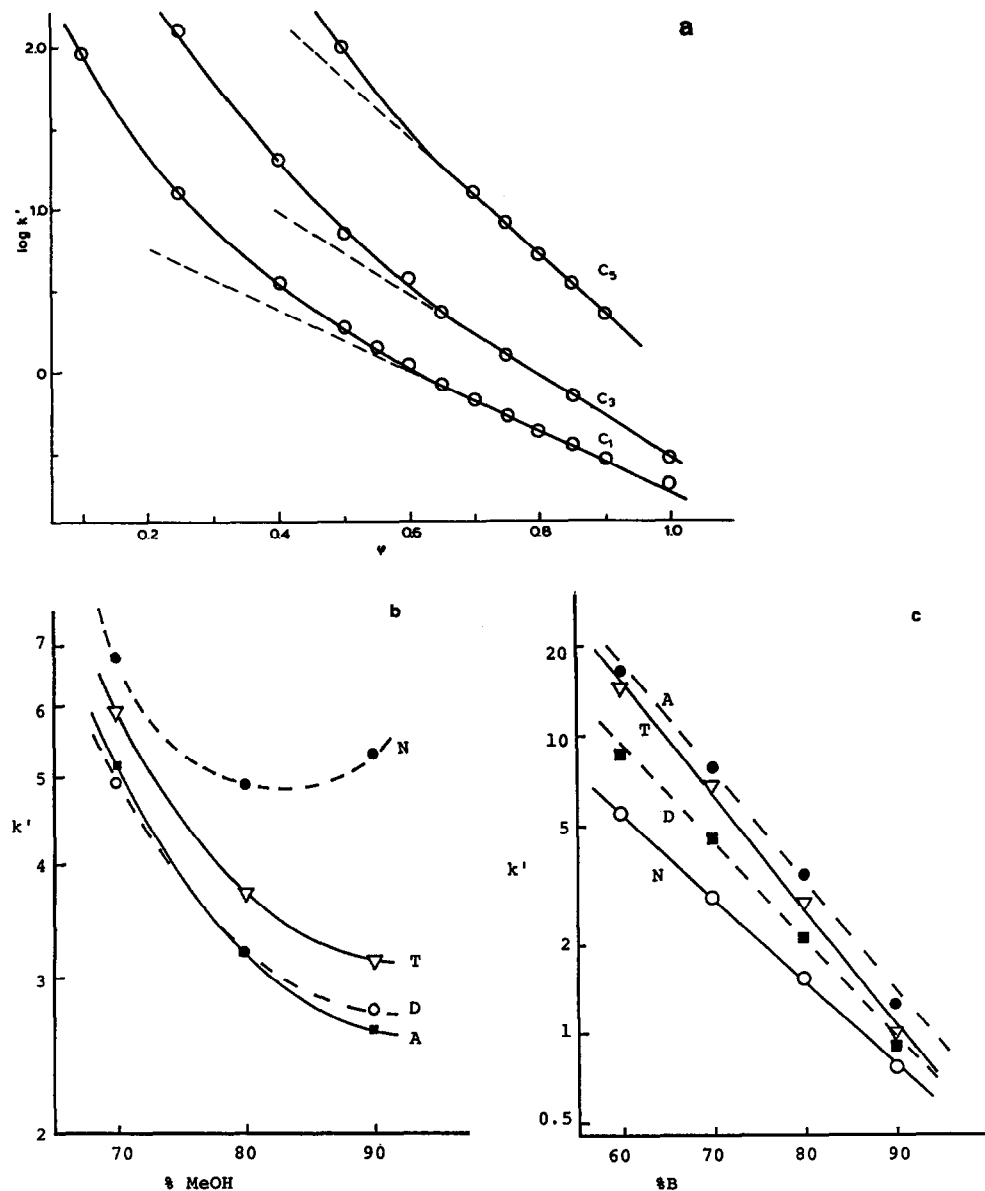
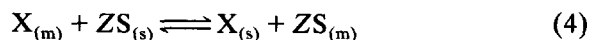


Fig. 1. Curvature of plots of  $\log k'$  vs.  $\phi$  in reversed-phase HPLC. (a) Separation of di-*n*-alkyl phthalates of varying carbon number (shown in figure) as a function of  $\phi$ ;  $C_{18}$  column, acetonitrile–water as mobile phase, 35°C. From ref. 24. (b) Separation of tricyclic antidepressants on a Zorbax CN column (Rockland) as a function of  $\phi$ ; methanol–buffer as mobile phase (buffer is 25 mM potassium phosphate plus 25 mM triethylamine at pH 6.2); 30°C. Solutes are nortriptyline (N), trimipramine (T), doxepin (D) and amitriptyline (A). Unpublished data from one of the author's (L.R.S.) laboratories. (c) Same as (b), except column is Stablebond  $C_8$  (Rockland).

at the interface between the solvated solute and the solvated bonded phase:



Here (m) refers to a molecule in the mobile phase and (s) refers to a molecule in the stationary phase. It is further assumed that (a) solution interactions are unimportant or cancel and (b)

the stationary phase has a constant concentration of the organic solvent S (for  $\varphi > 0.3$ ). Under these conditions, the following relationship can be derived:

$$\log k' = \log I - z \log C_o \quad (5)$$

Here  $I$  is a constant for a given solute and reversed-phase system, and  $C_o$  is the organic solvent concentration (mol/l) in the mobile phase. Geng [10] and others [21] have shown that eqn. 4 is not a good approximation when  $\varphi < 0.30$  (as expected).

The stoichiometric displacement model (SDM) is diametrically opposed to the solubility parameter and solvatochromic models, in that the latter assume that solvent polarity dominates the retention process. The SDM (in its original form) assumes that these interactions are negligible, and that mass action effects (eqn. 4) are instead mainly responsible for sample retention.

#### 2.4. Solvophobic model

This model [22] has been widely used by many workers for the interpretation of reversed-phase retention. The solvophobic theory [82] was adapted to chromatography by Melander and Horváth [23]. It describes the retention process by energy changes. According to the solvophobic theory the retention of a compound is proportional to its hydrophobic volume (*i.e.*, the size of the cavity formed in the solvent), the volume of the stationary and the mobile phase, the difference of the partial electrostatic charge of complex and the ligate–ligand partial electrostatic charge, dielectric constant and the surface tension of the solvent. As relationships between the surface tension and the volume fraction of organic solvents are not linear, slightly different plots can be expected for the  $\log k'$  vs. organic phase concentration relationships. Due to the non-linearity of the surface tension vs. volume fraction plot the deviations from the linearity at extreme organic phase concentration values (less than 0.3 and more than 0.9) can be explained. It was proved experimentally [83] that for solutes without  $\pi$  electrons the plots of  $\log k'$  vs.  $\varphi$  in methanol–water or acetonitrile–water are correlated with the evolution of surface tension of

these mixtures. But for ligands with  $\pi$  electrons slight modification of these curves appeared due to the specific  $\pi$ – $\pi$  interactions added to the pure solvophobic effect.

### 3. THE FUNCTIONAL DEPENDENCE OF $k'$ ON %B

#### 3.1. Experimental relationships

The experimental dependence of retention on solvent strength  $\varphi$  in reversed-phase HPLC has received considerable attention. Numerous studies have been reported which purport to show the validity of eqn. 1, usually by means of correlation coefficients  $r \approx 1$  for various solutes and a range in values of  $\varphi$ . The only comprehensive study which has attempted to compare the accuracy of eqn. 1 with other fitting equations (eqns. 2, 3 and 5) is that of Johnson *et al.* [11]. On the basis of a large number of reported studies with methanol and acetonitrile as organic solvents, these authors concluded that eqn. 3 ( $r^2 = 0.971$ ) was more reliable than eqn. 1 ( $r^2 = 0.943$ ). A comparison of eqns. 1 and 3 for a particular solute and organic solvent is shown in Fig. 2, taken from ref. 11. For this one example,

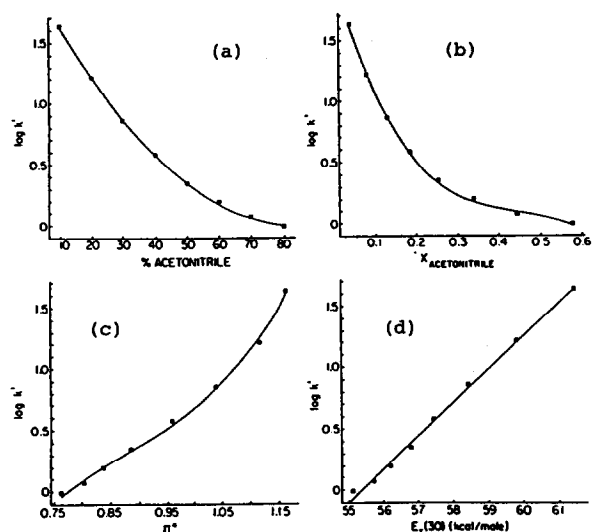


Fig. 2. Comparisons of eqns. 1, 3 and 5 for describing  $k'$  as a function of  $\varphi$  (%B). From ref. 11 for 4-nitrophenol as solute and acetonitrile as organic solvent. (a) eqn. 1; (b) eqn. 5; (c) see text; (d) eqn. 3.  $x_{\text{acetonitrile}}$  is the mole fraction of this solvent.

there is a good fit with eqn. 3 (Fig. 2d) and a less good fit for eqn. 1 (Fig. 2a). Another measure of solvent polarity ( $\pi^*$ , solvent dipolarity/polarizability) is seen in Fig. 2c to be less satisfactory vs.  $E_T(30)$ .

The conclusions drawn from ref. 11 and Fig. 2 with regard to the overall “goodness” of these various fitting questions must be qualified, however, in view of wide variations in the ability of a particular equation to fit different data sets. This is illustrated for eqn. 1 in Fig. 3 (same solute and column, different organic solvents). In this case, methanol and tetrahydrofuran (THF) give reasonably linear plots, but the data for acetonitrile show marked curvature. In general, it is observed that plots of  $\log k'$  vs.  $\phi$  are more nearly linear for methanol vs. acetonitrile, as is also apparent from the study of ref. 8. This can also be seen from the study of ref. 11, if we subdivide the data according to whether methanol or acetonitrile is used as solvent (Table 1), and also from ref. 23.

Table 1 indicates that eqn. 1 is actually a *better* fitting equation than eqn. 3 for methanol as solvent, but a much poorer fitting equation when acetonitrile is used. This is another way of recognizing the generally greater curvature of plots of  $\log k'$  vs.  $\phi$  for acetonitrile, as illustrated

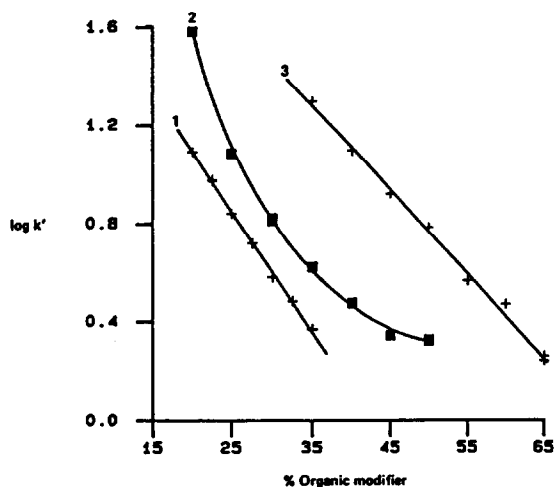


Fig. 3. Changes in the dependence of  $k'$  on  $\phi$  for different organic solvents. Solute is 4-ethylacetanilide eluted from a Spherisorb ODS-2 column. 1 THF; 2 acetonitrile; 3 methanol. From ref. 25.

TABLE 1  
ACCURACY OF EQN. 1 VS EQN. 3

Summary of data of ref. 11.

Solvent	Average correlation $r^2$ <sup>a</sup>	
	Eqn. 1	Eqn. 3
Methanol ( $n = 91$ )	$0.996 \pm 0.008$	$0.991 \pm 0.007$
Acetonitrile ( $n = 237$ )	$0.974 \pm 0.035$	$0.992 \pm 0.010$
All data ( $n = 328$ )	0.982	0.992
All data, ref. 11 ( $n = 332$ )	0.943	0.994

<sup>a</sup>  $r^2$  values are averages of values from Table I of ref. 11, with four outlying data sets excluded (Nos. 98, 108, 109 and 323; uncertainty values refer to standard deviations). The differences in values for the “all data” are due to the fitting of the entire data collection in ref. 11, whereas we simply report average values of  $r^2$ , with four outlier data-sets omitted.

in Figs. 1 and 3. Another study [8] reports values of the coefficients  $A$ – $C$  of eqn. 2 for 31 solutes and three organic solvents (methanol, acetonitrile and THF). Larger values of  $A/B$  imply greater curvature of plots of  $\log k'$  vs.  $\phi$ , and in this study average values of  $A/B$  for all solutes were: 0.27 (methanol), 0.42 (acetonitrile) and 0.44 (THF). This implies that methanol plots are less curved, and plots for acetonitrile and THF are more curved. However this is contradicted by the example of Fig. 3 and other data [23,25]; *i.e.*, plots of  $\log k'$  vs.  $\phi$  for THF as solvent are less curved. Correlation coefficients  $r$  for the application of eqn. 1 to these data were  $>0.980$  for methanol and THF and  $0.80$ – $0.99$  for acetonitrile. Most data suggest that eqn. 1 is generally a good approximation for methanol and THF as solvents, while acetonitrile generally gives somewhat curved plots of  $\log k'$  vs.  $\phi$ .

The relative curvature of plots of  $\log k'$  vs.  $\phi$  is also affected by the value of the column dead time  $t_0$  assumed for a given data set. Because there is considerable controversy over the best way of measuring  $t_0$ , and  $t_0$  also varies somewhat with  $\phi$  [24], this represents another uncertainty

in the evaluation of different equations as predictors of  $k'$  vs.  $\varphi$ .

Several other authors [26–29] have compared the applicability of various fitting equations for  $k'$  vs.  $\varphi$  in the case of a smaller number of different data sets (vs. the study of ref. 11). The results are in some cases contradictory and no compelling conclusions can be reached as to the superiority of a particular fitting equation. The data reviewed here suggest that no single equation will provide the best fit of  $k'$  vs.  $\varphi$  for all data sets (values of  $k'$  vs.  $\varphi$  for a given solute, solvent and other conditions).

### 3.2. Ternary and higher-order solvent systems

The use of mobile phases with more than one organic modifier originally stems from empirical observations of selectivity changes to improve separations. Initial work [3,30,31] demonstrated that the use of ternary systems could be used to optimize difficult separations in both isocratic and gradient elution modes. A systematic approach to optimization was proposed [4] for ternary and quaternary mobile phase systems which relied on a limited number of experiments to map and predict the retention of compounds as a function of solvent composition. Other workers proposed slightly different schemes for modeling retention [5], but all of these original works were an empirical approach to optimizing separations without fundamental basis in retention mechanism.

#### 3.2.1. Linear relationship

An extension of the linear relationship of eqn. 1 for higher-order solvents was proposed [12] and takes the general form of

$$\log k' = A + B(s_1\varphi_1 + s_2\varphi_2 + \dots) \quad (6)$$

where  $A$  and  $B$  are constants for a given solute, column and solvent system;  $s_1, s_2, \dots$  are the solvent strength values for each organic component of the mobile phase and  $\varphi_1, \varphi_2, \dots$  are the volume fractions of the organic components in the mobile phase. The value  $A$  in eqn. 6 is  $\log k_w$ , as in the simple binary solvent case of eqn. 1.

In the case of a ternary solvent system, eqn. 6 reduces to

$$\log k' = \log k_w + B(s_1\varphi_1 + s_2\varphi_2) \quad (7)$$

Since there are only two unknowns in eqn. 7 ( $k_w$  and  $B$ ), the implicit assumption is that the retention of a solute in this solvent system can be predicted by measuring the  $k'$  of that solute in two different solvent systems.

The assumption of a variation of eqn. 7 was tested with four data sets from different works [12] using non-linear least squares fitting to the experimental data. It was shown that for three of these data sets, the retention time predictions were generally within 3–8% of the measured values. The fourth data set showed much larger differences of up to 28% which were ascribed to additives in the mobile phases, such as heptane-sulfonic acid and acetic acid.

The work can be criticized because of some inconsistencies. The authors used data from three solvent systems and used the third to predict values for  $t_0$  (dead time). However, the data showed widely divergent values of  $t_0$  among the systems, and these values were often negative, which would be impossible in a real case. Also, the predictions showed some bias in direction (all predicted values greater than experimental for one system), rather than the normal scatter which would be expected. Also, the test cases used small data sets and solutes which were primarily aromatic hydrocarbons without much functionality or simple alcohols.

In fact, the work reported in ref. 12 is not consistent with the observations of others with regard to retention in ternary and higher-order solvent systems in many optimization studies. For example, Table 2 shows the  $k'$  values for nine substituted naphthalenes in six binary and ternary solvent systems [4]. In the case of 17 of the 27 solutes in ternary mobile phases, the  $k'$  values of the solute is outside the range of  $k'$  values from the corresponding binary solvent systems (examples in bold). A linear relationship of  $\log k'$  as a function of solvent composition (eqn. 6) cannot explain these observations.

#### 3.2.2. Solubility parameter model

Solubility parameter theory was described in eqn. 2 for a binary solvent system. The approach

TABLE 2

*k'* DATA FOR SIX BINARY AND TERNARY SOLVENT SYSTEMS

Data taken from ref. 4. Numbers in bold correspond to *k'* values of a solute which are outside the range of *k'* values from the corresponding binary solvent systems.

Compound No.	Solvent <i>k'</i>						
	MeOH <sup>a</sup>	MeOH, THF <sup>b</sup>	THF <sup>c</sup>	THF, ACN <sup>d</sup>	ACN <sup>e</sup>	ACN, MeOH <sup>f</sup>	MeOH, ACN, THF <sup>g</sup>
1	0.65	<b>0.74</b>	0.57	0.69	0.69	<b>0.52</b>	0.73
2	0.78	0.88	0.98	1.08	1.28	0.88	1.01
3	1.22	<b>2.55</b>	2.46	2.02	1.35	<b>1.13</b>	2.07
4	2.26	<b>2.55</b>	2.46	2.53	2.79	<b>2.25</b>	2.61
5	3.02	<b>4.60</b>	3.85	3.84	3.79	3.14	4.16
6	4.04	<b>5.56</b>	4.63	4.62	4.56	<b>3.87</b>	5.09 <sup>h</sup>
7	4.04	<b>6.00</b>	5.20	5.08	4.72	<b>3.87</b>	5.44
8	6.67	<b>9.16</b>	6.73	<b>7.05</b>	6.93	<b>6.40</b>	8.32
9	7.77	<b>10.36</b>	6.73	<b>8.09</b>	7.88	<b>7.32</b>	9.71

<sup>a</sup> MeOH–water (63:37).

<sup>b</sup> MeOH–THF–water (31.5:19.5:49).

<sup>c</sup> THF–water (39:61).

<sup>d</sup> THF–acetonitrile (ACN)–water (19.5:26:54.5).

<sup>e</sup> ACN–water (52:48).

<sup>f</sup> ACN–MeOH–water (26:31.5:42.5).

<sup>g</sup> MeOH–ACN–THF–water (31:17.3:13:38.7).

<sup>h</sup> Data corrected from misprint in ref. 4.

was extended to derive a more general relationship for ternary solvent systems between *k'* and  $\varphi$  [6]

$$\ln k' = A_1\varphi_1^2 + A_2\varphi_2^2 + B_1\varphi_1 + B_2\varphi_2 + C + D\varphi_1\varphi_2 \quad (8)$$

and the obvious expansion to quaternary solvent systems

$$\begin{aligned} \ln k' = & A_1\varphi_1^2 + A_2\varphi_2^2 + A_3\varphi_3^2 \\ & + B_1\varphi_1 + B_2\varphi_2 + B_3\varphi_3 + C + D_{12}\varphi_1\varphi_2 \\ & + D_{13}\varphi_1\varphi_3 + D_{23}\varphi_2\varphi_3 \end{aligned} \quad (9)$$

These authors presented the most thorough single study of retention in ternary solvent systems reported to date. The retention behavior of 32 solutes was studied in a methanol–THF–

water ternary system and 49 solutes in a methanol–acetonitrile–water ternary system. These data were then fit to a regression model to determine the coefficients in eqn. 8 for each solute. It is interesting to note that if a linear expression were possible for  $\ln k'$  as a function of  $\varphi_1$  and  $\varphi_2$ , then the coefficients  $A_1$  and  $A_2$  in eqn. 8 would have to be zero (or close to zero in a regression fit). This was clearly not the case for the data reported in [6] and provides further evidence that an equation such as 6 is clearly not appropriate to describe retention in a ternary solvent system.

In fact, the average deviation for all data points in this study was 12–13%; this was even larger than the 5–10% deviation reported by the same authors using the analogous eqn. 2 [8]. However, the deviations were generally <10% for *k'* values <10; larger deviations occurred for solutes with *k'* >10. This has interesting con-



sequences for practical separations and optimization as will be discussed later.

A similar equation was also derived by other authors [13] using interaction indices rather than solubility parameters, but the functional form of the retention equation was the same. These same authors concluded [32] that a simple linear interpolation of the  $k'$  values of two binary solvent systems gave equal or superior results to that using quadratic fitting and regression analysis. However, this work examined only eight solutes of similar functional type (dinitrobenzoates, benzene, and anisole) and while the linear fit was as good as other possibilities, the deviations noted were still  $\pm 8.7\%$  in  $k'$  for 32 solute predictions examined.

It appears from these systematic studies and other less extensive work that no adequate retention model has been proposed to describe  $k'$  vs.  $\varphi$  data for ternary and higher-order solvent systems. This is not surprising in view of the similar conclusion reached earlier for the more simple binary systems.

#### 4. VALUES OF $S$ (OR $Z$ ) AS A FUNCTION OF SOLUTE MOLECULAR STRUCTURE AND EXPERIMENTAL CONDITIONS

The parameter  $S$  in eqn. 1 has received considerable attention for both practical and theoretical reasons. From a practical standpoint, values of  $S$  for two adjacent bands determine selectivity as a function of a change in %B. A difference in  $S$  of as little as 2% can lead to useful changes in selectivity and sample resolution as %B is varied [33]; the ability to estimate values of  $S$  as a function of sample structure could therefore be useful in HPLC method development.

Values of  $S$  (or  $Z$ ) have also been used to gain insight into the retention process. Thus the observed correlation of  $Z$  with solute molecular size has been interpreted as favoring the stoichiometric displacement model [9,10]; similar studies [34] have used experimental values of  $Z$  to argue that more hydrophobic proteins have smaller contact areas—other factors equal. It is arguable that further information on the dependence of  $S$  on solute structure and experimental

conditions might lead to a better understanding of the reversed-phase retention process<sup>a</sup>.

Elsewhere it has been shown [34] that  $Z$  in eqn. 4 can be related to  $S$  as

$$Z = 2.3\varphi S \quad (10)$$

The following discussion of values of either  $S$  or  $Z$  will hence have a similar significance for both parameters<sup>b</sup>.

#### 4.1. $S$ ( $Z$ ) as a function of solute structure

Fig. 4 summarizes the dependence of  $S$  on alkyl carbon number  $n$  for various homologous

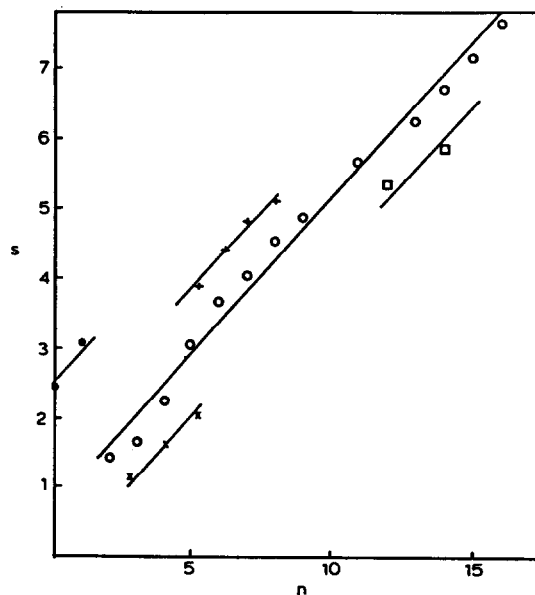


Fig. 4. Dependence of  $S$  (eqn. 1) on alkyl carbon number  $n$  for different homologous series of solutes.  $C_{18}$  column, methanol as organic solvent. ● = Alkyl benzenes; + =  $n$ -alkanes; ○ = carboxylic acids; × = dicarboxylic acids; □ =  $n$ -alkanols. From ref. 7.

<sup>a</sup> A reviewer has questioned the following discussion of values of  $S$  as a function of solute structure and experimental conditions, because the inexactness of eqn. 1 leads to a dependence of  $S$  on  $\varphi$ ; *i.e.*, it appears that values of  $S$  can only be compared for similar values of  $\varphi$ . Strictly speaking, this is correct. However our interest in values of  $S$  as a function of solute structure and separation conditions is largely satisfied by *comparisons* of  $S$  under similar conditions, including values of  $\varphi$ . The following discussion generally meets this requirement.

<sup>b</sup> When  $\varphi$  is between 0.2–0.7, eqn. 5 suggests that  $Z \approx S$ .

series of solutes ( $C_{18}$  column, methanol as organic solvent). Two generalizations can be drawn from Fig. 4: (i)  $S$  increases with  $n$  or molecular size, and (ii)  $S$  increases with increasing hydrophobicity (decreasing polarity) of the sample molecule: hydrocarbons (●, +) > carboxylic acids (○) > dicarboxylic acids (×) ≈ alcohols (□).

Since solute retention in reversed-phase HPLC also increases with increase in molecular size and hydrophobicity, this suggests that  $S$  should be greater for later-eluting solutes. Several studies confirm this behavior, which can also be expressed as

$$S = p + q \log k_w \quad (11)$$

where  $p$  and  $q$  are constant for a given study (same solutes, organic solvent, column, etc.). Table 3 summarizes the application of eqn. 11 to several data sets from the literature. Regardless of the retention process, eqn. 11 is expected to be applicable for solutes whose molecules are composed of repeating units; e.g., alkanes, aromatic hydrocarbons, synthetic oligomers, etc., and for this reason such data sets are omitted from Table 3.

The results of Table 3 (and related publications) can be summarized as follows: (i) eqn. 11 provides a good fit to data for methanol as solvent, but a less good fit for acetonitrile; data for THF are less abundant, but such data as are available suggest that THF is closer to methanol in this respect; (ii) the correlation with eqn. 11 (value of  $r$ ) improves for compounds of more similar structure; and (iii) values of  $q$  are generally (but not always) larger for acetonitrile than for methanol; i.e., values of  $S$  increase with solute hydrophobicity (as measured by  $k_w$ ) faster for acetonitrile as solvent; values of  $q$  for THF are less certain, but may be larger.

These observations can be rationalized as follows. The poorer correlation of eqn. 11 for acetonitrile as solvent may be due to the greater curvature of plots of  $\log k'$  vs.  $\varphi$  for acetonitrile (Fig. 3 and Table 1). A regular increase in  $S$  for larger values of  $k_w$  would be expected for related molecules such as homologues, benzologues or

oligomers, regardless of the retention model assumed. The increase in values of  $q$  from methanol to acetonitrile to THF parallels increases in elution strength for these solvents, and is expected if  $k_w$  for a given solute is the same for all solvents.

One study [52] for a group of substituted aromatics showed a clear trend toward larger  $S$  values (by +0.4 units in  $S$ ) for molecules with proton donor vs. acceptor substituents (buffered methanol–water as mobile phase). Another study [53] of substituted benzenes as solutes and all three organic solvents was able to correlate values of  $S$  with solute molar volume ( $V_w$ ), dipole moment ( $\tau^*$ ), basicity ( $\beta$ ) and acidity ( $\alpha$ ),

$$-S = q_1 + q_2(V_w/100) + q_3\pi^* + q_4\beta + q_5\alpha \quad (12)$$

as summarized in Table 4. These results show that solute molar volume and acidity ( $q_2, q_5$ ) are of major importance in influencing values of  $S$ ; larger, less acidic solute molecules have larger values of  $S$ . More dipolar and or basic solute molecules seem to have slightly *smaller* values of  $S$ , but only for THF as solvent. Other workers [38] claim that more polar solute molecules tend to have larger  $q$  values, implying larger values of  $S$  vs. values predicted by eqn. 11.

#### 4.2. Polymeric solutes

Values of  $S$  (or  $Z$ ) have been determined for a number of synthetic and biological polymers. The present special volume of the *Journal of Chromatography* largely excludes consideration of the reversed-phase retention of this class of solutes, in order to focus on the retention of smaller, more easily understood molecules. The retention of larger polymeric solutes is complicated by a number of factors not observed in the reversed-phase chromatography of molecules with molecular masses lower than ca. 5000. For a general discussion of this area, see refs. 9 and 54–58. One conclusion, however, is that  $S$  increases with solute molecular mass, and large solute molecules ( $M_r$  10 000–250 000) have large values of  $S$  (20–100).

TABLE 3

SUMMARY OF CORRELATIONS ( $r$ ) OF  $S$  AND  $k_w$  ACCORDING TO EQN. 11Data from the literature;  $C_{18}$  columns unless noted otherwise; temperature 20–35°C.

Sample	$r$	$p$	$q$
<i>Substituted aromatics [35]</i>			
Methanol (different $C_{18}$ columns)	0.93–0.98	0.99–1.57	0.69–0.79
Acetonitrile	0.06	–	–
THF	0.76	1.88	0.78
<i>Substituted acetanilides [25]</i>			
Methanol	0.995	1.21	0.91
Acetonitrile	0.964	1.03	1.26
THF	0.996	1.01	1.88
<i>Substituted nicotinates [36]</i>			
Methanol	0.99	1.47	0.80
<i>Hydroxyl aromatics [37]</i>			
Methanol	0.96	0.82	1.49
<i>Substituted aromatics [6]</i>			
Methanol	0.97	1.36	0.72
Acetonitrile	0.93	1.54	0.65
<i>Substituted aromatics [38]</i>			
Methanol	0.99	0.95	0.85
<i>Substituted anilines and phenols [39]</i>			
Methanol	0.90	0.37	1.46
<i>Substituted adenosine monophosphates [40]</i>			
Methanol	0.98	1.83	0.75
<i>Substituted barbiturates [41]</i>			
Acetonitrile	0.78	1.70	2.99
<i>Herbicides [42]</i>			
Methanol	0.95	1.98	0.72
<i>Pharmaceuticals [43]</i>			
Methanol	0.92	2.38	0.76
<i>Pharmaceuticals [44,45]</i>			
Acetonitrile	0.07	2.55	0.12
<i>Morphine derivatives [46]</i>			
Acetonitrile	0.18	1.48	0.34
<i>Tricyclic antidepressants [47]</i>			
Acetonitrile	0.86	0.20	0.96
<i>Benzodiazepines [48]</i>			
Acetonitrile	0.61	1.10	0.71
<i>Deoxyuridines [49]</i>			
Methanol	0.85	2.18	0.70
<i><math>\beta</math>-Lactam antibiotics [50]</i>			
Methanol	0.06	4.49	0.07
<i>Nitroaromatics [51]</i>			
Methanol	0.98	0.94	0.82
Acetonitrile	0.94	0.83	0.87

TABLE 4  
CORRELATION OF VALUES OF  $S$  WITH SOLUTE PROPERTIES (SEE EQN. 12 AND RELATED TEXT)

Solvent	$q_1$	$q_2$	$q_3$	$q_4$	$q_5$
Methanol	-1.0	-4.1	0.1	0.1	2.3
Acetonitrile	-1.1	-3.3	-0.2	0.0	2.3
THF	-0.9	-3.9	-1.1	-0.9	3.3
Solute property		Molar volume ( $V_w$ )	polarity ( $\pi^*$ )	basicity ( $\beta$ )	acidity ( $\alpha$ )

#### 4.3. $S$ as a function of pH

The primary effect of a change in pH on reversed-phase retention is the result of changes in solute ionization. A fully ionized solute is expected to be less retained, and this should result in correspondingly lower values of  $S$  for the ionized compound (eqn. 11). Neutral solutes typically show only minor changes in retention with change in pH, so that values of  $S$  should exhibit little change as pH is varied. One study [59] compared  $S$ -values for a series of neutral solutes in unbuffered and buffered (pH 2.15) methanol–water; as expected, values of  $S$  were found to be the same in both systems (average S.D.  $\pm 0.04$  units in  $S$ ). A similar study by Kaibara *et al.* [22] of neutral solutes showed very little change in  $S$  for a change in pH from 7.0 to 2.2.

Values of  $S$  as a function of pH and solute ionization have been reported for (a) substituted anilines [60] and (b) substituted benzoic acids [61]. Values of  $S$  divided by the  $S$  value for the un-ionized solute ( $S/S^0$ ) are plotted in Fig. 5 as a function of the fractional ionization ( $F^+$ ) of the solute. Data for the anilines exhibit a sharp decrease in  $S$  for progressive ionization of the molecule, while the benzoic acids show a much less pronounced decrease in  $S$  as solute ionization increases. Values of  $k_w$  for both anilines and benzoic acids typically show a decrease by about an order of magnitude for the ionized vs. un-ionized molecule, which (with the data of Table 3) suggests a decrease in  $S$  by about one unit. Values of  $S$  for the un-ionized molecules of Fig. 5 average about 3, so the predicted change in

( $S/S^0$ ) for Fig. 5 would be about 25% for  $F^+ = 0.8$ . The actual values are about 20% for the benzoic acids and 80% for the anilines. Therefore the benzoic acids appear to behave as expected, while the anilines show a much greater than expected decrease in  $S$  for the ionized compounds. Possibly this reflects the importance of silanol effects in the retention of the ionized anilines, with the involvement of ion exchange as well as hydrophobic interaction of solute with the stationary phase; see the discussion of ref. 15 and the related data of ref. 62. Alternatively, changes in pH and  $pK_a$  with  $\varphi$  may also be a factor in the trends of Fig. 5.

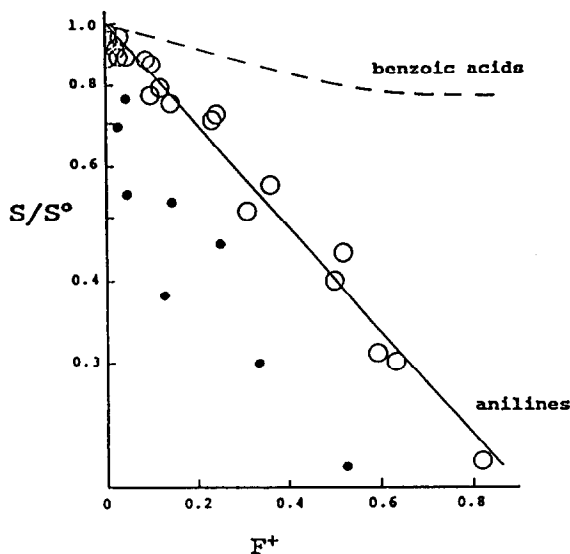


Fig. 5. Dependence of values of  $S$  on fractional ionization ( $F^+$ ) of the solute molecule (pH varied).  $\circ$ , — = Substituted anilines ( $\bullet$  refer to approximate values); --- = substituted benzoic acids.  $C_8$  column, 35°C. From ref. 61.

TABLE 5  
SUMMARY OF EFFECTS OF TEMPERATURE ON VALUES OF  $S$ ;  $C_8$  OR  $C_{18}$  COLUMNS

Sample	Solvent	Change in $S$ per °C increase
Alkyl phthalates [24]	Acetonitrile	–0.10%
Substituted benzenes [63]	Methanol	–0.49%
Phenylthiohydantoin-amino acids [64]	Methanol <sup>a</sup>	No change
Nitroaromatics [51]	Methanol	–0.63%
	Acetonitrile	–0.26%
Hydrocarbons [53]	Methanol	–0.38%

<sup>a</sup> Cyano column.

#### 4.4. $S$ as a function of temperature

Several studies have been reported for the effect of temperature on values of  $S$ . Table 5 summarizes these results. In general there is a small decrease in  $S$  with increasing temperature, but no obvious trends in this effect as a function of solute structure or solvent. Any change in  $S$  with temperature is usually quite regular; one study [59] suggests that the quantity  $ST_K$  is roughly constant as temperature ( $T_k$ , K) is varied. Since values of  $k'$  (and  $k_w$ ) decrease with temperature, this decrease in  $S$  is expected (eqn. 11).

### 5. VALUES OF $S$ (OR $Z$ ) AS A FUNCTION OF THE COLUMN

#### 5.1. $S$ as a function of column source and bonded-phase concentration

Several studies have compared values of  $S$  for substituted aromatics and  $C_8$  or  $C_{18}$  columns from different manufacturers [7,38,59]. Generally these data show little change in  $S$  (same solute, same solvent) from column to column. Comparisons of values of  $S$  for the same solute and solvent between *different* laboratories, however, often show considerable variation in values of  $S$ . Possibly this reflects a combination of experimental error, failure of eqn. 1, errors in the determination of  $t_0$  or special effects (*e.g.*, silanols).

Changes in the concentration of the bonded phase by a factor of more than two did not result in significant changes in values of  $S$  for several aliphatic and aromatic solutes (methanol solvent) [59]. A change in surface area by a factor of 2.5-fold had no effect on  $S$  values for phthalates and acetonitrile as solvent (24).

#### 5.2. $S$ as a function of bonded-phase type

Unless stated otherwise, all studies discussed in this paper are based on separations with  $C_8$  or  $C_{18}$  bonded-silica columns. Various studies have been carried out where values of  $S$  are compared as a function of the bonded-phase composition (other factors the same). Shi and Geng [65] reported values of  $Z$  (methanol solvent) for the same solutes on a  $C_1$ ,  $C_8$  and  $C_{18}$  column. Values of  $Z$  for less retained solutes decreased in the order  $C_{18} > C_8 > C_1$ , but  $Z$  was virtually the same for more strongly retained solutes. This may involve a failure of eqn. 4, since this relationship tends to give smaller values of  $Z$  for lower values of  $\phi$ , corresponding to the elution of less retained solutes from the weaker  $C_1$  column.

Several studies [66–68] have been reported which compare  $S$  values for columns of different functionality:  $C_{18}$ ,  $C_1$ , phenyl and/or cyano; see Table 6. There is a consistent trend with  $S$  decreasing slightly with decreasing column “strength” [84] or stationary phase polarity:  $S(C_{18}) > S(C_1) \approx S(\text{phenyl}) > S(\text{cyano})$ .

TABLE 6  
EFFECT OF BONDED-PHASE TYPE ON VALUES OF  $S$

Sample	Solvent	Average value of $S$			
		C <sub>18</sub>	C <sub>1</sub>	Phenyl	Cyano
<i>n</i> -Alkanes [66]	ACN	6.4			5.6
Glycosides [67]	ACN	3.2			2.6
Benzodiazepines [68]	MeOH		4.0	4.0	
Mixed solutes <sup>a</sup> [22]	MeOH	3.3	2.9	2.8	

<sup>a</sup> Values of  $Z$  (not  $S$ ).

## 6. DISCUSSION AND CONCLUSIONS

Our primary goal in this review is to summarize existing data for  $k'$  vs.  $\varphi$  as a function of solute structure, column type or other experimental conditions and to develop general conclusions that can be applied to certain questions discussed below.

### 6.1. The retention process

Previous workers [8–11,20,21,26–29,59] have attempted to show that experimental  $k'$ - $\varphi$  data sets are in agreement with various models of RP-LC retention or have interpreted such data in terms of these models. In this paper we have shown that comparisons of eqns. 1–5 as fitting equations for experimental  $k'$ - $\varphi$  data cannot result in a clear choice between the two main models of reversed-phase retention: (a) partitioning between the two phases as described by eqns. 2 or 3 and (b) stoichiometric displacement (eqn. 5). Thus there is no preferred equation that accurately describes  $k'$  vs.  $\varphi$  data from different studies (see Figs. 1 and 3, Table 1 and related discussion). Likewise, the extension of these simple models to ternary and higher-order solvent systems appears to oversimplify the actual retention behavior. It can be argued—in view of the considerable complexity of these RP-LC systems at the molecular level—that any of the retention models under consideration may be compatible with these experimental data.

The dependence of the parameter  $S$  (or  $Z$ ) on solute structure, column and experimental con-

ditions can be summarized as in Table 7. Both displacement and partition processes predict the observed increase in  $S$  for larger solute molecules. The lesser retention of more polar compounds—among molecules of similar size—seems to contradict a simple mass action effect as the sole determinant of the dependence of  $k'$  on  $\varphi$  (as was proposed in the original stoichiometric displacement model [9,10]). However it can also be argued that the presence of a polar functional group in the solute molecule might lead to exclusion of the more polar part of the solute molecule from the stationary phase—and a predicted decrease in  $Z$  or  $S$ .

Possible relevant to the present question is the dependence of  $S$  on bonded-phase concentration and alkyl-chain length:  $S$  does not depend on the former and decreases for shorter alkyl chains. However this decrease is rather modest and seems to be determined primarily by the greater polarity of a C<sub>1</sub> vs. C<sub>18</sub> phase. Concerning a distinction between displacement and partition retention processes, the absolute value of  $k'$  as a function of bonded-phase concentration and alkyl-chain length is probably of greater significance [69].

### 6.2. Estimates of $\log P_{o/w}$

The best equation for the extrapolation of  $k'$ - $\varphi$  data to  $\varphi = 0$  (for predictions of  $\log P_{o/w}$  values) is uncertain. While a particular equation may accurately describe an actual experimental data set over some range in  $\varphi$ , it is less obvious

TABLE 7

SUMMARY OF THE DEPENDENCE OF  $S$  (OR  $Z$ ) ON SOLUTE STRUCTURE, COLUMN AND EXPERIMENTAL CONDITIONS

Variable	Comment
Solute structure	(1) $S$ increases with solute retention or hydrophobicity (eqn. 11 and Table 3), and therefore generally with molecular size. Exceptions have been noted, usually for acetonitrile as solvent, but these may be due to the failure of eqn. 1 to apply to some RP-LC systems that involve acetonitrile. (2) $S$ is relatively greater for proton donor solutes (Table 4). (3) $S$ decreases for ionized solutes (as expected), but $S$ values for protonated bases are unexpectedly small. This is believed due to the ion-exchange retention of these compounds on ionized silanols.
Temperature	(1) Values of $S$ generally decrease with increasing temperature, by about 0.5% per °C for methanol as solvent. This effect is generally smaller (0.2%/°C) for acetonitrile, but the available data are too limited to say that this difference in solvents is really significant.
Column	(1) Values of $S$ do not appear to depend on the way in which the bonded phase is applied to the silica matrix. Values of $S$ are also unaffected by (a) the concentration of the bonded phase or (b) the surface area of the silica matrix. (2) Values of $S$ decrease (moderately) as column polarity increases (Table 6). Thus $S$ decreases in the sequence: $S(C_{18}) \approx S(C_8) > S(C_1) \approx S(\text{phenyl}) > S(\text{cyano})$ .

that the same equation will prove reliable for extrapolation. This is further discussed in ref. 1.

Some idea of the reliability of extrapolated  $k_w$  values can be obtained from studies where  $k'$  vs.  $\varphi$  data are provided for the same solutes and different solvents; values of  $k_w$  for a given solute should be the same, regardless of the solvent used—if the extrapolation procedure is reliable. Fig. 6 illustrates such a comparison of  $\log k_w$  values obtained for methanol and acetonitrile as solvents. Table 8 summarizes three such studies from the literature.

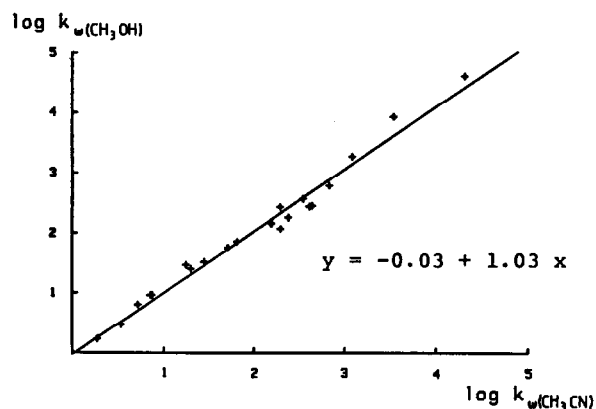


Fig. 6. Comparison of extrapolated values of  $\log k_w$  for methanol ( $j$ ) vs. acetonitrile ( $k$ ) as solvent (substituted nicotines); curve through data points is least-squares fit to  $\log(k_w)_j$  vs.  $\log(k_w)_k$ . From ref. 36.

For the case of the substituted nicotines (ref. 36 of Table 8), an excellent agreement is found between the two sets of  $\log k_w$  values for methanol vs. acetonitrile (slope  $b \approx 1$ ,  $a \approx 0$ ; see Fig. 6). In this case, eqn. 1 was used for the methanol data, and eqn. 2 was used for the acetonitrile data (which exhibited severe curvature of  $\log k'$  vs.  $\varphi$  plots). The substituted calvatic acids of Table 8 show a good correlation of  $\log k_w$  values for methanol vs. acetonitrile as solvents, but a somewhat poorer agreement ( $b = 1.28$ ). In this case, eqn. 1 was used for both solvents, despite some curvature in the plots of  $\log k'$  vs.  $\varphi$ . This suggests that the choice of eqn. 1 or 2 for the purposes of obtaining extrapolated values of  $k_w$  should be made on the basis of which equation gives the best fit to a particular data set.

The substituted aromatics of Table 8 show poorer correlation and agreement of  $\log k_w$  among these different solvents, despite the use of eqn. 2 for these extrapolations. Possibly this is due to (a) solutes of more varied structure and (b) extrapolation over a greater range of  $\varphi$ . Dorsey, Michels and Hsieh [70–72] have presented experimental data which suggest that the use of eqn. 3 is preferable to eqn. 1 in terms of yielding accurate (or consistent) values of  $\log k_w$  for different solvents (methanol, ethanol and

TABLE 8

COMPARISON OF VALUES OF  $\log k_w$  OBTAINED BY EXTRAPOLATION AS A FUNCTION OF  $\varphi$  WITH DIFFERENT ORGANIC SOLVENTS SEE TEXT

Sample	Solvent <sup>a</sup>	$\log (k_w)_j = a + b \log (k_w)_k$			
		a	b	r <sup>2</sup>	± S.D.
Substituted nicotines [36] <sup>b</sup>	MeOH/ACN	-0.03	1.03	0.98	± 0.15
Substituted calvatic acids [81] <sup>c</sup>	MeOH/ACN	-0.16	1.28	0.96	± 0.16
Substituted aromatics [35] <sup>d</sup>	MeOH/ACN	-4.0	2.0	0.75	± 0.72
	MeOH/THF	-6.3	2.4	0.46	± 1.12

<sup>a</sup> Solvents are listed as  $j/k$ ; data for solvent  $j$  are correlated with data for solvent  $k$  as shown.

<sup>b</sup> Eqn. 1 used for MeOH data, eqn. 2 for ACN data.

<sup>c</sup> Eqn. 1 used for both sets of data.

<sup>d</sup> Eqn. 2 used for both sets of data; data for anisole, benzophenone, biphenyl, naphthalene and quinoline omitted from correlation (large deviations and/or missing data).

acetonitrile—but not propanol). Similarly Jandera and Kubat [73] have put forth similar claims for the use of “lipophilic indices” in place of values of  $\varphi$ .

An interesting consequence of eqn. 11 for the purposes of predicting values of  $P_{o/w}$  from RP-LC data has to our knowledge not previously been pointed out. If this relationship is valid, then for any two solutes ( $i$  and  $j$ ) eqn. 11 will be obeyed, and it can be shown that the two plots of  $\log k'$  vs.  $\varphi$  will intersect at a value of  $\varphi = \varphi_i = 1/q$ . Since  $\varphi_i$  does not depend on the value of  $k_w$ , this means that all plots of  $\log k_i$  vs.  $\varphi$  will intersect at  $\varphi = \varphi_i$ . The corresponding value of  $k'$  at this intersection point is then given by  $\log k_i = -p/q$ . Fig. 7a illustrates a family of  $\log k' - \varphi$  plots for solutes where eqn. 11 is obeyed. Now values of  $\log k'$  for these solutes at any value of  $\varphi$  will be linearly related to values of  $\log k'$  at any other value of  $\varphi$ , including  $\varphi = 0$ . This means that a plot of values of  $\log k_w$  vs.  $\log k'$  at any value of  $\varphi$  (when  $\varphi < \varphi_i$ ) will then give a linear plot. Since it is assumed that  $\log P_{o/w}$  is linear in  $\log k_w$ , this means that a plot of  $\log P_{o/w}$  vs.  $\log k'$  (any value of  $\varphi$ ) should also be linear, as illustrated in Fig. 7b. This then represents a much simpler alternative to the extrapolation of  $k'$  to  $\varphi = 0$ , as discussed further in ref. 1.

For methanol as solvent (for which eqn. 11 is

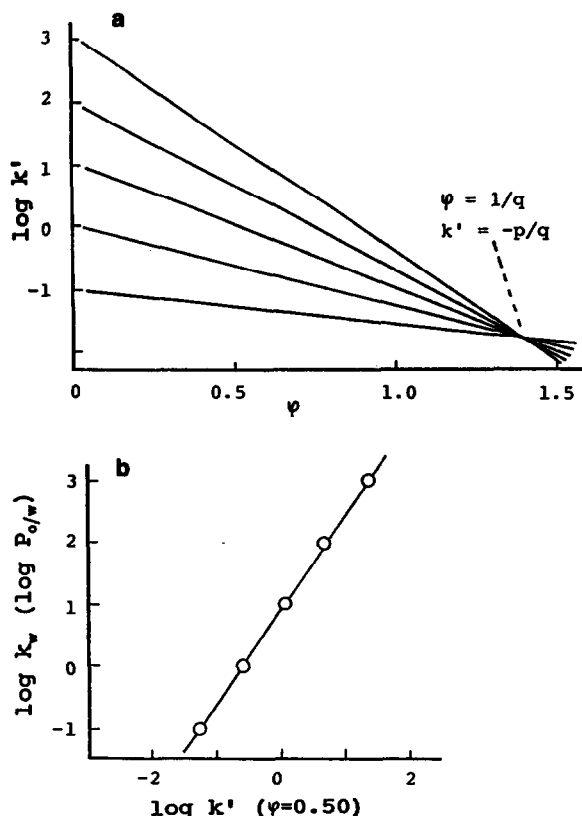


Fig. 7. Consequences of the applicability of eqn. 12. (a) Hypothetical plots of  $\log k'$  vs.  $\varphi$  for a system where eqn. 12 is obeyed; (b) resulting plots of  $\log k_w$  (or  $\log P_{o/w}$ ) vs.  $\log k'$  for any value of  $\varphi < \varphi_i$ .



most reliable), most values of  $\varphi_i$  and  $k_w$  from Table 3 fall in the range  $0.7 < \varphi_i < 1.4$  and  $-1 < \log k_i < -3$ . Chen *et al.* [74] have recently shown that eqn. 11 can be derived rigorously for homologues, benzologues, oligomers, etc. where the solutes under consideration differ by the number of some repeating unit.

### 6.3. Optimizing separation

Several computer programs are now available for predicting separation as a function of  $\varphi$  (%B) in a binary solvent system (other conditions constant). Usually eqn. 1 or 2 is used as the basis for these predictions, beginning with two or three experimental runs at different values of  $\varphi$  [75,76]. The use of eqn. 1 with two experimental runs leads to small errors for interpolation of values of  $\varphi$  and larger errors for extrapolation [77]. Interpolative errors increase as the difference in  $\varphi$  for the two experimental runs becomes larger, while extrapolative errors become smaller. Interpolated predictions for a wider range in  $\varphi$  can be made more accurate by the use of eqn. 2 and three experimental runs, *but this appears not to be the case for extrapolation*. Small errors in the experimental data used to define the coefficients of eqn. 2 or small deviations of experimental data from this model can lead (in our experience) to larger errors when extrapolation is used. As seen in Fig. 1a and c, curved plots of  $\log k'$  vs.  $\varphi$  are more likely when  $\varphi$  is either small ( $<0.3$ ) or large ( $>0.7$ ), and for these mobile phase compositions the use of eqn. 2 without extrapolation is recommended for predictions of  $k'$  as a function of  $\varphi$ .

One of the main advantages in varying  $\varphi$  for optimized separation is to take advantage of resulting changes in band spacing as a result of differences in  $S$  for adjacent solute bands [2]. The strict applicability of eqn. 11 would mean that values of  $S$  are always similar for compounds that have similar values of  $k'$ , so that the more closely eqn. 11 describes the retention of a given sample as a function of  $\varphi$ , the less likely are changes in  $\varphi$  to lead to changes in band spacing and improved separation. This would imply that acetonitrile as solvent is more likely to result in changes in band spacing as  $\varphi$  is varied,

since eqn. 11 is a poorer fitting equation for RP-LC separations with this solvent. In fact, available evidence [2] does not agree with this conclusion. It appears that eqn. 11 is sufficiently imprecise for all three commonly used solvents (methanol, acetonitrile, THF) to provide significant changes in band spacing as  $\varphi$  is varied.

Another aspect of optimizing a separation comes from the use of more than one organic modifier to change selectivity of adjacent solute bands. In theory, a predictable change of modifiers is more complicated than simply changing  $\varphi$  to achieve band spacing, since there are now more than two solvent variables to properly fit or model. This can be seen by analyzing the following version of the resolution equation:

$$R_s = N^{1/2} \frac{(k_2 - k_1)}{2(k_1 + k_2 + 2)} \quad (13)$$

where  $N$  is the column plate number and  $k_1$  and  $k_2$  are the  $k'$  values of solutes of interest. The effect on resolution due to uncertainty in the prediction of  $k'$  can be seen by examining the data in Table 9. The examples in this table assume a column plate count of 10 000. If  $k'_1$  is known, a 1% difference in the value of  $k'_2$  results in a  $R_s$  difference ( $\Delta R_s$ ) of 0.16 and 0.22, respectively, for  $k'_1$  values of 2.0 and 10.0. Since a real example would require prediction of both  $k'$  values, the actual uncertainty in resolution would be greater than these by a factor of  $2^{1/2}$ , or  $R_s$  difference values of 0.23 and 0.31, respectively. This level of precision of  $R_s$  prediction is approximately what is desired for practical optimization; this implies that an ability to predict  $k'$  to within 1% is necessary.

TABLE 9  
RESOLUTION AS A FUNCTION OF  $k'$

$k'_1$	$k'_2$	$R_s$	$\Delta R_s^a$
2.00	2.12	0.98	
2.00	2.14	1.14	0.16
10.00	10.45	1.00	
10.00	10.55	1.22	0.22

<sup>a</sup> Due to 1% error in predicted value of  $k'_2$ .

This issue of  $k'$  prediction was examined in some detail in ref. 78 where the authors sought to determine the effect of retention model inaccuracy on selectivity optimization procedures in reversed-phase systems. Their conclusion was also that the model needed to predict retention to within 1% or less to adequately predict optimal conditions. While the authors' own extensive data seem to support this conclusion, one of the prime examples was unfortunately based on a misprinted retention value from an earlier optimization study (see footnote in Table 2 of this paper). In fact, the predicted and actual resolution for the quaternary mobile phase No. 7 and the optimum mobile phase in this study [4] were quite close (within a few percent for  $R_s$  values). This occurred despite the fact that the actual  $k'$  values were actually greater than predicted in many cases (see Fig. 8 of ref. 78, for example).

A similar situation can be seen in the optimization of 12 sulphonamides in ref. 5. The prediction of  $t_R$  (and corresponding  $k'$  values) varied from 0.4–18.4% for the 12 solutes. Despite this, however, the experimental optimum separation gave a resolution of almost exactly 1.8, as predicted by the fitting equation.

One of us has also studied this for a series of 12 substituted anilines using ternary mixtures of methanol, acetonitrile, tetrahydrofuran and water [79] and a 3-point fit (eqn. 8). The % error in  $t_R$  predictions for these data were as follows:

Mobile phases	% error in $t_R$	% error in $R_s$
MeOH-ACN	±1.2%	±8%
ACN-THF	±2.1%	±12%
MeOH-THF	±3.6%	±37%

These errors in resolution prediction are larger than would be desired, especially for the MeOH-THF case, but still not approaching those which could be predicted by a strict interpretation of eqn. 13.

The reasons for the better apparent  $R_s$  predictions described above are not well understood, but they may relate to a few factors in measurement and prediction. The resolution of two adjacent solutes will only be negatively impacted by prediction errors if those errors are in opposite directions (and thus the peaks are actually

more poorly resolved than would be predicted). In many cases, such as the one in ref. 5 above, the prediction of retention time was inaccurate, but critical adjacent peaks were both inaccurate in the same direction. In effect, the uncertainties in prediction canceled out and the resolution was still sufficient for separation. In many cases, if retention uncertainties occur for widely separated solutes, the practical effect on the separation is negligible. Finally, with the possible exception of pH changes, most retention behavior in reversed-phase HPLC is fairly regular, particularly in small regions of solvent change. Therefore, it is unlikely that major uncertainties in resolution will occur if enough data are taken to properly describe retention in a given region. However, it is still desirable to more accurately model and predict solute retention to maximize the chance of obtaining the best separation in the most efficient manner.

It would be desirable to be able to predict when a change in  $\phi$  is likely to result in changes in band spacing for two compounds of known structure, and whether one solvent will be preferable to another for this purpose. This question is outside the scope of the present paper; see ref. 80 for further discussion.

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