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Application of solute and mobile phase partition coefficient to describe solute retention in reversed-phase high-performance liquid chromatography

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ABSTRACT

The calculated partition coefficient, log P_{sm} (logarithm of partition coefficient of solvent mixture), of aqueous mobile phases containing either methanol, acetonitrile or tetrahydrofuran, may be used as a quantitative parameter for solvent strength instead of the commonly used term, Φ (volume fraction of solvent in water). This has permitted the development of a new relationship that expresses the retention behaviour of non-ionised solutes based solely on the hydrophobic parameter of the solute (log P) and the mobile phase (log P_{sm}). This statistically derived relationship has not only given a highly significant fit to the data, but also provided regression coefficients that were consistent for different, but related chromatographic (reversed-phase liquid chromatography) systems. One unique advantage offered by this approach is that it allows all retention data, for all solutes analysed under different solvent systems, to be combined in a single expression. It is suggested that this expression could be used to determine the initial solvent conditions in the optimisation of chromatographic mobile phases or as a general model for quantitative structure–retention relationship.

INTRODUCTION

The prediction of the conditions required in reversed-phase high-performance liquid chromatography (RP-HPLC) for the adequate resolution of a particular group of analytes is still a difficult task for most chromatographers. The most commonly used 'trial-and-error' or systematic but empirical approach may be used to arrive at the optimum conditions so that adequate resolution is achieved with reasonable analysis time. Ideally, it should be possible to calculate the retention parameter of a given analyte in the chosen column–mobile phase system from the physico-chemical properties of the analyte, mobile phase and stationary phase. In practice, this has not yet been achieved although much progress is being made through the development of systematic methods. These approaches include the use of one or more gradient run(s) in order to select an optimum isocratic composition [1–3] or an iterative method [4]

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and automated sequential simplex approach [5,6]. These methods do not require *a priori* knowledge of the physico-chemical properties of the analytes.

Other workers have approached the problem by considering the physico-chemical properties of the analytes and using semi-deterministic or semi-empirical techniques, generally known as quantitative structure–retention relationship (QSRR) [7–12]. This type of modeling is commonly based upon the principles of linear free energy relationships, as used in quantitative structure–activity relationships (QSAR) in drug design. Commonly used parameters for the QSRR studies are: (1) hydrophobic parameter, π [9,12], (2) partition coefficient, P [9–11], (3) correlation factor, F [11,12], (4) molecular connectivity index, χ [10–12], (5) length over breadth shape parameter of a molecule, L/B [11] and (6) Van der Waals volume, V_W [10–12].

In an earlier study [13] we had shown that the logarithm of partition coefficient (1-octanol/water) of a solvent, $\log P_s$, could be used as a "solvent strength" parameter in RP-HPLC. Strength of a solvent refers to its eluotropic potential. Two solvents are iso-eluotropic if they elute a given solute with same elution volume (V_R) under identical chromatographic conditions.

In view of the well understood influence of hydrophobicity in RP-HPLC, the aim of this study was to examine whether the partition coefficients of the analytes as well as the mobile phase could be used to describe the retention behaviour of non-ionised analytes under those conditions.

THEORETICAL

Many physico-chemical parameters of a solute influence its retention in RP-HPLC, and some may be estimated from the retention parameter, k' [14]. However, it has been suggested that in RP-HPLC the interaction between solute and hydrocarbonaceous stationary phase is the major factor for the retention in absence of organic modifiers [15], *i.e.*, the process is governed by the so-called hydrophobic effect in which the cohesive density of water is responsible [16]. This theory has also been employed to describe the effect of solvents on certain chemical events [17,18] and does not restrict a solvophobic effect to aqueous media. Additionally, it has been observed that water is not a unique solvent in terms of its chromatographic property [19] and that the hydrophobic selectivity of different solvents is approximately independent of their chemical nature [20]. In other words, the total solvophobic force of solvent mixture is governing the solvation of molecule. Therefore, it is apparent that in the ion-suppressed mode of RP-HPLC, the solvophobic effect could be considered to be the most influential parameter.

The retention of solutes in RP-HPLC has been described by eqn. 1 [14,21].

$$\ln k' = a' + b' \log P \tag{1}$$

$$\ln k' = a + b\varphi + c\varphi^2 \tag{2}$$

where k' = peak capacity factor, $\log P$ = logarithm of octanol/water partition coefficient, φ = volume fraction of the organic modifier in mobile phase and a', b', a, b and c are system-dependent coefficients. These equations are applicable under certain conditions only, for example retention data of a given analyte for a given column analysed under *different* solvents may not be combined to obtain a single expression such as eqns. 1 and 2. Furthermore, a general expression is not available to describe the retention behaviour of a variety of solutes analysed employing different mixed solvents of different compositions in isocratic mode. The major difficulty for obtaining such a correlation lies in accurately defining the "solvent strength" of the mobile phase containing a mixture of solvents from chemically different classes.

A variety of approaches have been suggested for calculating solvent strength parameters. In our previous study [13] we have shown that partition coefficient of a solvent (log P_s, subscript "s" indicates that log P value refers to the solvent and not analyte) can be used as a "strength" parameter. In this study a number of currently available parameters were compared with log P_s. Solvent strength parameters, *viz.*, P' [22], S [23], δ_T [24] and log P_s [13], are listed in Table I and a comparison of the accuracy of prediction of solvent strength of mixed solvents using these parameters is given in Table II. It is apparent that parameter log P_s is able to predict solvent strength more accurately as compared to others for the experimental data under consideration. Therefore the log P_s parameter was employed to derive an expression (eqn. 3) for calculating the strength of a mixed solvent system, which is given below:

$$\log P_{sm} = \sum_{i}^{n} x_i \log P_{s,i} \qquad (3)$$

where, m indicates that the parameter is for solvent mixture, x_i is the mole fraction of the ith solvent component, log $P_{s,i}$ is logarithm of the partition coefficient of the ith solvent and n is the total number of pure solvents present in the solvent mixture. Therefore, according to eqn. 3, the eluotropic strength of a solvent mixture is a sum of the partition coefficient times the mole fraction of each solvent. Solvent strengths of water–methanol, water–acetonitrile and water–tetrahydrofuran were calculated and compared with experimental data as well as another study [21]. The experimental and predicted values compared very well [13]. It was found that the use of log P_{sm} as a strength parameter resulted in better predictions as compared to other parameters and approaches [13]. The major advantage of this method of strength parameter

TABLE I

STRENGTH PARAMETERS FOR SOME HPLC SOLVENTS

P' = polarity index [23]; S = solvent strength [24]; δ_T = total solubility [25]; log P_s = *n*-octanol/water log partition coefficients obtained from ref. 22.

Solvent	P'	S	δ_T	log P_s
Water	10.2	0.0	25.52	−1.38
Methanol	5.1	3.0	15.85	−0.82
Acetonitrile	5.8	3.1	13.15	−0.34
Ethanol	4.3	3.6	13.65	−0.31
Dioxane	4.8	3.5	10.65	−0.27
Acetone	5.1	3.4	10.51	−0.24
Propan-2-ol	3.9	4.2	12.37	+0.30
Tetrahydrofuran	4.0	4.4	9.88	+0.46

TABLE II

COMPARISON OF ACCURACY OF SOLVENT STRENGTH PREDICTIONS BY DIFFERENT PARAMETERS

Parameter	S.D.[a]	r^2 (%)[a]
S	5.938	66.1
P'	5.885	66.7
φ_T	4.997	76.0
Empirical[b]	4.572	79.9
log P_s	4.517	80.4

[a] Standard deviation (root mean square of the fit) and coefficients of determination (adjusted to degree of freedom) between experimental and predicted solvent compositions (% $\text{solvent}_{\text{exp.}} = c + m \cdot$ % $\text{solvent}_{\text{pred.}}$) [13].

[b] Empirical transfer equations of Schoenmaker *et al.* [3].

calculation is that a single quantitative value can be readily calculated for the solvent mixtures commonly employed in RP-HPLC. Our interest was then to see whether this parameter could be used as strength parameter instead of the φ term in eqn. 2. For the solvents water, methanol, acetonitrile and tetrahydrofuran it was found that a highly significant relationship existed between the volume fraction of solvent (φ) and reciprocal of computed solvent partition coefficient ($1/P_{\text{sm}}$) as compared to x_i and $1/P_{\text{sm}}$. Statistical analysis for the relationship between φ and $1/P_{\text{sm}}$ (eqn. 4) is presented in Table III.

$$\varphi = A' + B' \frac{1}{P_{\text{sm}}} \tag{4}$$

where A' and B' are regression coefficients. It is apparent from Table III that φ and $1/P_{\text{sm}}$ are highly correlated ($r^2 \geqslant 99.6\%$). This correlation suggests that the volume

TABLE III

STATISTICS FOR THE RELATIONSHIP BETWEEN φ AND $1/P_{\text{sm}}$ (SEE EQN. 4)

φ values were selected in the range of 0–1 with an interval of 0.01 and the corresponding P_{sm} values were back-calculated using eqn. 3.

Solvent	A	B	n[a]	r^2 (%)[b]	s[c]	F[d]
Methanol	1.40	−0.0569	101	0.998	0.015	42 816
Acetonitrile	1.07	−0.0439	101	0.999	0.005	>51 069
Tetrahydrofuran	0.92	−0.0378	101	0.999	0.066	>37 714

[a] Number of selected solvent compositions.

[b] Coefficient of determination.

[c] Standard deviation (root mean square of fit).

[d] F-ratio [28].

fraction term (φ) can be substituted by a solvent strength term ($1/P_{sm}$) in eqn. 2 to provide eqn. 5.

$$\ln k' = A'' + B'' \frac{1}{P_{sm}} + C'' \frac{1}{P_{sm}^2} \tag{5}$$

where A'' and B'' and C'' are regression coefficients. This equation has been examined for a relationship with eqn. 1 in order to express the well known dependence of solute retention in RP-HPLC upon solute log P and mobile phase strength. A number of equations (derived from eqns. 1 and 2) were generated and subjected to statistical analysis using two sets of experimental data from the literature (data sets 1 and 2) and two sets of experimental data (data sets 3 and 4) from our laboratory.

EXPERIMENTAL

Data set 1

Hypersil silica (Shandon, Cheshire, UK) was chemically bonded in house to form an *n*-octyl end-capped material and packed into a 150 mm × 4.6 mm I.D. column. It was used at 30°C to analyse sixteen non-ionic polar substances and non-polar aromatic compounds employing methanol–water (50:50, v/v), acetonitrile–water (30:70, v/v) and tetrahydrofuran–water (25:75, v/v) by Tanaka *et al.* [25]. The compounds were benzene, toluene, ethylbenzene, benzamide, benzyl alcohol, phenol, benzaldehyde, benzonitrile, 2-phenylethanol, phenyl methyl lactone, nitrobenzene, methoxybenzene, methyl benzoate, chlorobenzene, ethyl benzoate and isopropyl benzoate.

Data set 2

A 300 × 4.6 mm I.D. column was packed with Merck RP-18 material (Merck, Darmstadt, Germany) and used at 25°C and a flow-rate of 1.5 ml/min^{-1}, with 32 non-ionic aromatic compounds in aqueous systems containing 10–90% of methanol, acetonitrile or tetrahydrofuran as organic modifiers under isocratic conditions, by Schoenmakers *et al.* [26]. The analytes were acetophenone, anethole, aniline, anisole, anthracene, benzaldehyde, benzene, benzonitrile, benzophenone, benzyl alcohol, biphenyl, chlorobenzene, *o*-cresol, diethyl *o*-phthalate, N,N-dimethylaniline, 2,4-dimethylphenol, dimethyl *o*-phthalate, *m*-dinitrobenzene, diphenyl ether, ethylbenzene, N-methylaniline, naphthalene, *p*-nitroacetophenone, *o*-nitroaniline, nitrobenzene, *m*-nitrophenol, phenol, 1-phenylethanol, 2-phenylethanol, 3-phenylpropanol, quinolone and toluene.

Data sets 3 and 4

Equipments. Chromatographic studies were performed using either a Spectra-Physics Model SP8100 liquid chromatograph with Model SP8440 UV–VIS detector and Model SP4200 computing integrator (Spectra-Physics, St. Albans, UK) or a modular system assembled from a Constametric III pump (Milton Roy, Stone, UK), Rheodyne 7125 injection valve, and a UV–LC detector (Pye-Unicam, UK). Methanol, acetonitrile and tetrahydrofuran were HPLC grade (Fisons, UK) and HPLC-grade water was obtained from a Milli-Q water system (Millipore, Harrow, UK).

Data set 3. A 50 × 4.6 mm I.D. column was packed with 3 μm Hypersil-ODS (Shandon) and used at a flow-rate of 1.0 ml/min^{-1} and a temperature of 40°C, with UV detection at 254 nm. For the methanol–water mixture five compositions were used, *viz.*, 28, 42, 58, 74 and 90% (v/v) methanol. The five iso-eluotropic equivalent mobile phases containing acetonitrile and tetrahydrofuran were calculated [13] and used for analysis under the same conditions. Five non-ionisable solutes were chosen in order to give a range of log P values (log P values for solutes of data sets 1–4 were obtained from ref. 27, namely, benzyl alcohol (log P = 1.10), benzonitrile (1.56), benzene (2.15), chlorobenzene (2.84) and benzophenone (3.58). These solutes were dissolved in 25% methanol and injected individually, in triplicate.

Data set 4. A 150 × 4.6 mm I.D. column was packed with 6-μm Zorbax-ODS (Du Pont) and used at a flow-rate of 1 ml/min^{-1} and a temperature of 35°C, with UV detection at 254 nm. For the methanol–25 mM buffer (phosphate, pH 6.9) mixtures, three compositions were used, namely 50, 60 and 70% (v/v) of methanol, and their isoeluotropic equivalent solvents containing acetonitrile and tetrahydrofuran. The five related non-ionisable solutes were benzonitrile (log P = 1.56), benzene (2.15), toluene (2.72), naphthalene (3.2) and biphenyl (4.0), dissolved in 50% methanol and injected individually, in triplicate.

Data processing and computing was done using statistical software (MINITAB®) with Honewell 68 DPS Level 2 via RJE or GEC 63/40 computers.

RESULTS AND DISCUSSION

A number of relevant equations were statistically examined that incorporated relationships expressed by eqns. 1 and 5, using the four sets of data described in Experimental. In order to select the equation that was best able to describe the data, we compared F-ratio (weighted according to number of experimental data points), correlation coefficients (r) and standard deviation (s) about the regression line. On this basis, two equations were considered to be most suitable. These equations are given below.

$$\ln k' = A + B\frac{\log P}{P_{\mathrm{sm}}} + C\frac{1}{P_{\mathrm{sm}}^2} \tag{6}$$

$$\ln k' = A^0 + B^0\frac{\log P}{P_{\mathrm{sm}}} + C^0\frac{\log P}{P_{\mathrm{sm}}^2} \tag{7}$$

where A, B, C, A^0, B^0 and C^0 are regression coefficients. The results of statistical analysis are presented in Table IV. An examination of Table IV under "All data" shows that both equations gave significant "fit" to the experimental data as judged from the F-ratios ($\mathrm{F} > 337$) when compared to the tabulated [28] value ($F < 8.09$ at 99.9% confidence level). Since experimental data may include some accidentally large errors a "trimming" step was carried out in order to eliminate such observations. Those observations which showed Studentised Residual values greater than 2 were considered to be outliers. Statistical analysis was again performed for the "trimmed" data and the results appear in Table IV under "Data after outliers removed". It was

TABLE IV

RESULTS OF STATISTICAL ANALYSIS ON EQNS. 6 AND 7 FOR FOUR DATA SETS
Symbols *n*, *r*, *s* and *F* are defined in Table III.

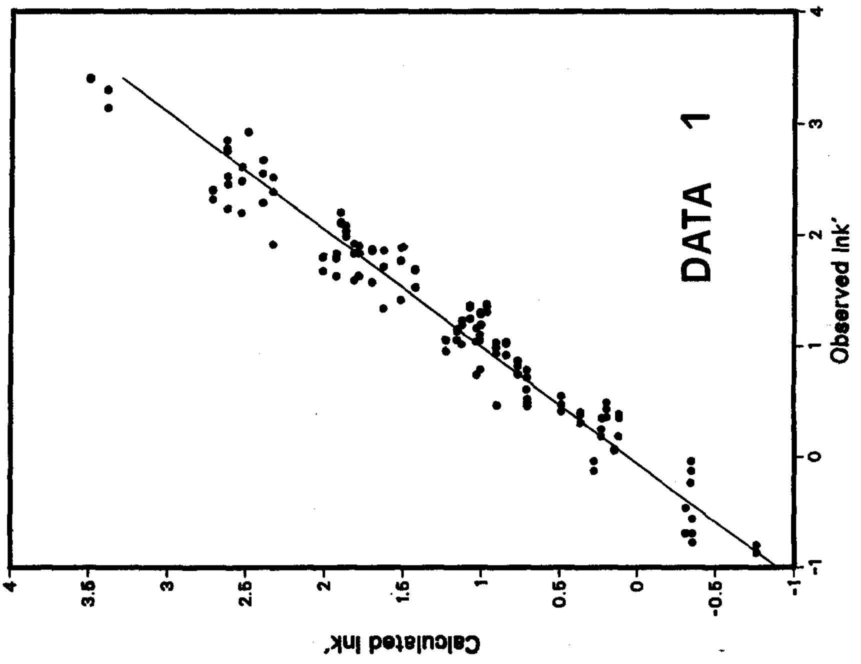
Eqn.	Data set	All data				Data after outliers removed			
		n	*r*	*s*	*F*	*n*	*r*	*s*	*F*
6	1	131	0.973	0.235	1113	127	0.976	0.222	1232
	2	1176	0.967	0.611	8054	1125	0.981	0.428	13 013
	3	145	0.969	0.311	1040	138	0.973	0.267	1211
	4	45	0.971	0.223	337	42	0.979	0.181	443
7	1	131	0.972	0.238	1112	129	0.974	0.231	1147
	2	1176	0.913	0.987	2869	1106	0.945	0.738	5043
	3	145	0.974	0.286	1313	138	0.984	0.226	2081
	4	45	0.972	0.217	360	41	0.987	0.150	694

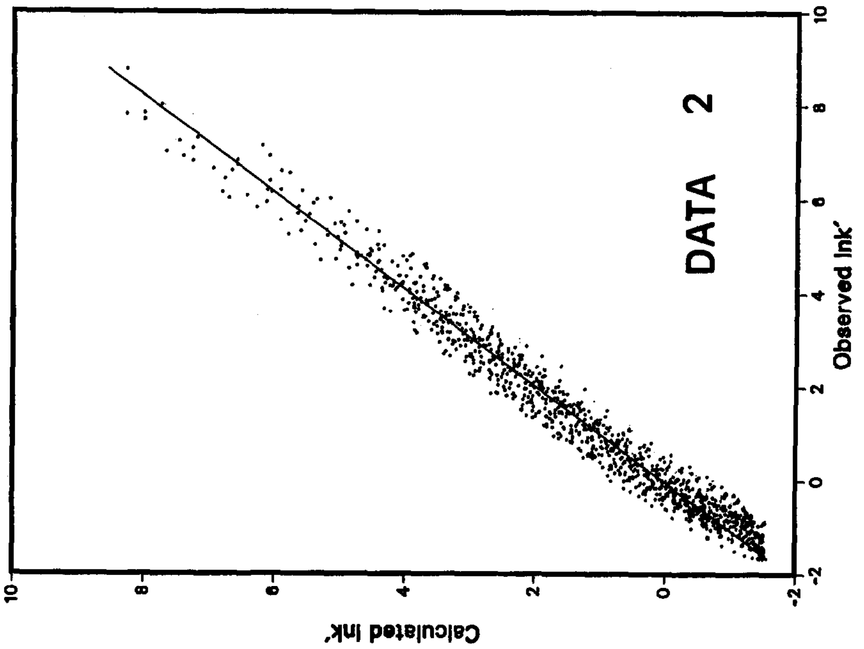
found that there were few outliers, and removing them did not change the correlation (*r*) and standard deviation (*s*) statistics considerably as compared to "untrimmed" data, but *F* ratios (*F*) were improved. Eqn. 6 was prefered over eqn. 7 because of higher *F*-ratios for larger data sets. Further support for eqn. 6 as more appropriate model comes from an examination of regression coefficients and the error associated with their estimation. These coefficients and corresponding *t*-ratios appear in Table V. It is evident that the coefficients (*A*, *B* and *C*) for eqn. 6 are consistent in magnitude and sign for all data sets, in contrast to those for eqn. 7. Such consistency of the estimated coefficients implies that a common "trend" of chromatographic process is occurring in different but related systems, as measured by the hydrophobic parameters of the solutes and solvents. Further evidence in favour of eqn. 6 over eqn. 7 is provided by the greater values of *t*-ratio (t_a, t_b and t_c) for each coefficient (see Table V). This indicates that the coefficients for eqn. 6 can be estimated with less error as compared to those for eqn. 7, therefore a higher confidence limit could be assigned to

TABLE V

REGRESSION COEFFICIENTS AND *t* STATISTIC FOR EQNS. 6 AND 7 FOR FOUR DATA SETS
A, *B* and *C* are regression coefficients. t_a, t_b and t_c are *t*-ratio (ratio of coefficient and standard deviation) for coefficients *A*, *B* and *C*, respectively.

Eqn.	Data set	*A*	t_a	*B*	t_b	*C*	t_c
6	1	−2.0585	−17.77	0.0849	45.49	0.0024	05.84
	2	−1.5866	−70.34	0.0667	71.71	0.0057	55.11
	3	−0.7013	−13.13	0.0459	22.80	0.0061	24.51
	4	−1.2222	−08.52	0.0567	24.81	0.0055	08.05
7	1	−1.4205	−22.66	0.0459	05.65	0.0024	05.32
	2	−1.4974	−37.67	0.1050	93.93	−0.0009	−00.11
	3	0.2180	04.28	−0.0244	−05.05	0.0056	27.62
	4	−0.1854	−02.39	−0.0052	−00.77	0.0045	10.87


DATA 1
Calculated lnk'
Observed lnk'
4
3.5
3
2.5
2
1.5
1
0.5
0
-0.5
-1
-1
0
1
2
3
4


DATA 2
Calculated lnk'
Observed lnk'
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8
6
4
2
0
-2
-2
0
2
4
6
8
10

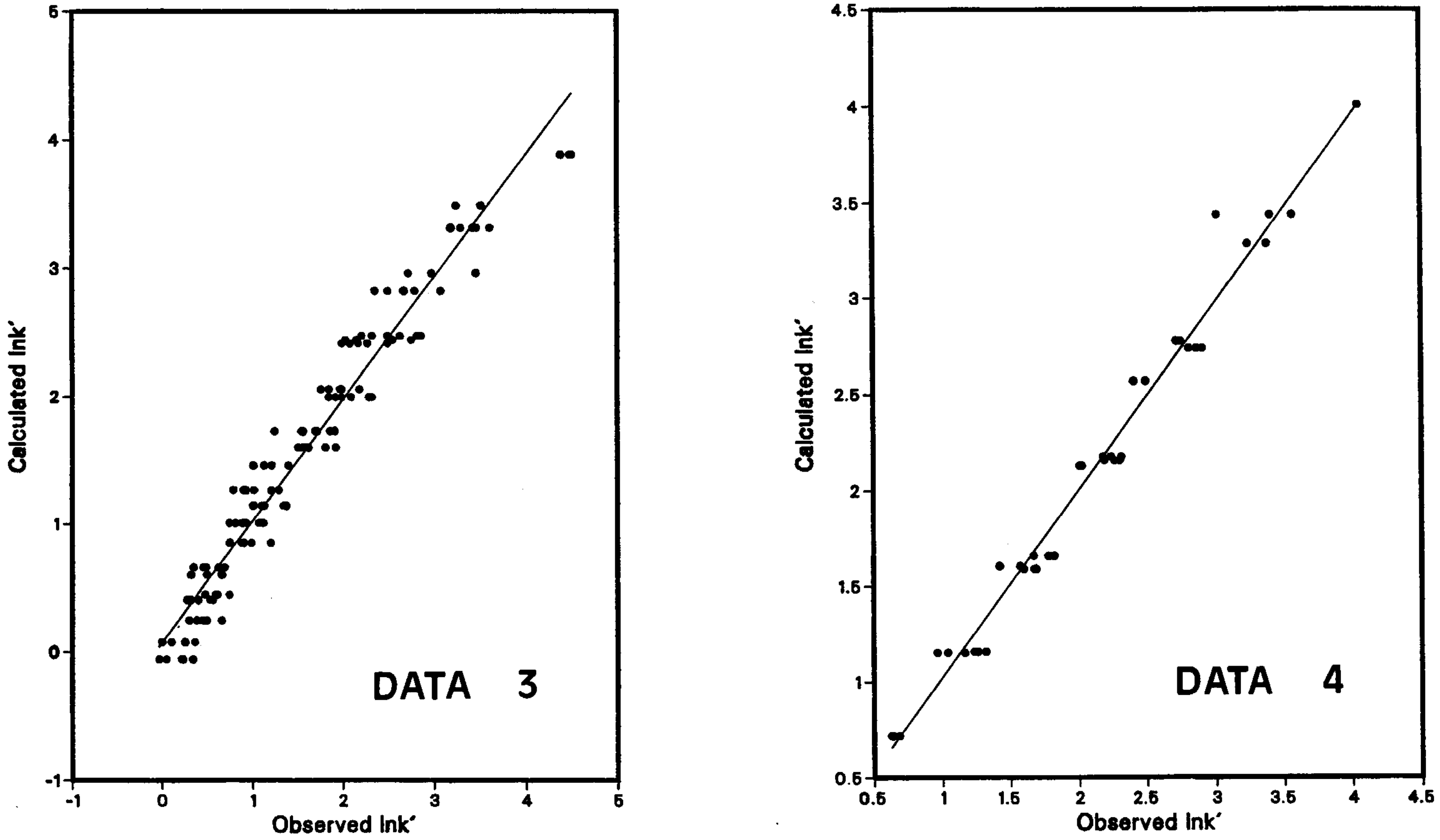

Fig. 1. Plots for the four data sets (1–4) showing the correlation between the observed and calculated ln k' values obtained using eqn. 6. The statistical analyses for these plots are given in Tables IV and V. Fractions of the organic solvents and their chemical nature used in the studies were: Data set 1, φ(MeOH) = 0.50, φ(ACN) = 0.30, φ(THF) = 0.25; Data set 2, φ(MeOH) = 0.10–0.90, φ(ACN) = 0.07–0.69, φ(THF) = 0.06–0.60; Data set 3, φ(MeOH) = 0.28–0.90, φ(ACN) = 0.20–0.69, φ(THF) = 0.18–0.60 and Data set 4, φ(MeOH) = 0.50–0.70, φ(ACN) = 0.37–0.52, φ(THF) = 0.32–0.46.

the predictions from eqn. 6. Plots of the experimental and calculated retentions (ln k'), according to eqn. 6, appear in Fig. 1 for the four data sets. It is evident that good correlation between observed and predicted solute retentions has been achieved. It is important to emphasise at this stage that though the predictions may not by very accurate, because of the use of only one physico-chemical parameter for solute (log P) and solvent (log P_{xm}), eqn. 6 does provide a general and consistent model relating retention and solute and solvent property for physically different but chromatographically similar reversed-phase systems.

Having established that eqn. 6 is a statistically sound, chromatographically appropriate and a general model, it was of interest to examine the significance of each term. The terms in eqn. 6 represent a number of familiar observations in RP-HPLC. For example, the direct proportionality of ln k' to log P shows the commonly observed phenomenon that retention is dependent on the hydrophobic parameter of the solute [14]. The quadratic dependance of ln k' on $1/P_{sm}$ reflects the relationship given in eqn. 2 which was derived semi-empirically based on regular solution theory [3]. Additionally, the inverse proportionality of ln k' to P_{sm} shows that P_{sm} is an appropriate solvent strength parameter, because the greater the solvent strength (P_{sm}), the shorter is the retention (ln k'). The interrelationship between solute log P and mobile phase strength, $1/P_{sm}$, expressed as the ratio (log P)/($1/P_{sm}$), again shows the commonly observed fact that the greater the hydrophobicity of the solute (log P), the greater is the solvent strength required to obtain the same retention.

The relationship provided by eqn. 6 may be used practically to obtain useful but approximate prediction of solute retention in many RP materials by the analysis of a few solutes sufficiently different in their log P values, under different strengths using the $1/P_{sm}$ scale, for different solvents. This will permit the estimation of the coefficients of eqn. 6 for a given chromatographic system. It is further suggested that eqn. 6 can be of value as a starting point in the development of solvent optimisation procedures, or in the development of other equations that are able to define solute retention more accurately by incorporating additional physico-chemical parameters. This study has shown that for non-ionic solutes chromatographed in a RP system, using data generated from four different RP materials, that the retention is very largely determined by solute hydrophobicity (log P) and mobile phase hydrophobicity (log P_{sm}).

CONCLUSIONS

(1) The calculated partition coefficient, log P_{sm}, of aqueous–organic mobile phases containing either methanol, acetonitrile or tetrahydrofuran has been found to be a quantitative parameter for eluotropic strength and to have a highly significant relationship with Φ, the volume fraction of solvent in water (eqn. 2).

(2) Eqn. 2 has permitted the development of a relationship (eqn. 6), using experimental data, that expresses the retention behaviour of non-ionic solutes based solely on the hydrophobic parameter of both the solute (log P) and the solvent (log P_{sm}).

(3) The relationship shown by eqn. 6 was found to be statistically sound and gave consistent regression coefficients when applied to four different experimental data sets, indicating a common type of retention and solute–solvent property relationship.

(4) A distinct advantage offered by eqn. 6 is that the retention data for different solutes analysed under different solvent compositions (qualitative and quantitative) may be combined in a single expression.

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