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Review

Extra-thermodynamic relationships in chromatography—study of the relationship between the slopes and intercepts of plots of ln *k′* *vs.* mobile phase composition in reversed-phase chromatography

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ABSTRACT

The chemical validity of the frequently reported linear relationship between the slopes (S) and intercepts ($\ln k'_w$) of plots of ln k' for various solutes against mobile phase volume fraction organic modifier in reversed-phase liquid chromatography (RPLC) has been investigated. We have shown that under certain conditions, the linear S–$\ln k'_w$ relationship for a chemically variegated set of solutes, even though it is mathematically quite real, is an artifact that results from statistical considerations and does not reflect chemical reality. However, S–$\ln k'_w$ correlations are chemically meaningful when the set of test solutes is restricted to a homologous series. Based on conventional extra-thermodynamic reasoning, such correlations can only be due to chemistry under two circumstances; *i.e.*, when only a single retention-governing solute property varies within the set of solutes studied, or when various retention governing processes have the same compensation composition (φ_{iso}). The existence of a real correlation is also shown to be at odds with the concepts of linear solvation energy relationships and other models of retention that describe retention in RPLC as the result of a variety of independent intermolecular interactions. Finally it is shown that if linearity between S and $\ln k'_w$ were universal, the well-known phenomena of changes in elution sequence with the volume fraction of organic modifier in the mobile phase would not be possible.

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1. INTRODUCTION

While the fundamental mechanism or mechanisms of reversed-phase liquid chromatography (RPLC) are still unresolved, a number of useful empirical observations of great practical significance have been established. One of the most important of these is the relationship between retention and mobile phase composition [1-8]. Based on various models of the retention process, many such relationships have been proposed. Due to its simplicity, one of the most useful, however approximate, of these is the logarithmic-linear relationship given in eqn. 1:

$$\ln k' = \ln k'_w - S\varphi \qquad (1)$$

where k' is the solute capacity factor at a specific mobile phase composition (φ) and k'_w is the extrapolated k' for pure water ($\varphi = 0$). Ln k'_w is the intercept of the relationship defined by eqn. 1, and S is the slope of the plot of ln k' *vs.* φ where φ is the volume fraction of organic modifier. In fact eqn. 1 is not particularly accurate. It has been shown that a quadratic form, given in eqn. 2, is generally better, at least from the point of view of statistical goodness of fit [8-10].

$$\ln k' = \ln k'_w + S_1\varphi + S_2\varphi^2 \qquad (2)$$

Eqn. 2 has the additional virtue of being derivable based on regular solution theory [11,12] and simple lattice models [13]. However, for practical work, eqn. 1 is usually preferred [1] and over a sufficiently narrow range in mobile phase composition it is usually quite satisfactory.

It is now well known that S is not solely a solvent-stationary phase parameter. Rather it varies considerably with solute structure especially with solute size [14-17]. Over the past few years there have been a number of papers [15,18-26] that have discussed a correlation between the slope (S) and intercept (ln k'_w) of eqn. 1 as the solute is varied.

$$S = p(\ln k'_w) + q \qquad (3)$$

Eqn. 3 has been used to estimate retention in RPLC [12,26-29].

From a fundamental perspective one wishes to know why a correlation between S and ln k'_w should exist. Assuming that the stationary phase is not altered by the mobile phase, which is known to be unrealistic for RPLC, and that eqn. 1 is valid over the range 0 to 100% organic modifier, then it is easy to show that S is proportional to the free energy of solute transfer from pure water to pure organic mobile phase. Similarly, ln k'_w is linearly related to the free energy of transfer of solute from pure water to the stationary phase. These two processes are only related by the fact that water is a common phase to both processes. Aside from the obvious problems caused by the sweeping assumptions stated above, S and ln k'_w will fail to be correlated to extent that the pure organic mobile phase and the environment established by the stationary phase are dissimilar.

The purpose of the present paper is to examine whether or not such correlations are in fact chemically meaningful or whether they result merely as a statistical artifact of the data analysis. If such correlations are chemically valid, then based on the extremely general formalism developed by Leffler and Grunwald [30] for examining solvent effects on chemical reactions, they have very significant implications as to the mechanism of RPLC (see below).

In a purely mathematical sense, the ln k'-φ relationship shown in eqn. 1 is analogous to the usual relationship used to relate capacity factors to temperature where $1/T$ in eqn. 4 plays the role of the solvent composition variable in eqn. 1:

$$\ln k' = A + B/T \qquad (4)$$

Making the assumption that the change in the heat capacity of the system upon transfer of solute from the mobile to the stationary phase is zero allows the derivation of simple equations between the enthalpy (ΔH) and entropy (ΔS) of transfer, and the capacity factor [31]:

$$\Delta H = -RB \qquad (5)$$

$$\Delta S = R(A - \ln \phi) \qquad (6)$$

where R is the gas constant and ϕ is the phase ratio of the column.

More generally, the same approach, that is the use of the temperature dependence of an experimental equilibrium constant or rate constant, has been applied to the measurement of the enthalpies and entropies of many chemical reactions and activation parameters [30].

It is frequently observed that for a series of different species undergoing a common process, plots of ΔS *vs.* ΔH are linear. This phenomenon is termed "enthalpy–entropy compensation" [30]. There have been many studies of "enthalpy–entropy compensation" in reversed-phase chromatography [31–33], and we recently explored in some detail whether or not it takes place in gas chromatography [34].

These studies are fundamentally important since when such compensation can be shown to be chemically valid then one can conclude, as per Leffler and Grunwald [30] and Boots and De Bokx [35,36], that there can be only *one* dominant retention governing interaction that varies as the solutes are varied. If there are two or more solute dependent variables that govern differences in retention, then each must have the *same* compensation temperature, an eventuality that is in general rather implausible. By analogy to the above terminology, we will refer to existence of a *chemically* valid correlation between S and $\ln k'_w$ as the phenomenon of "S–$\ln k'_w$ compensation".

Krug *et al.* [37–40] have made the point very strongly that many reports of "enthalpy–entropy compensation" are totally spurious, that is, they are devoid of any fundamental chemical significance. They have demonstrated that the propagation of random measurement error in a least squares determination of the slope and intercept of a plot of $\ln k'$ *vs.* $1/T$ must inexorably lead to some degree of correlation between the slope and intercept of eqn. 4 and thus an *apparent* "enthalpy–entropy compensation".

Because mathematically and statistically the enthalpy and entropy as determined from eqn. 4 are completely equivalent to $\ln k'_w$ and S as determined from eqn. 1, we hypothesized that spurious correlations between $\ln k'_w$ and S might result. Without going into the detailed statistical reasoning of Krug *et al.*, we show the result of a simple stochastic experiment (see Fig. 1). This result was obtained by using *fixed* values of $\ln k'_w$ (=8.0) and S (=3.0) and a fixed set of typical φ (=0.3, 0.4, 0.5, 0.6 and 0.7) values. A set of $\ln k'$ values corresponding to the set of φ values was generated. A small random noise of 5% was added to each $\ln k'$ and a least squares analysis was carried out to determine $\ln k'_w$ and S. A random noise of 5% was chosen because it is a typical standard deviation obtained by correlating experimental $\ln k'$ values *vs.* φ. The process of adding random noise to the data set was repeated and the set of resultant $\ln k'_w$ and S values is shown in Fig. 1. There is evidently a strong correlation ($r = 0.9666$) even though there is no real change in the basis data set. That is, there is no chemical information inherent in the correlation shown in Fig. 1. This stochastic experiment was done to demonstrate that a good correlation can result from purely statistical considerations, that is, it is completely unrelated to chemistry. Previously Balke made exactly the same point [41,42]. Upon comparing the range in $\ln k'_w$ and S values in Fig. 1 to those in real experiments, the range is evidently much greater in the real experiments. This suggests that in at

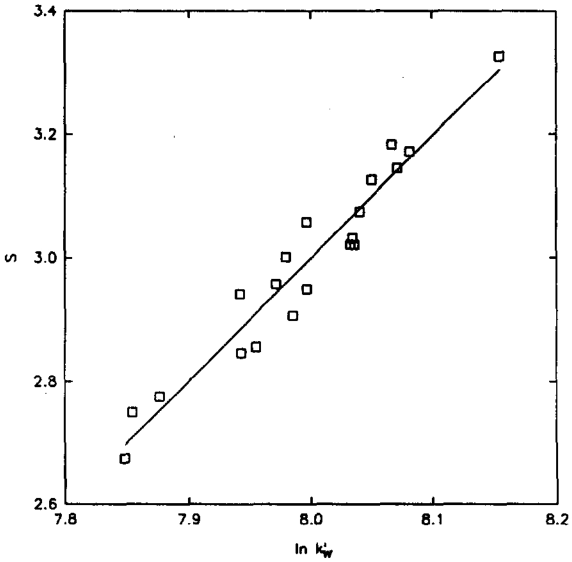

Fig. 1. Plot of S *vs.* $\ln k'_w$, where S and $\ln k'_w$ are the slope and intercept, respectively, from the least squares analysis of a series of equations $\ln k' = (8.00 - 3.00\ \varphi) + 0.1$ (random − 0.5). Random denotes computer-generated random number. φ ranges from 0.3 to 0.7.

least some situations the observed correlation is chemically meaningful.

Clearly based on the above stochastic experiment, the mere observation, in a statistical sense, of a correlation between ln k'_w and S ought not be interpreted as having any chemical significance even if the correlation coefficient is quite high. It is important to understand that we are not saying that no correlation exists in plots such as those shown in Fig. 1 or for the results of real experiments (see Fig. 2). It is plainly evident that S and ln k'_w are highly correlated in both Figs. 1 and 2. We, however, are saying that in many instances the correlation has no underlying chemical cause; rather it merely results from statistical considerations. Krug [40] has presented three tests that allow one to assess the existence of true, that is chemically significant, ΔS–ΔH compensation. By transposing his results to the present circumstances, we can validate whether a correlation between S and ln k'_w is chemically valid (real) or merely results from statistical driven covariance induced by the least squares analysis.

The purpose of the present paper is to examine several literature data sets in which "S–ln k'_w compensation" has been claimed, to determine conditions under which such are real, and to examine the conceptual consequences for chromatography.

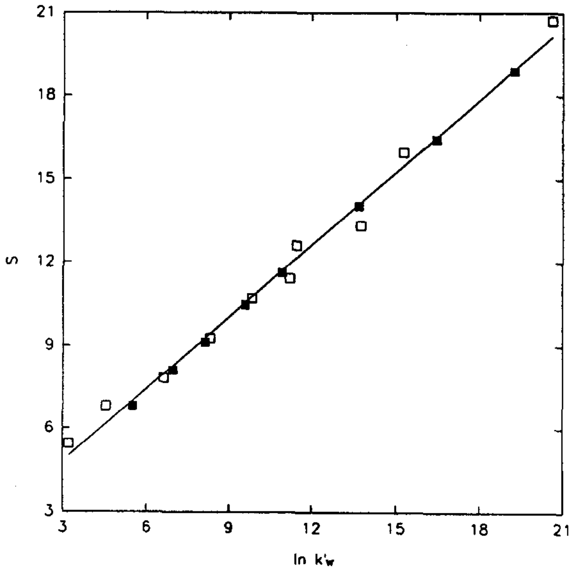

Fig. 2. Plot of S vs. ln k'_w for alkylbenzenes (■) and non-alkylbenzenes (□) listed in Table 1. The solid line is the least squares fit for the alkylbenzenes only.

2. EXPERIMENTAL

The S and ln k'_w values discussed in this paper were taken from references [18,20,26,43,44]. However, a detailed analysis will only be carried out on data taken from the work of Harnisch *et al.* [43]. We choose this data set because it shows a very strong correlation between S and ln k'_w and had the best goodness of fit among all the data sets reported. In addition, the data set includes enough members of a homologous series so that we can test other hypotheses. Further their data were acquired over a reasonably large number of mobile phase compositions and they reported an estimate of the precision of k'. From our perspective then the data set of Harnisch *et al.* is a very severe test of our hypothesis that many observations of "slope–intercept compensation" in RPLC merely result from statistical considerations and have no underlying chemical significance. In their work, ln k' values were obtained in organic aqueous mobile phase from 60 to 95% (v/v) methanol in 5% increments. It was reported that the ln k' values obtained for each solvent mixture were, in most instances, calculated from the mean value of 3–5 retention time measurements with relative standard deviations less than 1%. Therefore 1% random noise was incorporated into each ln k' value calculated from the S and ln k'_w values given.

3. RESULTS AND DISCUSSION

3.1. *Statistical tests for the validity of the correlation*

Table 1 shows the ln k'_w and S values for all the compounds studied by Harnisch *et al.* [43]. First, we show a plot of S vs. ln k'_w (Fig. 2). The least squares relationship is:

$$S = 2.190 + 0.870 \ln k'_w$$

$$n = 18, \text{S.D.} = 0.396, r = 0.9960 \quad (7)$$

TABLE 1

ln k'_w AND S VALUES FOR ALL SOLUTES

Solute	ln k'_w [a]	S [a]	V_x [b]	β_2 [c]	α_2 [d]	π_2^* [d]
Benzene	5.50	6.79	71.64	0.10	0.00	0.29
Toluene	6.97	8.10	85.73	0.11	0.00	0.29
Ethylbenzene	8.14	9.13	99.82	0.12	0.00	0.30
n-Propylbenzene	9.61	10.47	113.91	0.12	0.00	0.30
n-Butylbenzene	10.96	11.65	128.00	0.12	0.00	0.30
n-Hexylbenzene	13.70	14.05	156.18	0.12[e]	0.00[f]	0.30[f]
n-Octylbenzene	16.46	16.45	184.36	0.12[e]	0.00[f]	0.30[f]
n-Decylbenzene	19.26	18.89	212.54	0.12[e]	0.00[f]	0.30[f]
Aniline	3.22	5.44	81.62	0.50	0.20	0.76
Biphenyl	9.85	10.71	132.42	0.20	0.00	0.96[h]
Dibutylphthalate	11.45	12.60	227.42	0.80[e]	0.00[f]	0.66[e,h]
1,2-Dichlorobenzene	8.32	9.27	96.12	0.03	0.00	0.57
p,p'-DDT	15.28	15.98	221.80	0.24[e]	0.00[f]	1.63[e,h]
Di(2-ethylhexyl)phthalate	20.62	20.71	340.14	0.88[e]	0.00[f]	0.63[e,h]
Fluoranthene	11.23	11.43	158.46	0.25[e]	0.00[f]	1.06[e,h]
Hexachlorobenzene	13.76	13.33	145.08	0.00	0.00	0.53[h]
4-Nitrophenol	4.54	6.78	94.93	0.32	0.86[g]	0.94[h]
Trichloroethylene	6.62	7.81	71.46	0.05	0.07[g]	0.37[h]

[a] ln k'_w and S are the intercept and slope of plot of ln k' *vs.* mobile phase composition (see eqn. 1), respectively, obtained from ref. 43.

[b] V_x is intrinsic molar volume, obtained from ref. 45.

[c] β_2 is solute hydrogen bond basicity, obtained from ref. 46 unless otherwise specified.

[d] π_2^* and α_2 are solute dipolarity/polarizability and solute hydrogen bond acidity, respectively, obtained from ref. 47 unless otherwise specified.

[e] Estimated using estimation rules set in ref. 46.

[f] Estimated using rules set in ref. 47.

[g] Estimated from eqn. 8 of ref. 47.

[h] Estimated from eqn. 7 of ref. 47.

In the above equation, and elsewhere, n is the number of the data points, while S.D. and r indicate the average standard deviation and the correlation coefficient of the fit, respectively. Inspection of Fig. 2 and the excellent correlation coefficient clearly support the view that S and ln k'_w are strongly linearly correlated and we are readily seduced into the belief that there must be something of fundamental chemical significance in this observation. However, Krug *et al.* [37–40] have shown that enthalpy–entropy correlations with as high correlation coefficients as reported in eqn. 7 were, in fact, spurious, that is, devoid of any chemical information.

To determine whether S–ln k'_w compensation really takes place in this data set, we examined it using the mathematical and statistical tests formulated by Krug [40], as transposed to the present circumstances.

Krug has shown that the statistical effect that induces the spurious correlation between the slope and the intercept has no effect on the least squares computed value of the average data coordinates [40]. Based on this he suggests plotting the slope *vs.* the average value of the dependent variable. In the present case, this means that we should plot the least squares determined slope (S) against the values of ln k' computed at the average solvent composition (φ_{ave}) from the least squares S and ln k'_w values. The average solvent composition is defined as:

$$\varphi_{\text{ave}} = \frac{\sum_{i}^{n} \varphi_i}{n} \qquad (8)$$

For the set of mobile phase compositions studied, the average mobile phase composition, φ_{ave}, is 0.775.

The S–ln k'_{ave} plot is shown in Fig. 3. As a whole, the plot shows a weaker correlation than that shown in Fig. 2. As expected, the data for the alkylbenzenes show an almost perfect linear relationship (see closed squares). The S–ln k'_{ave} relationship for all compounds is represented by the following equation:

All compounds:

$$S = 6.978 + 2.540 \ln k'_{\mathrm{ave}}$$

$$n = 18, \ \mathrm{S.D.} = 1.216, \ r = 0.9615 \qquad (9)$$

Note that this correlation should be compared directly to that given in eqn. 7. The correlation coefficient is considerably reduced and the average standard deviation of the fit has increased more than 3-fold. This results solely from the elimination of the statistically induced correlation between S and ln k'_{w} that is absent in the correlation in S and ln k'_{ave}. Inspection of Fig. 3 clearly supports the idea that there is a correlation. However, this correlation is primarily due to the presence of the alkylbenzene homologues in the data set. When we correlate S *vs.* ln k'_{ave} for just the alkylbenzenes, we find a regression that is quite excellent even though we used ln k'_{ave} and not ln k'_{w} (see eqn. 9a).

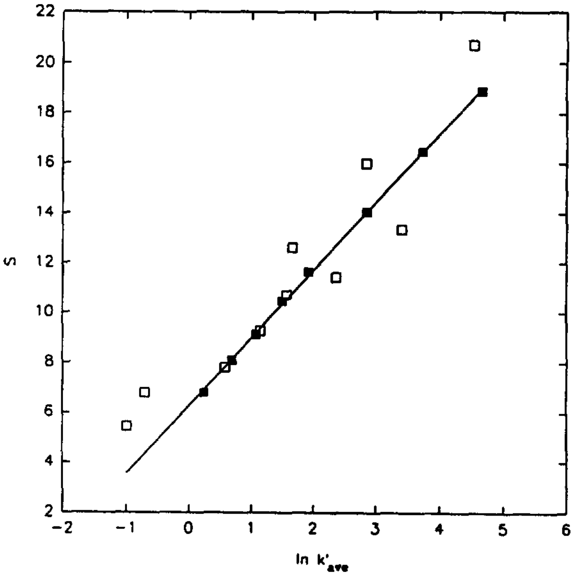

Fig. 3. Plot of S *vs.* ln k'_{ave} for alkylbenzenes (■) and non-alkylbenzenes (□) listed in Table 1. The solid line is the least squares fit for the alkylbenzenes only (eqn. 9a).

Alkylbenzenes:

$$S = 6.255 + 2.727 \ln k'_{\mathrm{ave}}$$

$$n = 8, \ \mathrm{S.D.} = 0.108, \ r = 0.9997 \qquad (9a)$$

Finally if we eliminate the alkylbenzenes from the data set and correlate the remaining solutes we find a much poorer correlation. Note the average standard deviation of eqn. 9b is about 15 times worse than that in eqn. 9a.

Non-alkylbenzenes:

$$S = 7.360 + 2.470 \ln k'_{\mathrm{ave}}$$

$$n = 10, \ \mathrm{S.D.} = 1.631, \ r = 0.9416 \qquad (9b)$$

The reason that S and ln k'_{ave} correlate in eqn. 9b is primarily due to the fact that both S and ln k'_{ave} correlate with molecular size even for the alkylbenzene excluded data set. When a second solute descriptor, namely its hydrogen bond basicity, is introduced into the correlation between S and ln k'_{ave} the correlation is greatly improved (see below).

In view of the extremely small S.D. obtained with the alkylbenzenes (see eqn. 9a), the tremendous scatter in eqns. 9 and 9b cannot be due to either random experimental errors in the measurement in k' or to poor fits of ln k' *vs.* φ. We conclude that the poor fits are due to chemically systematic deviations or scatter about the "true" correlation line that would result if the data set contained solutes that only differ from one another by a single molecular parameter that controls the molecules retention. The above results are consistent with the work of Quarry *et al.* [25] who show that deviation from the overall regression (eqn. 9) depended on the solute's chemical functionality. As a final comment, we emphasize that the correlation as presented in eqns. 9, 9a and 9b are chemically legitimate, they are not due to spurious statistical effect, and can be used for predictive purposes. However, the precision of prediction especially for non-alkylbenzenes is not going to be very impressive given the large average standard deviation (see eqn. 9b). Based on the average standard deviation in eqn. 9b, the average predicted k' will be in error by 17, 37 and 61% when φ differs from φ_{ave} by 10, 20 and 30% (v/v), respectively. We advise that equations relating S to $\ln k'_{\text{w}}$ not be used for predictive purposes.

Another very simple, graphical test of the chemical validity of a compensation relationship, developed by Krug [40], is based on the fact that when compensation takes place, then algebraically all plots of $\ln k'$ *vs.* φ must intersect at a *single* concurrence point called the "isoelution point", φ_{iso}, or in the language of Leffler and Grunwald [30], the "isokinetic point". That this is true is easily shown by substituting eqn. 3 in eqn. 1. This leads to:

$$\ln k' = -q/p + S(1/p - \varphi) \tag{10}$$

It is evident that the "isoelution point" is found at:

$$\varphi_{\text{iso}} = 1/p \tag{11}$$

and all solutes elute with the same k', denoted k'_{iso}, as follows:

$$\ln k'_{\text{iso}} = -q/p \tag{12}$$

Fig. 4 shows the $\ln k'$–φ plots for the compounds listed in Table 1; the broken lines show the alkylbenzenes and the solid lines are for non-alkylbenzenes. Overall, these various lines do intersect but they do so over a considerable range in mobile phase composition. We see several points at which there are changes in elution sequence as is commonly observed in RPLC.

The plots for the alkylbenzenes do, in fact, converge at a single intersection point and this suggests, as does the high quality correlation seen in eqn. 9a, a chemically meaningful "S–$\ln k'_{\text{w}}$ compensation" for this limited set. The "isoelution point", φ_{iso}, for the alkylbenzenes occurs at about 114% methanol.

The fact that the lines intersect over a fairly wide range for a variegated solute set coincides with common experience in RPLC. There is not a single φ_{iso} for such a set of compounds. If there were, optimization of the mobile phase composition in RPLC would be trivial; one should merely avoid this φ_{iso} mobile phase composition. There would be no need for the extremely useful "resolution maps" [25] and "window diagrams" [48] that have been shown to be so helpful in RPLC.

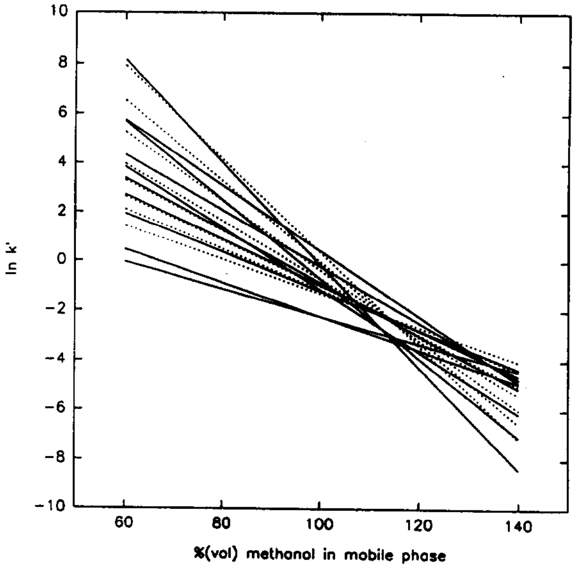

Fig. 4. Plot of $\ln k'$ *vs.* percentage volume of methanol in mobile phase for all alkylbenzenes (······) and non-alkylbenzenes (——) listed in Table 1.

As a last test as to whether a compensation phenomena is chemically real or is due to statistical effects, Krug devised a detailed analysis of variance (ANOVA) that allows the variance in the slope and intercept to be compared to the random experimental variance so that an F-ratio test can be used to test for correlation. From the information supplied by the data in ANOVA tables, such as shown in Table 2, probabilities can be assigned for different effects by evaluation of the F-statistic. Readers are referred to Krug [40] for details of the analysis. Tables 3 and 4 show the ANOVA summaries of the set for all compounds and for the alkylbenzene subset, respectively. For the analysis of all compounds, the probability for concurrence (MS_{con}) is higher than that for non-concurrence (MS_{noncon}) at the 99% significance level.

TABLE 2

GENERAL ANOVA TABLE

From ref. 40.

Source of variation	*DF*[a]	*SS*[b]	*MS*[c]
Total	$pq-1$[d]	SS_T	MS_T
Rows (solutes)	$p-1$	SS_R	MS_R
Column (mobile phase composition)	$q-1$	SS_C	MS_C
Interactions	$(p-1)(q-1)$	SS_{RC}	MS_{RC}
Slopes	$p-1$	SS_S	MS_S
Concurrence	1	SS_{con}	MS_{con}
Non-concurrence	$p-2$	SS_{noncon}	MS_{noncon}
Residuals	$(p-1)(p-2)$	SS_ϵ	MS_ϵ

[a] *DF* is the degree of freedom.
[b] *SS* is the sum of squares.
[c] *MS* is the mean sum of squares, $MS = SS/DF$ for each source of variation.
[d] p is the number of solutes and q is the number of mobile phase compositions.

$$MS_{con}/MS_{noncon} = \frac{30.2}{0.147}$$
$$= 205 > F(1,16,1- = 0.99) = 8.6$$

However, the probability for non-concurrence (MS_{noncon}) is also higher than the precision of the data (MS) at the same level of significance.

$$MS_{noncon}/MS = \frac{0.147}{0.001}$$
$$= 147 > F(16,102,1- = 0.99) = 2.2$$

Therefore, to within the precision of the data, no concurrence can be detected. However, for the analysis of the alkylbenzene subset, the probability for concurrence is higher than that for non-concurrence and the probability for non-concurrence is low compared to the precision of the data at the 99% significance level.

$$MS_{con}/MS_{noncon} = \frac{13.2}{0.002}$$
$$= 6600 > F(1,6,1- = 0.99) = 1.37$$

TABLE 3

ANOVA TABLE FOR RETENTION OF ALL SOLUTES

Data from ref. 43. *DF*, *SS* and *MS* as in Table 2.

Source of variation	*DF*	*SS*	*MS*
Total	143	645	4.51
Rows (solutes)	17	358	21.1
Columns (mobile phase compositions)	7	254	36.3
Interactions	119	32.6	0.274
Slopes	17	32.6	1.92
Concurrence	1	30.2	30.2
Non-concurrence	16	2.36	0.147
Residuals	102	0.084	0.001

TABLE 4

ANOVA TABLE FOR RETENTION OF ALKYLBENZENES

Data from ref. 43. *DF*, *SS* and *MS* as in Table 2.

Source of variation	*DF*	*SS*	*MS*
Total	63	263	4.18
Rows (solutes)	7	130	18.6
Columns (mobile phase compositions)	7	120	17.1
Interactions	49	13.3	0.271
Slopes	7	13.2	1.89
Concurrence	1	13.2	13.2
Non-concurrence	6	0.011	0.002
Residuals	42	0.037	0.001

$$MS_{\text{noncon}}/MS = \frac{0.002}{0.001}$$

$$= 2 < F(6{,}42{,}1- = 0.99) = 3.3$$

Thus, to within the precision of the data, concurrence is detected for the alkylbenzenes. The F values used above were obtained from Ostle [49].

To summarize, based on the results of the statistical tests, "S–ln k'_w compensation" exists only in the subset of alkylbenzene homologues but not in the whole data set. Consequently most of the good correlation seen in Fig. 2 results from the statistical complications that results from the use of least squares to determine both S and ln k'_w from a single data set.

3.2. *Relevance to models of RPLC*

The existence of a real linear correlations between the slope (S) and the intercept (ln k'_w) of eqn. 1 implies that the compounds under study must coelute at a single φ_{iso}. This phenomenon is seldom observed in practice when a variegated set of solutes is studied.

Extra-thermodynamic consideration, as elegantly shown by the very general model of "enthalpy–entropy compensation" developed by Boots and De Bokx [35,36], indicates that exact S–ln k'_w compensation behavior can only be observed when the differences in retention in any given set of solutes is governed by differences in a *single* type of *solute-dependent* intermolecular parameter. The existence of true, *i.e.* chemically valid, "S–ln k'_w compensation" in RPLC is at odds with the fundamental basis for the use of linear solvation energy relationships (LSERs) and other models of chromatographic retention such as multi-component solubility parameters [50–52] which fundamentally represent solute–solvent interactions as a suite of processes (dispersion, dipolar, hydrogen bonding, etc.). To be specific, application of LSERs to RPLC has shown that there are two principal contributors to solute retention [53–56]. These are the endoergic energy needed to make a cavity and the exoergic process of forming hydrogen bonds with the solvent acting as a donor to the solute acting as a hydrogen bond base. LSER studies of RPLC have shown that the solute's ability to interact via dipole-dipole and dipole-induced dipole interactions and its ability to donate hydrogen bonds are distinctly less important. Thus variations in retention from solute to solute are governed by two characteristic solute parameters, namely a measure of solute size and solute basicity (see below).

In addition to the results of LSER analysis, it is difficult to imagine how a system governed by a single solute-dependent characteristic interaction could display the very large variation in relative retention and even retention sequence commonly observed in RPLC as the type and amount of mobile phase modifier are varied. It is therefore very important to understand the phenomenon of the S–ln k'_w linear relationship in detail so as to assess its limits. We next examine the phenomenon based on the LSER model. However, we point out that qualitatively similar results will be obtained using other models of retention.

The methodology of LSERs has been used in conjunction with solvatochromic parameters to describe retention in RPLC [53–56]. According to this approach, variation in capacity factors with solute structure can be related to their potential for intermolecular interactions as follow:

$$\ln k' = SP_0 + mV_x + b\beta_2 + a\alpha_2 + s\pi_2^* \qquad (13)$$

where V_x, β_2, α_2 and π_2^* are parameters that describe the solute, and m, b, a and s are the corresponding regression coefficients. SP_0 is the intercept of the relationship defined by eqn. 13. The V_x term measures the intrinsic molar volume; β_2 and α_2 denote the hydrogen bond basicity and acidity, respectively; while π_2^* is a measure of solute dipolarity/polarizability. The solute parameters of the 18 compounds used in this study are listed in Table 1.

We mentioned earlier that S is not solely a solvent–stationary phase parameter but rather it depends on solute structure [14–17,26]. Therefore both $\ln k'_w$ and S can be explained by the same solute parameters used to describe $\ln k'$. Zou *et al.* [17] showed that eqn. 14 and 15 are valid for mobile phases containing methanol, acetonitrile or tetrahydrofuran as the organic modifier. The subscripts w and s designate fitting coefficients for $\ln k'_w$ and S, respectively.

$$\ln k'_w = SP_w + m_w V_x + b_w \beta_2 + a_w \alpha_2 + s_w \pi_2^* \quad (14)$$

$$S = SP_s + m_s V_x + b_s \beta_2 + a_s \alpha_2 + s_s \pi_2^* \quad (15)$$

The LSER equations of $\ln k'_w$ and S for the data listed in Table 1 are as shown in eqns. 16 and 17. The $a\alpha_2$ terms for both equations are not included because they are not significant. They will be further discussed below.

$$\ln k'_w = (1.49 \pm 0.35) + (8.87 \pm 0.25)V_x/100 - (10.97 \pm 0.71)\beta_2 - (1.70 \pm 0.38)\pi_2^*$$

$$n = 18, \text{S.D.} = 0.554, R = 0.9947 \quad (16)$$

$$S = (3.24 \pm 0.34) + (7.62 \pm 0.24)V_x/100 - (8.38 \pm 0.68)\beta_2 - (1.28 \pm 0.36)\pi_2^*$$

$$n = 18, \text{S.D.} = 0.528, R = 0.9938 \quad (17)$$

Based on the LSER model, we now consider which conditions must obtain such that S values will be linearly correlated with the $\ln k'_w$ values. When "$\ln k'_w$–S compensation" is chemically valid, then all compounds must coelute at φ_{iso}, where they will have the same $\ln k'$ value, *i.e.*,

$$\begin{aligned}\ln k' &= \ln k'_w - S\varphi_{\text{iso}} \\ &= (SP_w - \varphi_{\text{iso}} SP_s) + (m_w - \varphi_{\text{iso}} m_s)V_x \\ &\quad + (b_w - \varphi_{\text{iso}} b_s)\beta_2 + (a_w - \varphi_{\text{iso}} a_s)\alpha_2 \\ &\quad + (s_w - \varphi_{\text{iso}} s_s)\pi_2^* = \text{constant} \quad (18)\end{aligned}$$

There are two extreme cases in which eqn. 18 can be mathematically satisfied. First, if we assume that the retention process is such that only one molecular property of the solute influences retention, then in fact exact compensation must be observed. For the sake of argument and to be specific, suppose that all the coefficients in eqn. 18 other than m were zero. Furthermore, let us assume that the term SP_0 or $(SP_w - \varphi_{\text{iso}} SP_s)$ in eqn. 18 is independent of mobile phase composition. This would then lead to the equation:

$$\ln k' = SP_0 + (m_w - \varphi_{\text{iso}} m_s)V_x \quad (19)$$

It follows from eqn. 19 that all solutes will be equally retained when:

$$\varphi_{\text{iso}} = m_w/m_s \quad (20)$$

This result is in complete accord with classical extra-thermodynamic considerations that lead to "enthalpy–entropy compensation", that is, compensation phenomena in chromatography can and *must* take place when a single characteristic type of property of the solute governs changes in retention as the solute is varied. As stated above in RPLC, linear solvation energy relationships have shown that there are two very important terms in eqn. 13. Both m and b coefficients are large and thus changes in V_x and β_2 strongly influence retention. The a and s coefficients in RPLC are generally much smaller, and in many cases the a coefficient is seen to be statistically zero [53–56]. The LSER analysis of $\ln k'_w$ and S based on data in Table 1 (see eqns. 16 and 17) also show that there is more than a single explanatory parameter. V_x and β_2 are the two most important parameters while π_2^* is less significant, and the $a\alpha_2$ term is not significant.

The results of LSER analysis of $\ln k'_w$ and S based on data taken from other literature are also given in Table 5. The data includes RPLC analysis carried out with three different organic modifiers: methanol, acetonitrile and tetrahydrofuran. It is important to note that in accord with analysis of $\ln k'$ data at a single composition, two or more solute parameters are needed to explain

TABLE 5

LSER ANALYSIS OF ln k'_w AND S FROM LITERATURE

Ref.		m[a]	b[a]	a[a]	s[a]	n	r[b]	S.D.[b]
Modifier = methanol								
43	ln k'_w	8.87 ± 0.25	−10.97 ± 0.71		−1.71 ± 0.38	18	0.995	0.554
	S	7.62 ± 0.24	−8.38 ± 0.68		−1.28 ± 0.36	18	0.994	0.528
18	ln k'_w	4.69 ± 0.19	−3.23 ± 0.32			25	0.982	0.277
	S	3.72 ± 0.16	−1.71 ± 0.26			25	0.981	0.226
20	ln k'_s	3.43 ± 0.28	−6.45 ± 0.64			14	0.983	0.171
	S	3.13 ± 0.26	−3.45 ± 0.59			14	0.976	0.158
26	ln k'_w	9.09 ± 1.22	−6.83 ± 1.97		−3.57 ± 1.41	20	0.918	0.933
	S	7.32 ± 1.25	−4.72 ± 2.02		−2.64 ± 1.45	20	0.956	0.865
44	ln k'_w	3.43 ± 0.36	−2.45 ± 0.31	−0.74 ± 0.13		27	0.931	0.184
	S	3.63 ± 0.39	−1.53 ± 0.34	−0.67 ± 0.14		27	0.918	0.204
Modifier = acetonitrile								
26	ln k'_w	2.96 ± 0.25	−3.40 ± 0.32	−0.91 ± 0.24		21	0.974	0.219
	S	1.19 ± 0.49	0.79 ± 0.63	1.62 ± 0.47		21	0.750	0.433
44	ln k'_w	3.04 ± 0.18	−2.85 ± 0.20	−0.69 ± 0.10	−0.51 ± 0.11	40	0.977	0.147
	S	3.77 ± 0.22	−2.88 ± 0.24	−0.44 ± 0.12	−0.46 ± 0.14	40	0.970	0.176
Modifier = tetrahydrofuran								
26	ln k'_w	3.15 ± 0.32	−5.08 ± 0.41			21	0.962	0.293
	S	3.57 ± 0.63	−2.74 ± 0.81			21	0.825	0.586
44	ln k'_w	2.43 ± 0.19	−3.90 ± 0.22			40	0.957	0.192
	S	3.57 ± 0.29	−5.03 ± 0.33			40	0.945	0.295

[a] m, b, a and s denote m_w, b_w, a_w and s_w in eqn. 14 and m_s, b_s, a_s and s_s in eqn. 15, respectively.

[b] n is the number of data points, while r and S.D. denote correlation coefficient and average standard deviation of the LSER equations, respectively.

ln k'_w and S in all instances, with V_x and β_2 being the two most important parameters. Of course, the LSER approach is not exact and in any case there are many other models of retention in RPLC. Lochmüller *et al.* [57] have used model-independent chemometric approaches to explore retention in RPLC, and conclude that there are two principal solute dependent abstract factors that control retention.

The fact that we did not observe "S–ln k'_w compensation" for the full data set indicates that there is more than one retention controlling parameter that varies as the solute is changed. However, when we consider the alkylbenzene subset we see that "S–ln k'_w" compensation is really observed (see Fig. 4). In fact several research groups have pointed out that for a given homologous series of solutes, plots of ln k' *vs.* φ do converge and intersect at a single point [58,59]. However, the full significance of this observation was not considered in the context of "S–ln k'_w compensation". Thus the compensation observed with the alkylbenzenes is not a spurious statistical artifact. Classical extrathermodynamic considerations suggest the existence of a single retention controlling solute parameter for this subset of the data. This is in good agreement with the LSER approach. Based on the properties given in Table 1, we see that the only substantial difference between the various alkylbenzenes is their size. For all practical purposes, the variations in β_2, α_2 and π^*_2 are negligible. Thus we expect compensation to be observed for this subset. Given that solute size is so influential in RPLC, it is unlikely that one could find a set of solutes in which there were substantial differences in any one other solute parameter without also changing a second solute parameter. Consequently in accord with our analysis of "enthalpy–entropy compensation" in gas chromatography [34], we conclude that "S–ln k'_w compensation" will always be observed when dealing with a homologous series and unlikely to be observed in chemically variegated datasets.

In the context of a specific model of retention, such as the LSER model, we can also test whether "S–ln k'_w compensation" is chemically valid by introducing additional solute explanatory parameters into the correlation between S and ln k'_{ave} and seeing if the correlation improves. Notice we use S–ln k'_{ave} instead of S–ln k'_w correlation to get rid of the spurious statistical effect on the goodness of fit. We added the solvatochromic parameters one by one to eqn. 9 and found that addition of β_2 did improve the correlation significantly. The dependence of S on β_2 is significant and rather strong (see eqn. 21). The S.D. in eqn. 21 is statistically better than that in eqn. 9, at the 90% confidence level. This again suggests that S–ln k'_w compensation is not rigorously true and S depends on solute functionality, in accord with the qualitative finding of Quarry *et al.* [25]. This improvement makes good sense with the underlying LSERs for RPLC in that solute size and solute hydrogen bond basicity are found to be the major retention governing factors in RPLC [53–56].

$$S = (6.22 \pm 0.35) + (2.50 \pm 0.12) \ln k'_{ave} + (3.55 \pm 0.81)\beta_2$$

$$n = 18, \text{S.D.} = 0.832, R = 0.9833 \tag{21}$$

The second case in which "S–ln k'_w compensation" could, in principle, be observed is when the ratio of all coefficients in eqns. 14 and 15 are the same:

$$m_w/m_s = b_w/b_s = a_w/a_s = s_w/s_s = \varphi_{iso} \tag{22}$$

Note that each ratio must be equal to φ_{iso}.

This is equivalent to Leffler and Grunwald's [30] postulate that there can be several solute-dependent characteristics that influence the process of interest and "enthalpy–entropy compensation" can still be observed. However, all the processes must have a *common* compensation temperature. In the case of "S–ln k'_w compensation", all processes are required to have the *same* compensation composition (φ_{iso}). This is evidently an exceedingly stringent condition for the general existence of the linear relationship between S and ln k'_w. It is hard to believe that this can take place in general when one considers the very different processes that are involved in each of the individual terms in the LSERs. Table 6 lists the coefficient ratios obtained in the LSER analysis of the data in Table 5, and the goodness of fit of the compensating S–ln k'_w correlations. In most cases, the coefficient ratios are not at all similar, as expected.

However, of all the data sets examined, Harnish *et al.*'s data [43] show the best correlation of S and ln k'_w, and the coefficient ratios (m_w/m_s, b_w/b_s, etc.) are startlingly similar (see Table 6). The near equality of the m and b ratios for this data contributes to the high quality of the relationship between S and ln k'_w for Harnisch *et al.*'s data. However, as shown by all the above mathematical and statistical analysis as per Krug [40], the apparent S–ln k'_w correlation for Harnisch *et al.*'s data set is in fact not chemically rigorous. The goodness of this correlation is largely due to the large fractional population of the data set by a homologue series and secondarily due to the near equality of the ratio of the coefficients as shown in Table 6. Therefore, we are quite sure that the S–ln k'_w correlation in other data sets which have poorer S-ln k'_w correlation and coefficient ratio which are very different are not chemically valid.

There is a third, chemically uninteresting, way in which one could conclude that "S–ln k'_w compensation" is real. Suppose we had a data set in which the goodness of fit of the data to eqn. 1 were poor due either to inherent random error or curvature in plots of ln k' *vs.* mobile phase composition. Furthermore suppose this data set was loaded with many solutes where molecular size was the principal but not sole difference. It is possible in such cases to find concurrence and thus conclude that compensation is not an artifact. Notice that almost half of the data points from Harnisch *et al.* [43] are alkylbenzenes. These help to give the entire data set an impressive S–ln k'_w correlation. However, a good experiment design that included a signifi-

TABLE 6

RATIOS OF LSER COEFFICIENTS

Ref.	m_w/m_s [a]	b_w/b_s [a]	a_w/a_s [a]	s_w/s_s [a]	n [b]	r [b]	S.D. [b]
Modifier = methanol							
43	1.16 ± 0.05	1.31 ± 0.14		1.34 ± 0.48	18	0.996	0.396
18	1.26 ± 0.08	1.89 ± 0.34			25	0.982	0.206
20	1.09 ± 0.13	1.87 ± 0.37			14	0.974	0.158
26	1.24 ± 0.27	1.45 ± 0.75		1.35 ± 0.91	20	0.985	0.311
44	0.94 ± 0.14	1.60 ± 0.41	1.10 ± 0.29		27	0.930	0.180
Modifier = acetonitrile							
26	2.48 ± 1.03	−4.28 ± 3.42	−0.56 ± 0.22		21	0.007	0.618
44	0.81 ± 0.07	0.99 ± 0.11	1.58 ± 0.50	1.12 ± 0.42	40	0.982	0.131
Modifier = tetrahydrofuran							
26	0.88 ± 0.18	1.85 ± 0.57			21	0.796	0.611
44	0.68 ± 0.08	0.77 ± 0.07			40	0.986	0.150

[a] Ratios of coefficients for LSER equations from Table 5.

[b] n is the number of data points, while r and S.D. denote the correlation coefficient and average standard deviation of the S–ln k'_w correlation, respectively.

cant number of chemically distinct solutes that span the space of important chemical variables would circumvent this problem.

Based on the above analysis we conclude that for a variegated set of molecules, that differ in many molecular properties, S and ln k'_w cannot be truly correlated. The apparent S–ln k'_w correlation from such a data set, albeit widely use as the basis of approaches to mobile phase optimization in RPLC [26,28,60], are not fundamentally rigorous.

3.3. *Overview of extra-thermodynamic relations in chromatography*

Above we discussed the two types of chemically valid, namely those that survive the analysis as per Krug [40], extra-thermodynamic relationships that have been observed in chromatography. These are "enthalpy–entropy compensation" and "S–ln k'_w compensation". Such behavior *must* be observed when there is only a single solute dependent parameter that controls retention. We suggest that chromatographic systems and data sets that exhibit this property be termed "*iso-retentive*" systems. It should be recognized that a homologous series is a trivial case of an "iso-retentive" system. One must also recognize that both "enthalpy–entropy compensation" and "S–ln k'_w compensation" can exist when there is more than a single solute-dependent retention controlling parameter but in that case there are very strict additional relationships that must obtain (see *e.g.* eqn. 22). We suggest that those chromatographic systems and sets of solutes that exhibit this behavior be termed "*pseudo iso-retentive*" systems.

A third extra-thermodynamic relationship, termed the Collander equation [61,62], is also quite common in chromatography. This observation relates to linearity in plots of logarithmic capacity factors (or partition coefficients) for a set of solutes *vs.* logarithmic capacity factors on a different stationary phase or in a different mobile phase. For example, the linear correlation between log k' for RPLC and logarithmic partition coefficients for the distribution of a solute between octanol and water (K_{ow}) is a well-known example of a Collander relationship. This is actually a linear free energy relationship since the logarithmic partition coefficients and capacity factors are easily converted to free energies by multiplication by RT. Some thought will show that accurate Collander relationships are only possible when the chromatographic systems and sets of solutes under study are in fact "iso-retentive" systems.

In fact, in view of the relationship between S and the free energy of solute transfer from water to pure organic modifier, and the relationship between ln k'_w and the free energy of solute transfer from water to the stationary phase, it is evident that "S–ln k'_w" compensation is simply a specific form of the Collander equation subject to the additional restrictions previously outlined.

In addition, out results agree with the general model of enthalpy–entropy compensation developed by Boots and De Bokx [35,36]. In their model, they describe a suitably defined Gibbs free energy change as the product of a temperature-dependent, system-independent factor $y(T)$ and a temperature-independent, system-dependent factor $\sigma(\{f\})$:

$$\Delta G = y(T)\sigma(\{f\}) \tag{23}$$

They concluded that enthalphy–entropy compensation occurs only when ΔG depends on one parameter. Transposing Boots and De Bokx's model to the present system, where the logarithmic retention capacity factor (ln k') depends only on one solute-dependent parameter

$$\ln k' = SP_0 + v_1(\varphi)v_2(f_2) \tag{24}$$

$v_1(\varphi)$ in eqn. 24 is a mobile phase-dependent, solute-independent factor that is analogous to $y(T)$, while $v_2(f_2)$ is a mobile phase-independent, solute-dependent factor that is analogous to $\sigma(\{f\})$. As per our LSER model, $v_2(f_2)$ can be any of the solute dependent parameter V_x, β_2, α_2 or π_2^*, while $v_1(\varphi)$ is the corresponding dependent of the solute parameter, namely m, b, a or s.

4. CONCLUSIONS

As a minimal first step in avoiding chemically meaningless correlations, we urge that instead of examining plots of S *vs.* ln k'_w that plots of S *vs.* ln k'_{ave} be tested instead. If one is satisfied with the precision of the recommended correlations, then further work including method development should be based on this correlation and not the correlation between the slope and least squares determined intercept.

The existence of "S–ln k'_w compensation" really implies that retention in RPLC is dominated by a single structural/chemical property of a solute, a situation which we term "iso-retentive". Alternatively, "S–ln k'_w compensation" can be observed when the ratio of effects for each retention process for the stationary and mobile phase must be very similar. We call this case "pseudo iso-retentive". The second situation is experimentally much too stringent to be frequently observed. Exactly the same extrathermodynamic logic applies to the observation of "enthalpy–entropy" compensation and Collander type relationships.

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