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ESTIMATION OF THE REVERSED-PHASE LIQUID CHROMATOGRAPHIC LIPOPHILICITY PARAMETER $\log k'_w$ USING ET-30 SOLVATOCHROMISM

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SUMMARY

The estimation of the reversed-phase liquid chromatographic lipophilicity parameter $\log k'_w$ has been studied using the solvatochromic $E_T(30)$ polarity scale. Over 200 sets of chromatographic retention data were used to compare the $\log k'_w$ estimates made from the volume percent of organic modifier (%) and $E_T(30)$ models of mobile phase strength. It was found that linear extrapolations of $\log k'$ versus $E_T(30)$ plots to the polarity of unmodified aqueous mobile phase gave a more reliable value of $\log k'_w$ than linear regressions of $\log k'$ versus volume percent. This evaluation was based upon the relative value of the 95% confidence interval about $\log k'_w$, the point of intersection of $\log k'$ versus solvent strength plots for different modifiers, the scatter of estimations with different modifiers and the goodness-of-fit of the data to the linear model. Estimations of $\log k'_w$ using the solvatochromic approach are found to give a more sensitive lipophilicity description than the volume percent approach. Recommended procedures for carrying out lipophilicity estimations with reversed-phase liquid chromatography are also discussed.

INTRODUCTION

A growing application of reversed-phase liquid chromatography (RPLC) is the estimation of the physico-chemical properties of chemical compounds, also known as quantitative structure-retention relationships (QSRR). QSRR constitutes a large subset of quantitative structure-activity relationships (QSAR). One such physico-chemical property, lipophilicity, is a descriptor of the hydrophobic partitioning character of a compound and has many uses in the environmental and biological sciences^{1,2}. A recent *Chemical Abstracts* database search performed by this laboratory revealed that since 1975 the number of papers published each year regarding chromatography and QSAR has increased steadily. In 1975, the percentage of those papers concerning high-performance liquid chromatography (HPLC) was approximately 12% and as of 1988 that portion has expanded to about 25%.

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Recent reviews have covered research studying the use of RPLC retention data as an estimator of lipophilicity³⁻⁷. The major advantages of using RPLC over shake-flask methods are that it is faster, shows a larger dynamic range of $\log P$, is more convenient to perform experimentally, is extremely reproducible and can be automated. RPLC also works well because precise peak-height or -area quantitation is not necessary and the sample need not be 100% pure. An experiment is usually carried out by obtaining the logarithm of the capacity factor for a solute, $\log k'$. Since the capacity factor is directly proportional to the chromatographic partition coefficient⁸, capacity factors have been used to estimate P .

Various approaches have been devised to obtain capacity factors for lipophilicity information, but one that has received much attention is the parameter $\log k'_w$, the logarithm of the capacity factor using only an aqueous phase as the eluent. The advantage of using $\log k'_w$ is that it is independent of any organic modifier effects, reflects polar-non-polar partitioning in a manner similar to shake-flask methods^{9,10} and is dependent on the solute's structure and polar functionalities¹¹.

The $\log k'_w$ parameter, however, is difficult to measure directly in an RPLC experiment because of poor partitioning kinetics across the stationary phase-mobile phase interface and prohibitively long retention times. It is arduous both to detect and locate a peak centroid because of the skewing of the peaks.

$\log k'_w$ can be estimated by the intercept of the regression equation¹²:

$$\log k' = S\% + \log k'_w \quad (1)$$

where S is Snyder's strength value and $\%$ is the volume percent of organic modifier in the mobile phase. The retentivity of the chromatographic system with respect to changes in mobile phase "strength", denoted here by $\%$, is inferred by S . It has been suggested that $\log k'_w$ itself be a direct measure of lipophilicity for neutral solutes because it minimizes hydrogen bonding effects¹³ and has been found to be reproducible between C_{18} columns used¹⁴. These columns must be from the same manufacturer, however, since $\log k'_w$ should vary from column to column because of bonding density and phase ratio differences.

Despite the utility of $\log k'_w$ as a lipophilicity descriptor, a problem exists in the fundamental detail of its estimation. While eqn. 1 holds on a qualitative basis, curvature exists in these plots¹⁵⁻¹⁷ and could lead to erroneous extrapolation results. A solution to this problem of mobile phase strength characterization for QSRR has been to describe retention data with the equation^{18,19}

$$\log k' = A\%^2 + B\% + \log k'_w \quad (2)$$

where A and B are the mobile phase- and solute-dependent first and second coefficients of the second-order polynomial regression. While a second-order or higher polynomial will almost always give a better fit to the data, additional uncertainty in an extrapolation occurs due to the $A\%^2$ term and that uncertainty can only be reduced by measuring extra data points closer to the point of extrapolation.

One possible solution to the problem of describing mobile phase strength for lipophilicity studies lies in empirical solvatochromic solvent polarity scales. These methods are useful for this purpose because they quantify some of the significant

intermolecular interactions experienced by a solute in the chromatographic system. One such scale, $E_T(30)^{20}$, is attractive for use in RPLC because the ET-30 molecule is readily soluble in RPLC solvents, is spectrally influenced by interactions characteristic to those solvents such as hydrogen bonding, dipolar and charge-transfer interactions, and is extremely sensitive to small changes in organic modifier. It has been shown previously²¹ with 332 sets of RPLC retention data that when solute retention is modeled by the equation

$$\log k' = m[E_T(30)] + b \quad (3)$$

a more linear relationship is found than when using eqn. 1.

If eqn. 3 provides a more linear description of the retention process than eqn. 1, a more accurate estimation of $\log k'_w$ should result upon extrapolation to the polarity of mobile phase containing no organic modifier. It has been reported⁹ that values of $\log k'_w$ estimated by eqn. 1 are dependent on the modifier used. Also, since the $E_T(30)$ polarity scale shows a linear relationship with $\log k'$, eqn. 3 should produce results rivaling or surpassing results from using eqn. 2. A prior study using homologous alcohols and acetonitrile as RPLC organic modifiers²² has shown two important conclusions. When the retention data for solutes were plotted with both eqns. 1 and 3 using methanol, ethanol and acetonitrile as single organic modifiers, (i) the average difference between estimated $\log k'_w$ values between two different modifiers was improved by 40% with the $E_T(30)$ model compared to volume % modifier and (ii) $E_T(30)$ plots for all three modifiers converged toward the polarity of water while volume percent plots diverged at 0% modifier. These conclusions imply that a solvatochromic model of eluent strength, like $E_T(30)$, may yield reliable values of $\log k'_w$ in more than just one modifier.

These types of convenient linearizations are likely the best application of solvatochromic measurements in liquid chromatography as it is not clear that any type of true fundamental information can be obtained from them. This paper compares the estimation of $\log k'_w$ by the $E_T(30)$ and % models of solute retention through the calculation of comparative figures of merit. A brief discussion of recommended execution of lipophilicity experiments using estimated $\log k'_w$ values is also presented.

EXPERIMENTAL

Solvatochromic measurements

Solvatochromic solvent polarity measurements were made on binary solutions of pure organic solvent and aqueous buffer using ET-30 (Reichardt's dye) (Aldrich, Milwaukee, WI, U.S.A.). The organic solvents were Fisher (Austin, TX, U.S.A.) HPLC-grade methanol and acetonitrile and all water used was purified using a Barnstead (Newton, MA, U.S.A.) purification system. One buffer was composed of 0.02 M 3-morpholinopropanesulfonic acid (MOPS) (Kodak, Rochester, NY, U.S.A.) and 0.2% (v/v) *n*-decylamine (Aldrich) and another made up of 66.6 mM ACS certified sodium phosphate monobasic (Fisher). Both buffers were adjusted to a pH of 7.4 with aqueous sodium hydroxide. Binary organic-buffer solutions were prepared by mixing additive volumes of pure organic solvent and buffer solution in increments ranging from 0 to 100% organic modifier for MOPS buffer and from 0 to 70% for phosphate

buffer. ET-30 was added to the simulated mobile phases to a final concentration of approximately 100 mg/l. Samples were placed into a Fisher 5-cm path length glass cell and spectra obtained with an IBM Instruments (Danbury, CT, U.S.A.) Model 9420/9430 UV-visible spectrophotometer. Six spectra were acquired for each sample and the $E_T(30)$ values averaged. Maximum absorbance wavelengths were determined using a first-derivative algorithm on the instrument. The $E_T(30)$ data were taken every 10% organic and fit to an appropriate degree polynomial using the Crickett Software (Philadelphia, PA, U.S.A.) program STATWORKS run on an Apple (Cupertino, CA, U.S.A.) Macintosh SE microcomputer. Any unmeasured $E_T(30)$ values (*i.e.*, 45% methanol in MOPS buffer) were determined by interpolation. $E_T(30)$ polarity values for methanol-water, acetonitrile-water and ethanol-water mobile phases were the same as those used in previous studies²¹⁻²³.

Retention measurements

All retention measurements were taken from the literature^{18,19,21,22,24,25}. Each reference employed a C₁₈ column and Table I summarizes the pertinent experimental details.

Calculations

All computations were done on the Apple Macintosh SE computer with the exception of the polynomial confidence intervals, which were done on an Apple II 48K microcomputer using the program POLYCONFINT written in this laboratory. Linear and polynomial regression were performed with STATWORKS and all other calculations accomplished with the Microsoft (Redmond, WA, U.S.A.) spreadsheet EXCEL.

TABLE I
EXPERIMENTAL CONDITIONS FOR REVERSED-PHASE RETENTION DATA TAKEN FROM THE LITERATURE FOR LOG k'_w STUDY

| Ref. | Column | Modifiers | t_0 method | Solutes |
|------|---------------------|---|---------------------------|---|
| 22 | Ultrasphere ODS | Methanol Ethanol Acetonitrile | Solvent elution | Nitro-, amino-, alkyl-, keto- and polycyclic aromatics |
| 18 | LiChrosorb RP-18 | Methanol Acetonitrile MOPS ^a <i>n</i> -Decylamine | Solvent elution | Nicotinate esters |
| 21 | Ultrasphere ODS | Methanol Acetonitrile | Solvent elution | Alkylbenzenes |
| 24 | Spherisorb S5 ODS-2 | Methanol Ethanol | NaNO ₃ elution | 5-Dimethylamino- 1-sulfonyl derivatives |
| 25 | Nucleosil 10-RP-18 | Methanol Acetonitrile Tetrahydrofuran | Set $t_0 = 125$ s | Nitro-, amino-, alkyl-, keto-, halo- and polycyclic-aromatics, alcohols, heterocyclics |

^a Morpholinopropanesulfonic acid.

RESULTS AND DISCUSSION

Solvatochromic solvent polarity measurements for neutral electrolyte solutions

Studies have been previously performed on the solvatochromic polarity of surfactant and electrolyte solutions. One study of ionic surfactant solutions²⁶ showed that the $E_T(30)$ polarity of a surfactant solution was dependent on the presence of added buffer salts. Using NMR data, it was shown^{26,27} that the charged groups of ET-30 are coulombically influenced and align with the other oppositely charged groups of the surfactants. Another study using Kosower's Z scale²⁸, which uses a probe similar to ET-30, has also shown solvatochromic polarity increasing with the addition of electrolyte. Table II presents our results showing the effects of increasing the amount of electrolyte on $E_T(30)$ polarity. $E_T(30)$ values were measured for mixtures of organic modifier with neutral buffers and sodium chloride solutions. Within an experimental error of about 0.10 kcal/mole, an increase in polarity is observed with increase in electrolyte concentration. $E_T(30)$ values of electrolyte solutions, however, should be viewed with caution when directly compared to values taken in non-electrolyte solutions because of the added coulombic interactions between the charged functional groups of the dye and the electrolytes. This is evident when comparing the water-organic to electrolyte-organic mixtures.

The $E_T(30)$ polarity of a solution is also influenced by acidity. Langhals²⁹ has shown that $E_T(30)$ values cannot be measured in acidic electrolyte solutions because the phenoxide group of ET-30 becomes protonated and the character of the charge-

TABLE II

DEPENDENCE OF $E_T(30)$ POLARITY ON THE CONCENTRATION OF ELECTROLYTE IN NEUTRAL AQUEOUS-ORGANIC MIXTURES

| <i>Solution</i> ^a | $E_T(30)$ (kcal/mole) | <i>Solution</i> ^a | $E_T(30)$ (kcal/mole) |
|--|-----------------------|---|-----------------------|
| Water- 50% (v/v) methanol | 58.30 | 99 mM phosphate- 40% (v/v) acetonitrile | 57.76 |
| 1 mM NaCl- 50% (v/v) methanol | 58.03 | Water- 60% (v/v) methanol | 57.46 |
| 10 mM NaCl- 50% (v/v) methanol | 58.03 | 10 mM MOPS- 0.2% (v/v) DA- 60% (v/v) methanol | 58.08 |
| 100 mM NaCl- 50% (v/v) methanol | 58.25 | 20 mM MOPS- 0.2% (v/v) DA- 60% (v/v) methanol | 58.73 |
| Water- 40% (v/v) acetonitrile | 57.46 | 31 mM MOPS 0.2% (v/v) DA- 60% (v/v) methanol | 59.29 |
| 31 mM phosphate- 40% (v/v) acetonitrile | 57.40 | | |
| 66 mM phosphate- 40% (v/v) acetonitrile | 57.48 | | |

^a NaCl = sodium chloride; phosphate = sodium phosphate monobasic; MOPS = morpholinopropansulfonic acid; DA = *n*-decylamine.

transfer functionality is destroyed. There are no problems of complete protonation of the dye with the work presented here on account of ET-30 spectra being obtained in mobile phases buffered at pH 7.4. The pK_a of the dye has been reported to be 8.4 in 50% methanol–water²⁶. Based on its pK_a and the pH of the solutions used, ET-30 can sense acidic interactions. Reichardt³⁰ has defined polarity as the total solvating power of the solvent and thus these forces can be included in the $E_T(30)$ “polarity” of basic solutions.

The relationships between the $E_T(30)$ polarity and the volume percent of methanol and acetonitrile added to the buffer solutions are shown in Figs. 1 and 2. The methanol–MOPS buffer curve in Fig. 1 expressed non-linear behavior from 0 to 100%. One explanation of the shape of this plot could be that the probe is more specifically solvated by the MOPS and *n*-decylamine molecules at the high and low percentage methanol regions. Furthermore, the random mixing approximation is likely to fail at these composition extremes. Mobile phases of compositions between 30 and 70% methanol give almost a linear polarity response with respect to %. The acetonitrile–MOPS buffer curve in Fig. 1 shows the polarity decreasing non-linearly from 0 to 80% acetonitrile and rapidly decreasing from 80 to 100%. This behavior is similar to what has been seen with acetonitrile–water mixtures²¹.

Mixtures of phosphate buffer with both methanol and acetonitrile were studied and their polarity profiles are presented in Fig. 2. The $E_T(30)$ polarity for these solutions also exhibited non-linear behavior in the range of % organic compositions studied for both solvent systems. Due to problems with solubility of the phosphate buffer salt with the organic modifiers, the maximum mobile phase compositions allowed were 70% methanol and 60% acetonitrile, so no polarity measurements could be made at organic compositions greater than these.

Estimation of $\log k'_w$ by extrapolation methods

Values of $\log k'_w$ were estimated by extrapolating linear regression plots of $\log k'$ versus % organic for each solute to 0% organic modifier and plots of $\log k'$ versus $E_T(30)$ to the polarity of pure water. If a buffer solution was used as the aqueous component of the mobile phase, the $E_T(30)$ plots were extrapolated to the measured $E_T(30)$ polarity of the pure buffer (63.36 kcal/mole for the MOPS–decylamine buffer).

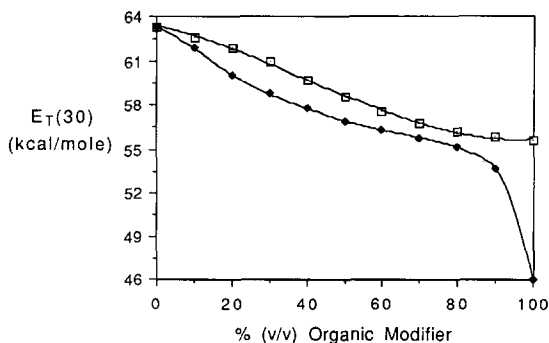


Fig. 1. $E_T(30)$ polarity change as a function of the volume % of organic modifier in a mixture with 0.02 M MOPS (pH 7.4)–0.2% (v/v) *n*-decylamine buffer. □, methanol; ◆, acetonitrile.

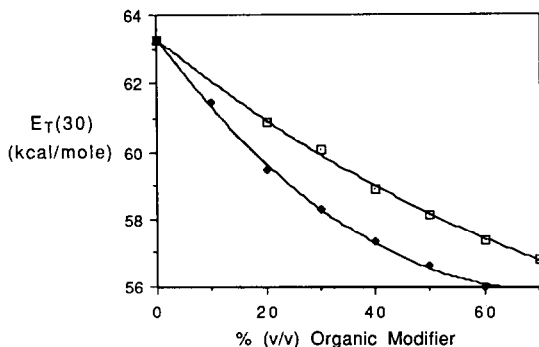


Fig. 2. $E_T(30)$ polarity change as a function of the volume % of organic modifier in a mixture with 66.6 mM phosphate buffer (pH 7.4). □, methanol; ◆, acetonitrile.

Literature data sets were used that had reported $\log k'$ versus % organic on a C_{18} column for both methanol and a second solvent modifier, such as acetonitrile or ethanol^{18,21,22,24,25}. A data set refers to a collection of $\log k'$ values for one solute taken at different mobile phase conditions using one modifier. A summary of the experimental conditions from all of the references can be found in Table I.

Three restrictions were placed on each set of $\log k'$ data used. The first restriction was to not use $\log k'$ values above 90% methanol or 80% of the second modifier. It has previously been discussed^{21,22} that a limitation of the $E_T(30)$ polarity scale used as a measure of mobile phase "strength" for RPLC is the occurrence of specific solvation effects between the probe molecule and the solvent components at high percentages of organic modifier. Changes in the solvent polarity above these % values were found not to relate linearly to $\log k'$; this is no problem, however, because $\log k'$ values measured with more aqueous-rich mobile phases should be used to extrapolate to $\log k'_w$. This reasoning will be explained later in the discussion dealing with confidence intervals. A second restriction was not to use $\log k'$ values much less than -0.30 to try to minimize measurement errors associated with small values of $\log k'$. The last restriction is directly related to the first two such that if a data set contained less than four $\log k'$ values, it was not used. This will also be explained by the confidence interval discussion.

In order to compare the reliability of the $\log k'_w$ values estimated by both the % and $E_T(30)$ models, four figures of merit (FOM) were calculated using a total of 204 data sets. Final results for each reference based entirely on linear extrapolations are summarized in Table III. The first FOM was the correlation coefficient, r , which is a descriptor of the "goodness-of-fit" of a linear model to a set of data. It would be desirable to use a model that gives the most linear description to the data to minimize errors associated with forcing a line through data points that do not express linear behavior. In the previous study of linearity of $\log k'$ versus $E_T(30)$ ²¹, only ten data sets on a C_{18} column were obtained, so r was monitored in this study as well to check if similar linearity improvements over the % model are obtained. From Table III, it can be seen that the average correlation coefficient was greater using the $E_T(30)$ model with the only exception being where the average r for both models was the same. The overall apparent correlation improvement using $E_T(30)$ over % may only be consid-

TABLE III
COMPARATIVE FIGURES-OF-MERIT FOR LOG k'_w STUDY BASED ON LINEAR REGRESSION FOR BOTH THE $E_T(30)$ AND % ORGANIC MODELS

| Ref. | r^a | | $\log k'_w$ | | $E_T(30)$ | | Intersection ^c | | RCI ^d | |
|-------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|------------------------------|-----------------------------|-----------------------------|------------------|--|
| | % | | % | | $E_T(30)$ | % | (v/v) | $E_T(30)$ (kcal) | % | |
| 22 | 0.9966 | 0.9972 | 0.3939 | 0.2435 | 216.95 | 65.65 | 12.41 | 10.39 | | |
| | ± 0.0036 ($N = 30$) | ± 0.0021 ($N = 30$) | ± 0.3170 ($N = 30$) | ± 0.2123 ($N = 30$) | ± 584.37 ($N = 29$) | ± 11.60 ($N = 30$) | ± 7.50 ($N = 27$) | ± 4.01 ($N = 30$) | | |
| 18 | 0.9936 | 0.9986 | 0.5551 | 0.2864 | - 93.22 | 68.75 | 22.28 | 9.82 | | |
| | ± 0.0045 ($N = 36$) | ± 0.0014 ($N = 36$) | ± 0.4395 ($N = 18$) | ± 0.4048 ($N = 18$) | ± 938.95 ($N = 18$) | ± 16.86 ($N = 18$) | ± 10.76 ($N = 35$) | ± 4.78 ($N = 35$) | | |
| 21 | 0.9972 | 0.9991 | 0.5530 | 0.4740 | - 81.94 | 59.70 | 9.58 | 7.06 | | |
| | ± 0.0021 ($N = 10$) | ± 0.0006 ($N = 10$) | ± 0.2405 ($N = 5$) | ± 0.2562 ($N = 5$) | ± 630.13 ($N = 5$) | ± 0.78 ($N = 5$) | ± 5.72 ($N = 9$) | ± 3.17 ($N = 10$) | | |
| 24 | 0.9908 | 0.9983 | 0.9741 | 0.7749 | 182.72 | 60.21 | 11.08 | 4.72 | | |
| | ± 0.0064 ($N = 36$) | ± 0.0011 ($N = 36$) | ± 0.2293 ($N = 18$) | ± 0.2311 ($N = 18$) | ± 107.77 ($N = 18$) | ± 0.28 ($N = 18$) | ± 3.79 ($N = 36$) | ± 1.47 ($N = 35$) | | |
| 25 | 0.9952 | 0.9952 | 0.3931 | 0.4144 | 80.90 | 67.84 | 15.60 | 14.56 | | |
| | ± 0.0034 ($N = 92$) | ± 0.0036 ($N = 92$) | ± 0.2744 ($N = 46$) | ± 0.3552 ($N = 46$) | ± 620.55 ($N = 46$) | ± 62.28 ($N = 46$) | ± 8.02 ($N = 86$) | ± 7.74 ($N = 92$) | | |
| Total | 0.9945 | 0.9968 | 0.5144 | 0.4089 | 96.68 | 65.13 | 15.24 | 11.04 | | |
| | ± 0.0047 ($N = 204$) | ± 0.0030 ($N = 204$) | ± 0.3675 ($N = 117$) | ± 0.3532 ($N = 117$) | ± 625.80 ($N = 116$) | ± 39.90 ($N = 117$) | ± 8.69 ($N = 193$) | ± 6.91 ($N = 202$) | | |

^a r = correlation coefficient.

^b $\log k'_w$ = difference between $\log k'_w$ values estimated using two different modifiers.

^c Intersection = % or $E_T(30)$ intersection point between regressions using two different modifiers.

^d RCI = relative confidence interval about $\log k'_w$.

ered marginal, however, since all of the average r values are greater than 0.99. It was found that retention plots using methanol as the modifier were comparable between the two models but acetonitrile and ethanol showed much better linearity with the $E_T(30)$ model as compared to volume %. An F -test³¹ of 95% confidence performed on the variances of the averaged correlation coefficients for both models, however, determined the two average r values ($n = 204$) to be significantly different. These results solidify previous findings on linearity improvements and support the observation that a solvatochromic polarity scale such as $E_T(30)$ provides a more linear description of the strength of the mobile phase than the bulk organic modifier composition.

A second FOM was $\Delta \log k'_w$, the difference between the $\log k'_w$ values estimated by two different modifiers. Since it would often be useful to employ another modifier, the difference between estimations by the two modifiers should be minimized. Furthermore, agreement of $\log k'_w$ values from two (or more) modifiers lends confidence to the accuracy of the extrapolated value. Previous work has shown that for 27 data sets a 40% decrease in the $\Delta \log k'_w$ occurred when using the $E_T(30)$ model over %²². Table III shows a $\Delta \log k'_w$ improvement of roughly 21% when using the $E_T(30)$ model for all reference data sets ($N = 117$) [$\Delta \log k'_w = 0.4089$ for $E_T(30)$ and 0.5144 for %].

The third FOM is the extrapolated intersection point of linear regressions for a solute using two different modifiers. This intersection determines if two different retention plots converge toward a point indicating an unmodified aqueous mobile phase. If a given retention model describes the strength of the mobile phase in a useful manner, then % plots should converge to 0% organic or the $E_T(30)$ plots to 63.11 kcal/mole (the polarity of pure water). It will be defined here that % intersections less than 50% organic and $E_T(30)$ intersections greater than a polarity of 58 kcal/mole (the approximate polarity of 50% organic mobile phase) will be considered converging toward $\log k'_w$. From Table III it can be seen that all of the average intersections of the $E_T(30)$ data sets converged toward the polarity of water while it is difficult to infer the same about the % data. Some of the average % intersections were negative, some were positive and the standard deviations of those averages were large.

In order to extract more meaning out of the intersection results, frequency histograms were constructed to demonstrate the distribution of these values. Fig. 3 shows the spread of the % intersections covering a range from -200 to 500% organic for 97 of the 114 total data sets. Some intersections could not be calculated because the two retention plots of interest were parallel. It was found that 70% of those intersections occurred at values greater than 50% organic, with 41% of the total sets being in the range between 100 and 200% organic. Fig. 4 illustrates the spread of the $E_T(30)$ intersections in the range from 55 to 70 kcal/mole for 94 of the 117 total data sets. It was found that 78% of these intersections were greater than 58 kcal/mole, with 42% of the total sets occurring between 60 and 63 kcal/mole. The mentioned intersection results indeed show that linear regressions of $\log k'$ versus $E_T(30)$ do converge toward the polarity of a pure aqueous mobile phase while $\log k'$ versus % plots diverge at 0% organic modifier. Based on these intersection results, a more useful estimation of the partitioning processes occurring in a chromatographic system at 100% aqueous mobile phase may be obtained with the $E_T(30)$ solvent strength model.

A last FOM is the relative confidence interval, RCI. The RCI for a solute using a particular organic modifier indicates the relative uncertainty in a $\log k'_w$ value and

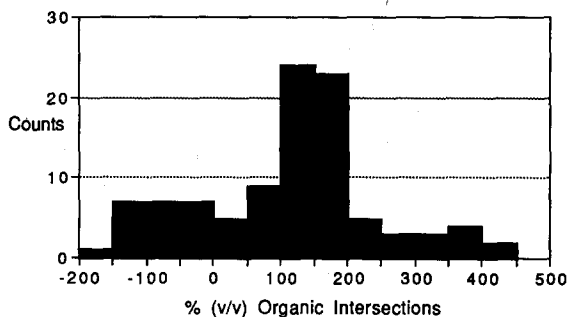


Fig. 3. Frequency histogram of the distribution of linear-linear intersections between plots of $\log k'$ versus % methanol and $\log k'$ versus % acetonitrile or ethanol.

can be compared with other solutes in the same and another retention model. For a given solute this figure is calculated by the equation

$$RCI = [(95\% \text{ CI})/\log k'_w] \cdot 100 \quad (4)$$

where 95% CI is the 95% confidence interval at $\log k'_w$ and is the product of Student's t -value and the standard deviation of the regression at $\log k'_w$ ^{31,32}. The 95% confidence interval is a function of the residual error of the regression, the number and spread of the data points on the ordinate (or x) axis and the difference between the centroid of the x values and the predicting x [0% organic or the aqueous $E_T(30)$]. A minimum in an RCI would occur for a predicted y value that was positioned near the mean of the x values of a regression model having a correlation coefficient near 1 and consisting of a large number of data points ($N > 10$) spanning a wide range of x values. Based on these statistical criteria, the data points taken to perform the extrapolation to $\log k'_w$ should be done with water-rich mobile phases as close to 0% modifier added as possible. This is why the limitation of the $E_T(30)$ polarity scale at very high organic compositions does not become a limiting factor, because at high % organic the extrapolated value is much less reliable. Also, at least four $\log k'$ values are needed for a linear extrapolation (where the degrees of freedom for the regression equal the number of data points minus the number of parameters in the regression

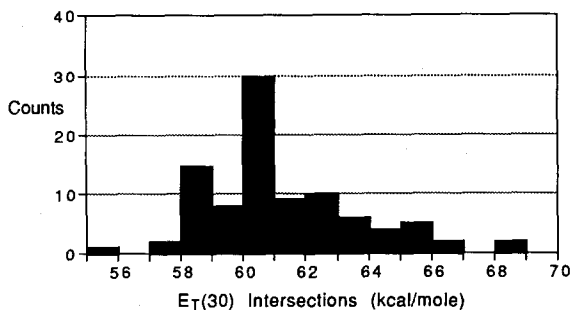


Fig. 4. Frequency histogram of the distribution of linear-linear intersections between plots of $\log k'$ versus $E_T(30)$ for methanol-water mixtures and $\log k'$ versus $E_T(30)$ for acetonitrile-water or ethanol-water mixtures.

model, $N - P$) because the Student's t -value for less than four data points increases significantly³².

The RCI results are shown in Table III. For all data sets ($N = 193$ for % and 202 for E_T), the overall relative uncertainty in the estimated $\log k'_w$ values is lower when using the $E_T(30)$ model. An F -test statistically verified this observation. The greatest difference in RCI between the two retention models is with the data sets of Raymond *et al.*¹⁸ and Lehtonen²⁴ because data points were taken as low as 10% organic in many cases and the $E_T(30)$ plots were very linear. The average of the RCI data of Johnson *et al.*²¹, Michels and Dorsey²² and Schoenmakers *et al.*²⁵ did not favor the $E_T(30)$ model to as large an extent, but the standard deviation of those RCIs was also lower. As was seen with $\Delta \log k'_w$, the magnitude of improvement in RCI varied with the data reference because some experiments were done with more $\log k'$ data points than others but in all cases the $E_T(30)$ data was more favorable than the % data.

The four figures of merit in this study were also recalculated for the data sets using the second modifiers acetonitrile and ethanol in which a polynomial regression was fit to the data. Table IV presents a comparison of the polynomial % and the $E_T(30)$ results from Table III. The r and RCI results are only for the polynomial regressions of acetonitrile and ethanol and the $\Delta \log k'_w$ and intersection results are for linear methanol data used with polynomial acetonitrile or ethanol data. As statistically expected, the polynomial regression yielded correlation coefficients of almost 1. Another positive aspect of using the polynomial over the linear regression is seen in the $\Delta \log k'_w$ and intersections data, where the scatter in the $\log k'_w$ values was reduced and the regression models intersected near 0% organic. Fig. 5 exhibits the frequency histogram for the intersections between the linear methanol plots and the polynomial acetonitrile or ethanol plots. All of these data were found to converge to 0% organic with 82% of them occurring between -10 and 20% organic.

Relative confidence intervals were also calculated for the polynomial-extrapolated $\log k'_w$ values. It was found that the RCIs for the % polynomial fits were still larger than those for $E_T(30)$ and determined to be statistically different by an F -test. An added level of uncertainty is contributed to the % organic retention model by the squared-term of the second-order polynomial equation. The number of degrees of freedom when calculating a second-order polynomial will be a value of one larger than that for a linear regression because the value of P increases by 1. A solution with which to improve the RCI for polynomial estimated $\log k'_w$ is to measure at least five or six points on the $\log k'$ versus % plot toward 0% organic and thus increase the number of degrees of freedom of the regression. While this a worthy solution, the time of analysis would be greatly increased over using a linear solvatochromic model which only requires four or five points.

Procedures for the estimation of $\log k'_w$

Certain procedures should be followed to ensure reliable results from the estimation of $\log k'_w$ by extrapolation methods. Both the solvatochromic and linear-polynomial % models give reasonable statistical results. It is recommended that only polynomial fits should be used for acetonitrile or ethanol % plots. The $\log k'_w$ values estimated by both models, however, are different and deciding which model gives the most accurate estimation of the lipophilic properties of solutes could only be done by

TABLE IV
 COMPARATIVE FIGURES OF MERIT FOR LOG k'_w STUDY BASED ON LINEAR REGRESSION FOR $E_T(30)$ AND % MODELS USING METHANOL AS MODIFIER AND SECOND-ORDER POLYNOMIAL REGRESSION FOR % MODEL USING ETHANOL AND ACETONITRILE AS MODIFIERS (ONLY % MODEL POLYNOMIAL DATA SHOWN FOR r AND RCI)

| Ref. | r^a | | $\Delta \log k'_w$ | | Intersection ^c | | RCI ^d | |
|-------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|-----------------------------|------------------------------|-----------------------------|-----------------------------|
| | % | $E_T(30)$ | % | $E_T(30)$ | % (v/v) | $E_T(30)$ (kcal) | % | $E_T(30)$ |
| 22 | 0.9996 ±0.0005 (N = 20) | 0.9972 ±0.0021 (N = 30) | 0.3207 ±0.2029 (N = 30) | 0.2435 ±0.2123 (N = 30) | 9.54 ±16.55 (N = 24) | 65.65 ±11.60 (N = 30) | 19.74 ±14.77 (N = 20) | 10.39 ±4.01 (N = 30) |
| 18 | 0.9999 ±0.0000 (N = 18) | 0.9986 ±0.0014 (N = 36) | 0.1241 ±0.1011 (N = 18) | 0.2864 ±0.4048 (N = 18) | -1.24 ±5.73 (N = 18) | 68.75 ±16.86 (N = 18) | 11.71 ±11.58 (N = 18) | 9.82 ±4.78 (N = 35) |
| 21 | 0.9998 ±0.0001 (N = 5) | 0.9991 ±0.0006 (N = 10) | 0.1599 ±0.0420 (N = 5) | 0.4740 ±0.2562 (N = 5) | 6.00 ±1.60 (N = 5) | 59.70 ±0.78 (N = 5) | 8.03 ±1.03 (N = 5) | 7.06 ±3.17 (N = 10) |
| 24 | 0.9993 ±0.0009 (N = 18) | 0.9983 ±0.0011 (N = 36) | 0.4198 ±0.1597 (N = 18) | 0.7749 ±0.2311 (N = 18) | 9.33 ±3.24 (N = 18) | 60.21 ±0.28 (N = 18) | 7.73 ±2.82 (N = 18) | 4.72 ±1.47 (N = 35) |
| 25 | 0.9991 ±0.0018 (N = 47) | 0.9952 ±0.0036 (N = 92) | 0.1739 ±0.1381 (N = 46) | 0.4144 ±0.3552 (N = 46) | 4.13 ±12.78 (N = 45) | 67.84 ±62.28 (N = 46) | 13.53 ±6.33 (N = 46) | 14.56 ±7.74 (N = 92) |
| Total | 0.9995 ±0.0008 (N = 107) | 0.9968 ±0.0030 (N = 204) | 0.2411 ±0.1838 (N = 117) | 0.4089 ±0.3532 (N = 117) | 5.37 ±12.03 (N = 110) | 65.13 ±39.90 (N = 117) | 13.15 ±9.68 (N = 107) | 11.04 ±6.91 (N = 202) |

^a r = correlation coefficient.

^b $\Delta \log k'_w$ = difference between $\log k'_w$ values estimated using two different modifiers.

^c Intersection = % or $E_T(30)$ intersection point between regressions using two different modifiers.

^d RCI = relative confidence interval about $\log k'_w$.

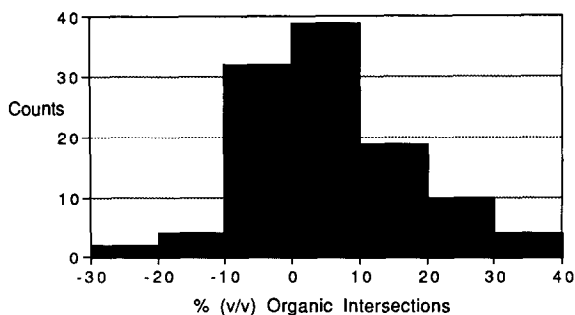


Fig. 5. Frequency histogram of the distribution of intersections between linear plots of $\log k'_w$ versus % methanol and polynomial plots of $\log k'_w$ versus % acetonitrile or ethanol.

comparative QSSR or QSAR studies with real biological and environmental partitioning data. This work is currently underway in our laboratory. Table V shows the results of correlation analysis between $\log k'_w$ and $\log P_{ow}$ for the data of Reymond *et al.*¹⁸, Michels and Dorsey²² and Schoenmakers *et al.*²⁵. The values of $\log P_{ow}$ were taken from shake-flask measurements³³ and calculation¹⁸. A separate correlation was done for each mobile phase system and the slopes, intercepts and correlation coefficients are reported. Fig. 6 shows an example correlation between $E_T(30)$ -estimated $\log k'_w$ from the data of Schoenmakers *et al.*²⁵ and $\log P_{ow}$. It can be seen from Table V that for all solvent systems the $E_T(30)$ -estimated $\log k'_w$ values give larger slopes and comparable correlation coefficients. This implies that the solvatochromic method gives a more sensitive scale with which to measure solute lipophilicity, but not a direct measure of $\log P_{ow}$ values. $\log k'_w$ has been proposed to be a useful lipophilicity descriptor by itself¹⁴ and these results support this belief.

A recent paper by Minick *et al.*³⁴ reported attempting the use of the $E_T(30)$ polarity scale for $\log k'_w$ extrapolations. The authors, however, did not carry out any

TABLE V

RESULTS OF THE CORRELATION ANALYSIS OF $\log k'_w$ VERSUS $\log P_{ow}$ USING DIFFERENT BINARY HYDROORGANIC MOBILE PHASES FOR THE $E_T(30)$ AND % MODELS

| Parameter | Methanol-water | | Ethanol-water | | Acetonitrile-water | |
|------------------------|----------------|-----------|---------------|-----------|--------------------|-----------|
| | % (v/v) | $E_T(30)$ | % (v/v) | $E_T(30)$ | % (v/v) | $E_T(30)$ |
| Slope ^a | 0.936 | 1.41 | 0.979 | 1.25 | 0.871 | 1.11 |
| Intercept ^a | 0.203 | -0.166 | 0.366 | 0.187 | 0.338 | 0.700 |
| r^a | 0.971 | 0.970 | 0.924 | 0.937 | 0.976 | 0.983 |
| Slope ^b | 0.880 | 1.28 | | | 0.832 | 0.958 |
| Intercept ^b | 0.148 | -0.622 | | | 0.243 | -0.040 |
| r^b | 0.982 | 0.976 | | | 0.970 | 0.982 |
| Slope ^c | 0.814 | 1.53 | | | 0.769 | 1.10 |
| Intercept ^c | 0.536 | -0.287 | | | 0.662 | 0.510 |
| r^c | 0.960 | 0.959 | | | 0.907 | 0.881 |

^a $N = 10$ from ref. 22.

^b $N = 18$ from ref. 18 (ethanol-water not used).

^c $N = 34$ from ref. 25 (ethanol-water not used).

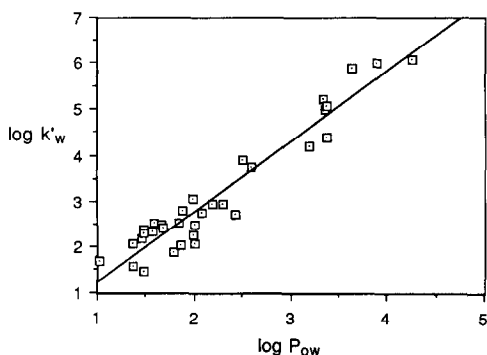


Fig. 6. Correlation between $E_T(30)$ -estimated $\log k'_w$ from the data of Schoenmakers *et al.*²⁵ and octanol-water partition coefficients³³.

extrapolations of $\log k'$ versus $E_T(30)$ plots because of observed non-linearity. A normalization procedure producing " $\Delta E_T(30)$ " values was used as the solvent strength descriptor instead of directly measured $E_T(30)$. Using any type of normalized $E_T(30)$ values, such as Reichardt's E_T^N values³⁰, distorts the behavior of the retention model and is not recommended. They did show graphically, however, that the extrapolated $\log k'_w$ values give a more accurate measure of lipophilicity than using isocratic values. The elution order of solutes can invert at lower % organic ranges to yield incorrect lipophilicity assignments.

Following sound statistical practice is important for estimating $\log k'_w$ values of minimal uncertainty. Data points on a plot of $\log k'$ versus mobile phase strength should be taken in water-rich eluents as close as possible to the value of unmodified aqueous mobile phase. The variance in the regression model is dependent on the centroid as well as the spread and number of the data points along the ordinate axis. Ideally, $\log k'$ values should be taken down to 10% modifier, but the chromatographic partition coefficient limits this process so that the lowest modifier composition for the elution of some compounds is roughly 50% methanol. Since the solvatochromic $E_T(30)$ model yields linear retention behavior for modifiers that are slightly stronger than methanol, like acetonitrile and ethanol, and $E_T(30)$ plots for all three of these modifiers converge toward the polarity of unmodified mobile phase, a solvatochromic model would be useful for this type of experiment. Using organic modifiers stronger than acetonitrile or ethanol, such as propanol or tetrahydrofuran, is not desirable, however, because of specific solute³⁵ and stationary phase^{22,36} solvation effects that produce a different mechanism of retention. It is also crucial to measure accurately the void time of the chromatographic system since t_0 is dependent on the bulk composition of the mobile phase, but there is much debate over the best way to measure this parameter³⁷⁻⁴¹.

To study statistically how the reliability of a $\log k'$ versus solvent strength extrapolation changes with the range and centroid of the data points, a data set with twelve points covering a wide range of solvent compositions was used. In Table VI the data point closest to pure water was removed in succession until five data points remained in the high organic portion of the plot. As the range and number of data points became smaller and more removed from water, the value of the RCI became larger. Also note that for both solvent strength models the value of $\log k'_w$ became

TABLE VI

CHANGE IN THE RELATIVE CONFIDENCE INTERVAL (RCI) ABOUT $\log k'_w$ ESTIMATED WITH METHANOL-WATER MOBILE PHASES FOR METHYLAMINO-5-DIMETHYLAMINO-1-SULFONATE²⁴ AS A FUNCTION OF THE SPANNING OF $\log k'_w$ VALUES TOWARD PURE WATER

| Lowest % (v/v) | N | $E_T(30) \log k'_w$ | $E_T(30) RCI$ | % $\log k'_w$ | % RCI |
|----------------|----|---------------------|---------------|---------------|----------------|
| 35 | 12 | 3.5781 | 4.48 | 2.7730 | 12.92 |
| 40 | 11 | 3.4789 | 3.57 | 2.6267 | 12.53 |
| 45 | 10 | 3.4775 | 4.53 | 2.4895 | 12.08 |
| 50 | 9 | 3.4390 | 5.53 | 2.3742 | 13.50 |
| 55 | 8 | 3.4415 | 8.04 | 2.2246 | 14.60 |
| 60 | 7 | 3.3964 | 14.15 | 2.0761 | — ^a |
| 65 | 6 | 3.2276 | 16.78 | 1.8999 | 25.24 |
| 70 | 5 | 3.0366 | 29.74 | 1.6880 | 32.39 |

^a Calculation yielded an empty set result.

smaller when the data points were in the organic-rich region. Table VII shows the effects of shifting the centroid of the ordinate axis further from water when the range of the data points is kept constant. As the centroid reaches a value of 75% organic, the uncertainty in the extrapolation with both models becomes very large. These results illustrate that $\log k'_w$ values must be measured in water-rich mobile phases to keep the uncertainty about the extrapolated $\log k'_w$ value at as low a level as possible.

Of the 117 intersections calculated in this paper using the $E_T(30)$ model, fourteen were found not to converge to the polarity of unmodified mobile phase. Of those fourteen divergent sets, ten included a nitrogen-containing functional group that could be highly interactive with a surface silanol (eighteen nitrogen-containing sets altogether). We have also investigated the use of $E_T(30)$ -estimated $\log k'_w$ values for the calculation of solute-solvent contact free energy in RPLC³⁶ and have found the same problem of nitrogen-containing compounds interacting with surface silanols.

Employing a stationary phase that provides the most possible non-polar surface would be desirable since the influence of residual silanols would be minimized. Silanol interactions can be negated by using masking agents in the mobile phase or by

TABLE VII

CHANGE IN THE RELATIVE CONFIDENCE INTERVAL (RCI) ABOUT $\log k'_w$ ESTIMATED WITH METHANOL-WATER MOBILE PHASES FOR METHYLAMINO-5-DIMETHYLAMINO-1-SULFONATE²⁴ AS A FUNCTION OF THE MOVEMENT OF THE ORDINATE CENTROID FROM PURE WATER

Four values of $\log k'$ are taken in each % range, with each $\log k'$ taken every 10% organic.

| % (v/v) range | $E_T(30) RCI$ | Average $E_T(30)$ | % RCI | Average % |
|---------------|----------------|-------------------|-------|-----------|
| 35-65 | 10.18 | 58.28 | 22.91 | 50 |
| 40-70 | 6.29 | 57.94 | 16.27 | 55 |
| 45-75 | — ^a | 57.56 | 15.84 | 60 |
| 50-80 | 11.13 | 57.24 | 25.20 | 65 |
| 55-85 | 8.67 | 56.93 | 29.83 | 70 |
| 60-90 | 33.08 | 56.64 | 49.70 | 75 |

^a Calculation yielded an empty set result.

using monomeric stationary phases of very high alkyl ligand bonding density. Studies using masking agents like tetraethylamine or *n*-decylamine in the mobile phase^{42,43} have observed that for hydrogen-bond accepting solutes, the masking agent binds to the silanol and allows the polar solute to interact primarily with hydrophobic ligands. A disadvantage with using these agents, however, is that they can form ion pairs with specific solutes and alter the measured hydrophobicity. Another method of minimizing silanol interactions is by exhaustively end-capping and/or producing a high bonding density C₁₈ phase. It has been shown that exhaustive end-capping with a C₁ functional group deactivates surface silanols⁴⁴ and for small, polar solutes, directly measured log k'_w values are more reproducible⁴⁵.

CONCLUSION

The RPLC estimation of solute lipophilicity, in the form of the parameter log k'_w , has been discussed using the solvatochromic $E_T(30)$ solvent polarity scale. Comparative figures of merit were calculated for 204 data sets to contrast the % and $E_T(30)$ models of mobile phase strength. Results show that the $E_T(30)$ model is more useful than the linear % model because of increased statistical confidence in log k'_w values. Calculations done with a polynomial % model on retention data generated with acetonitrile and ethanol modifiers found log k'_w values statistically comparable to those estimated by linear % extrapolations of methanol-generated data. The $E_T(30)$ -estimated log k'_w values, however, were still statistically more certain than polynomial % estimated values. Correlations relating log k'_w and log P_{ow} have shown $E_T(30)$ -estimated log k'_w values producing a more sensitive scale of hydrophobicity than %-estimated values.

Suggested guidelines for the execution of RPLC lipophilicity estimations have been presented. The most desirable chromatographic system would be an end-capped high bonding density C₁₈ stationary phase and an aqueous mobile phase modified with methanol, ethanol or acetonitrile. A high bonding density stationary phase would minimize interactions between the solute and surface residual silanols and maximize the partitioning capabilities of the chromatographic system. Using organic modifiers stronger than acetonitrile or ethanol such as propanol or tetrahydrofuran would be undesirable because the mechanism of retention changes with the stronger solvents. Retention measurements for a solute of interest should be taken over a minimal range of 40% organic modifier with the most water-rich mobile phase used being as close to unmodified eluent as possible. Qualitative structure-activity relationships for solutes of biological and environmental interest also need to be performed using log k'_w values estimated by $E_T(30)$, and this work is ongoing in this laboratory.

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