

# Column selection for liquid chromatographic estimation of the $k'_w$ hydrophobicity parameter

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## Abstract

Newer reversed-phase column technologies that incorporate polar groups either by an endcapping procedure or by embedding them into the stationary phase ligand have been receiving much attention in the literature for their robustness when highly aqueous conditions are used. We investigated their ability to accurately determine the chromatographic hydrophobicity value  $\log k'_w$ . The non-linear deviations of retention data as mobile phase conditions approach zero percent modifier are a large source of error when extrapolating to  $\log k'_w$  values using the linear solvent strength model. Here, we compare a conventional reversed-phase stationary phase with others that have incorporated either polar embedded or polar endcapped phases, along with a hybrid-based particle derivatized with a polar embedded ligand. Our results show that polar endcapped phases perform very similarly to the conventional phase and do not show any improved ability for determining  $\log k'_w$ , but polar embedded phases have reduced curvature in the data, and therefore result in less error in extrapolation. We also investigated the solubility parameter model and the  $[E_T(30)]$  model for their extrapolation efficiency, and have concluded that the  $[E_T(30)]$  model shows the least error when extrapolating the data.

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## 1. Introduction

### 1.1. Reversed-phase liquid chromatographic estimations of hydrophobicity

There have been numerous publications describing reversed-phase chromatographic retention as a model of hydrophobicity and correlations to the octanol/water partition coefficient [1–12]. Chromatographic methods offer numerous advantages including speed, reproducibility, greater dynamic range, online detection, and reduced sample handling and sample sizes; when compared to traditional octanol/water partitioning measurements ( $\log P$ ). Also, chromatography is inherently a method of separation so impurities and degradation products are typically non-problematic. The correlation of these two models is based on the assumption that retention in RPLC is due primarily to hydrophobic partitioning between the station-

ary and mobile phases. If so, a linear relationship will exist between the partition coefficients of the two different systems through the Collander equation [13]. This linear relationship will exist for any two-phase aqueous-organic partitioning system as long as hydrophobic interactions are the dominant driving force [6]. The presence of organic modifiers in the mobile phase may lead to breakdown of the Collander relationship because the modifier may disrupt the matrix of hydrogen bonding present in pure water, therefore decreasing the hydrophobic effect. Modifiers can also preferentially solvate non-polar solutes, creating a different environment for the solute compared to the bulk mobile phase. The breakdown of the Collander equation coupled with the unstandardized multitude of data sparked the need for homogeneity in chromatographic conditions used to derive these relationships. The reversed-phase chromatographic retention in pure water ( $k'_w$ ) is generally agreed to be the most useful retention factor for estimating hydrophobicity in RPLC because it has no effects due to organic modifiers, so that the only solvophobic effect is the hydrophobic effect. In RPLC, using a pure water mobile phase most truly represents the polar/non-polar partitioning

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that it is trying to model. The problem with using pure water mobile phases is that the elution strength is too weak for most solutes. To solve this problem,  $\log k'_w$  values are often extrapolated from the plot of the log of the retention factor ( $\log k'$ ) versus fraction of organic modifier ( $\Phi$ ) by use of Snyder's linear solvent strength (LSS) model [14–16]:

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

where  $S$  is the slope of the equation (which is dependent on the system and solute),  $k'$  is the retention factor and  $k'_w$  is the retention factor in pure water. Using typical reversed-phase columns, investigation of the entire range of  $\Phi$  has revealed that most solutes have non-linear behavior at extreme mobile phase compositions, such that linear behavior is limited to approximately 20–80% organic modifier ( $0.2\text{--}0.8\Phi$ ). Schoenmaker's solubility parameter model [17,18] attempts to better describe the curvature seen in the  $\log k'$  versus  $\Phi$  plots by describing retention in terms of the differences in the solubility parameters of the solute, mobile phase and stationary phase:

$$\log k' = A\Phi^2 + B\Phi + E\sqrt{\Phi} + \log k'_w \quad (2)$$

where  $\Phi$  is the fraction of organic modifier  $A$ ,  $B$ ,  $E$ , are fitting coefficients and  $\log k'_w$  is the intercept. These coefficients are all expressed in terms of solubility parameters (for detailed derivations see ref. [18]). The  $A\Phi^2$  term in this equation accounts for the curvature seen at high concentrations ( $\Phi > 0.8$ ) of organic modifier, whereas the  $E\sqrt{\Phi}$  term accounts for curvature due to low concentrations ( $\Phi < 0.2$ ) of organic modifier. This model can fit experimental data with greater accuracy throughout the entire range of mobile phase composition, and the more data points taken at the extreme ranges of  $\Phi$ , the better the fitting parameters will accurately model the observed data and the less error upon extrapolation to a  $\log k'_w$  value. If no chromatography is performed below  $0.2\Phi$ , there will be little or no gain in using the solubility parameter model versus the LSS model, since curvature is not encountered. The closer data are taken to pure water mobile phases (i.e. shorter extrapolation to the intercept), the better accuracy in those values.

Another model used to find the values of retention in pure water mobile phases is the  $[E_T(30)]$  polarity scale [19]. This is based on the solvatochromic shift of the probe molecule 2,6-diphenyl-(2,4,6-triphenyl-*N*-pyridino)-phenolate (Fig. 1). The  $[E_T(30)]$  polarity scale is based on the charge transfer energy of the probe molecule, and the values of this scale were set as:

$$[E_T(30)] = \frac{28,592}{\lambda_{\max}} \quad (3)$$

where the constant (28,592) is the product of the speed of light, Planck's constant and Avogadro's number,  $\lambda_{\max}$  is the wavelength of maximum absorption, and the  $[E_T(30)]$  units are kcal/mol. This scale was used by Dorsey's group [20–22]

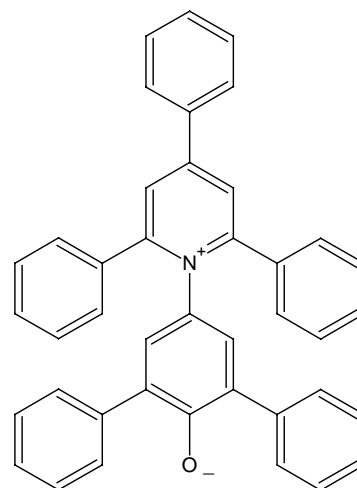


Fig. 1. Structure of ET-30 molecule.

to show that plots of  $\log k'$  versus  $[E_T(30)]$ , were often more linear than plots of  $\log k'$  versus  $\Phi$ , and they examined the estimation of the  $\log k'_w$  hydrophobicity parameter by use of this scale.

Lately there has been interest in using micellar and microemulsion electrokinetic chromatographies for correlations to  $\log P$  [10,23–27]. It has been shown that these techniques are better than RPLC for modeling octanol/water values. They state that the variables controlling retention in RPLC are similar but not identical to those controlling partitioning between the bulk phases, whereas micellar and microemulsion electrokinetic chromatographies have more similarities [10]. Although the  $\log k'_w$  value is not as closely correlated to  $\log P$  as those from electrokinetic techniques, it does not diminish its usefulness. There are those that believe  $\log k'_w$  values alone are an adequate estimation of hydrophobicity [6]. These values also find uses in other QRAR and QSRR applications as well as for defining selectivities. Regardless, faster more accurate methods for determining this parameter still need to be investigated.

## 1.2. Stationary phase considerations

Non-linear retention behavior as  $\Phi$  approaches zero is a common phenomenon and it is believed that highly aqueous environments create some pore exclusions in the stationary phase particles. As the mobile phase is altered (at low  $\Phi$ ) the stationary phase environment changes, creating the non-linear trends observed. These curvatures are both solute and column dependent [28].

There have been numerous attempts to improve the aqueous stability of  $C_{18}$  stationary phases. The reduction, blocking, or elimination of residual surface silanols, which are believed (in part) responsible for the observed non-linear behavior, has been the focus of many. This research has not been solely for the development of phases that have aqueous stability but also for improved peak symmetry, pH stability, and column longevity. Newer column technologies

have shown promise for high aqueous stability and include polar-encapped phases, polar-embedded phases, and hybrid particle phases. The polar-encapped phases use a secondary reaction to bond a short carbon chain (usually C<sub>3</sub>–C<sub>4</sub>) with a polar end, to reduce the surface silanols. The polar head of the small ligand increases the polarity near the surface of the silica and should allow highly aqueous mobile phases better penetration into the stationary phase.

Polar-embedded phases exploit polar groups in a different fashion. These phases are designed so that the polar group is within the C<sub>18</sub> ligand. The polar groups used in this arrangement are usually ureas, carbamates, or amides [29–32]. These bonded ligands are then able to hydrogen bond to each other via the polar group, creating a stabilizing network in highly aqueous environments. The hydrogen bonding of the polar groups should hinder solutes from diffusing to the surface of the silica, therefore reducing the exposure to residual silanols [33]. The internally bound polar groups should also provide improved stationary phase wetting with highly aqueous mobile phases.

In an attempt to control the heterogeneous surface of the silica particles, Waters<sup>®</sup> has developed a hybrid organic-inorganic porous particle synthesized by a mixture of organosilanes that form siloxane and methylsiloxane groups throughout the particle. This arrangement produces a stationary phase surface that has silanol and methylsiloxane units on the surface of the particle which helps to eliminate the inhomogeneity of the surface [34]. This surface is then the anchor point for derivation with the C<sub>18</sub> ligand. An advantage of this procedure is that the hybrid matrix can be modified for optimum ratio of surface silanols available for derivatization.

The present study focuses on reversed-phase chromatographic hydrophobicity estimations, specifically with newer technology C<sub>18</sub> stationary phases. Our goal is to understand if the newer generation of water-friendly stationary phases are better at estimating hydrophobicity ( $\log k'_w$ ), that is, are plots of retention versus  $\Phi$  more linear than with conventional reversed-phase columns. If so, these columns should have less error in extrapolation resulting in higher assurance in that data. We have investigated four different types of C<sub>18</sub> stationary phases consisting of a polar encapped, polar embedded, hybrid-based polar embedded, and conventional C<sub>18</sub> column (see Fig. 2). We used test solutes of differing polarity to see their effects on the curvature as suggested by Hsieh and Dorsey [28] and used methanol and acetonitrile as modifiers. All the data were fit to the three equations described above to obtain the best fitting function.

## 2. Experimental

### 2.1. Instrumentation

The HPLC system consisted of a Spectra-Physics Model 8800 ternary mixing pump (Spectra-Physics, Mountain

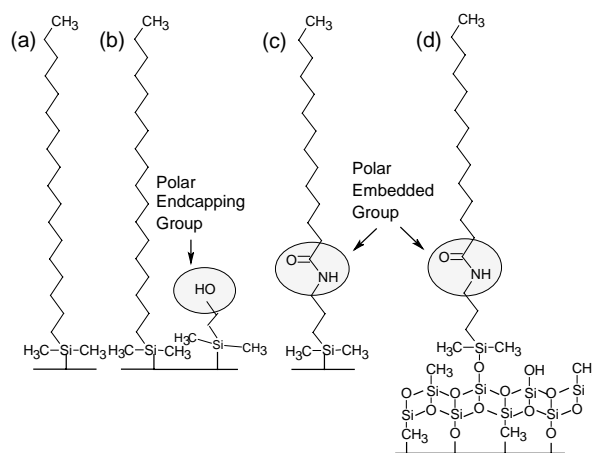


Fig. 2. Schematic representation of the four reversed-phase ODS binding chemistries studied. (a) Typical mono-functional ODS stationary phase. (b) Polar encapped ODS (here shown as C<sub>3</sub> encapping ligand). (c) Polar embedded ODS (here shown with amide as polar group). (d) Hybrid-based particle (Xterra manufactured by Waters<sup>®</sup>) bonded with the polar embedded ligand.

View, CA, USA), a Valco six port injector (Valco, Houston, TX, USA) equipped with a 20  $\mu$ L sample loop, and a Spectroflow 757 variable UV–vis detector (Kratos Analytical, Chestnut Ridge, NY, USA). Chromatograms were recorded with TurboChrom 4.0 software (Perkin Elmer, Wellesley, MA, USA). All columns were thermostated with a water-jacket and TE-7 Tempette (Techne, Cambridge, UK) temperature controller.

### 2.2. Columns

The columns used in this study are listed in Table 1 with specifications provided by the manufacturers. All columns are reversed-phase C<sub>18</sub> columns measuring 15 cm in length and 4.6 mm i.d. containing 5  $\mu$ m diameter particles. The four columns studied differ in their bonding chemistries. The Zorbax SB-C<sub>18</sub> column (Hewlett Packard, Wilmington, DE, USA) is a conventional C<sub>18</sub> bonded phase, the YMC ODS-AQ column (YMC Inc., Wilmington, NC, USA) is a polar (hydrophilic) encapped C<sub>18</sub> phase, the Symmetry Shield RP-18 column (Waters Corp., Milford, CT, USA) is a polar embedded bonded phase, and the Xterra RP-18 (Waters Corp., Milford, CT, USA) is a hybrid particle made of an organic/inorganic matrix which is bonded with polar embedded ligands.

### 2.3. Chemicals

Acetonitrile and methanol were HPLC grade (Fisher Scientific, Fair Lawn, NJ, USA). The water used for mobile phases was purified with a Barnstead NANOPure II system (Barnstead International, Dubuque, IA, USA). All test analytes are reagent grade or better. Acetone, methyl–ethyl

Table 1  
Properties of columns used

Column type	Dimensions	Pore size (Å)	Surface area (m <sup>2</sup> /g)	Carbon (%)	Bonding density (μmol/m <sup>2</sup> )
Zorbax SB-C <sub>18</sub> (mono-functional)	4.6 × 150 mm 5 μm dp	80	180	10	2.66
YMC-Pack ODS-AQ (polar endcapped)	4.6 × 150 mm 5 μm dp	120	300	14	~3.2–3.5 <sup>a</sup>
Symmetry Shield RP-18 (polar embedded)	4.6 × 150 mm 5 μm dp	90	332	17.38	3.31
Xterra RP-18 (hybrid-polar embedded)	4.6 × 150 mm 5 μm dp	120	175	14.76	2.31

Data obtained from manufacturers.

<sup>a</sup> Estimated total bonding density as a sum of C<sub>18</sub> density (2.0–2.2 μmol/m<sup>2</sup>) and endcapping agent density (1.2–1.4 μmol/m<sup>2</sup>), assuming a three carbon chain on endcapping agent.

ketone, diethyl ketone, benzyl alcohol, and benzene were from Fisher Scientific (Fisher Scientific, Fair Lawn, NJ, USA), nitro-ethane was from Sigma–Aldrich (Sigma–Aldrich, Milwaukee, WI, USA), and deuterium oxide from Cambridge Isotope Labs (Cambridge Isotope Labs, Andover, MA, USA). 1-Propanol (Fisher Scientific, Fair Lawn, NJ, USA) was used as an additive to help solvate the test analytes.

#### 2.4. Test procedures

Analytes were chromatographed at mobile phases containing 50, 40, 30, 20, 15, 10, 8, 6, 4, 2 and 0% (v/v) organic modifier. The HPLC system was operated at 1 ml/min with the detector set at 254 nm and an injection volume of 20 μL. All columns were thermostated at 30.0 ± 0.2 °C. The void time (*t*<sub>0</sub>) of the system was measured by using

Table 2  
Fitting parameters for Zorbax column

Solute	Modifier	Formula <sup>a</sup>	Equation	log <i>k'</i> <sub>w</sub>	Correlation ( <i>r</i> <sup>2</sup> )
Diethyl ketone	ACN	N/A	Chromatographically determined	1.63	N/A
		A	−0.029Φ + 1.30	1.30	0.9061
		B	0.2248 [E <sub>T</sub> (30)] − 12.806	1.37	0.9481
		C	0.0024Φ − 0.24Φ <sup>0.5</sup> + 1.65	1.65	0.9978
Nitroethane	ACN	N/A	Chromatographically determined	0.49	N/A
		A	−0.011Φ + 0.45	0.45	0.9879
		B	0.0847 [E <sub>T</sub> (30)] − 4.8684	0.47	0.9729
		C	−0.0096Φ − 0.012Φ <sup>0.5</sup> + 0.47	0.47	0.9895
Benzyl alcohol	ACN	N/A	Chromatographically determined	1.54	N/A
		A	−0.034Φ + 1.32	1.32	0.9500
		B	0.2678 [E <sub>T</sub> (30)] − 15.48	1.40	0.9832
		C	−0.0097Φ − 0.20Φ <sup>0.5</sup> + 1.61	1.61	0.9921
Benzene	ACN	N/A	Chromatographically determined	1.97	N/A
		A	−0.026Φ + 1.89	1.89	0.9941
		B	0.2053 [E <sub>T</sub> (30)] − 10.999	1.94	0.9902
		C	−0.022Φ − 0.037Φ <sup>0.5</sup> + 1.95	1.95	0.9940
Diethyl ketone	MeOH	N/A	Chromatographically determined	1.60	N/A
		A	−0.029Φ + 1.48	1.48	0.9817
		B	0.3015 [E <sub>T</sub> (30)] − 17.532	1.52	0.9935
		C	−0.016Φ − 0.11Φ <sup>0.5</sup> + 1.63	1.63	0.9983
Nitroethane	MeOH	N/A	Chromatographically determined	0.52	N/A
		A	−0.017Φ + 0.43	0.43	0.9753
		B	0.1751 [E <sub>T</sub> (30)] − 10.615	0.45	0.9863
		C	−0.0083Φ − 0.071Φ <sup>0.5</sup> + 0.53	0.53	0.9978
Benzyl alcohol	MeOH	N/A	Chromatographically determined	1.56	N/A
		A	−0.029Φ + 1.46	1.46	0.9894
		B	0.2921 [E <sub>T</sub> (30)] − 16.96	1.50	0.9957
		C	−0.019Φ − 0.078Φ <sup>0.5</sup> + 1.57	1.57	0.9991
Benzene	MeOH	N/A	Chromatographically determined	1.85	N/A
		A	−0.022Φ + 1.85	1.85	0.9961
		B	0.2199 [E <sub>T</sub> (30)] − 12.026	1.87	0.9849
		C	−0.024Φ + 0.014Φ <sup>0.5</sup> + 1.83	1.83	0.9966

<sup>a</sup> Formulas: (A) log *k'* = *S*Φ + log *k'*<sub>w</sub>; (B) log *k'* = *m*[E<sub>T</sub>(30)] + *b*; (C) log *k'* = *B*Φ + *E*Φ<sup>0.5</sup> + log *k'*<sub>w</sub>.

D<sub>2</sub>O. Samples were prepared in water with 1% 1-propanol added for solvation. All chromatograms were run in triplicate and these data were used to construct plots of the logarithm of the retention factor ( $\log k'$ ) versus percent organic modifier. The fitting functions were calculated using the regression tools in Microsoft Excel 2000 (Microsoft, Redmond, WA, USA) and Scientific Data Analysis Software (Prentice Hall, Upper Saddle River, NJ, USA) add-on for Excel.

### 3. Results and discussion

#### 3.1. Column comparison

For the column comparison, we will use a modified version of Schoenmaker's solubility parameter model eliminating the quadratic term ( $A\Phi^2$ ) from the equation. The quadratic term most accurately models the curvature at high fractions of modifier concentrations ( $\Phi > 0.8$ ). Baczek et al. [12] showed that there was only a statistical difference between the linear and quadratic models for acetonitrile systems, not methanol systems for the 20–95% modifier range. In this work, we are investigating the low range of modifier concentrations and therefore do not require the quadratic term. This can be seen when examining data containing a high degree of curvature and inspecting the differences in the correlation coefficients ( $r^2$ ). The correlations shown are the average of all four columns for the solute diethyl ketone

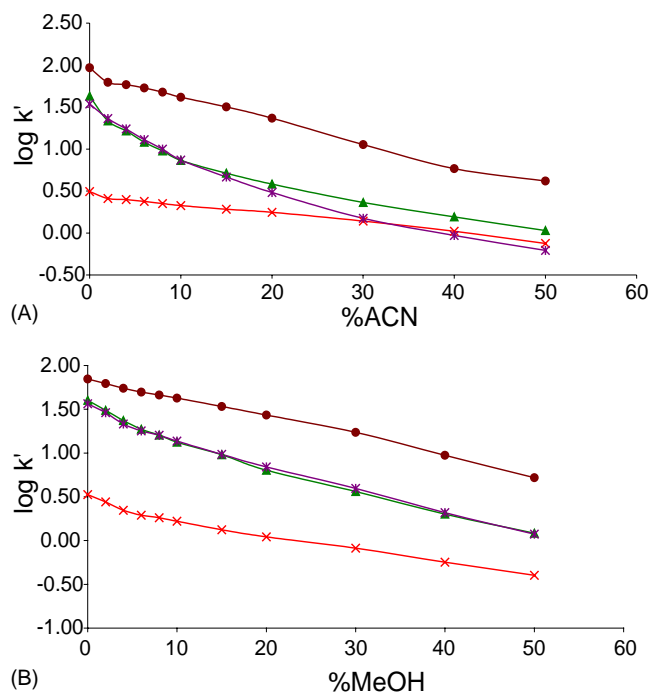


Fig. 3. Chromatographic data for the conventional Zorbax column. (A) Acetonitrile as the organic modifier and (B) methanol as modifier. Legend: (x) nitroethane; (Δ) diethyl ketone; (X) benzyl alcohol; and (●) benzene.

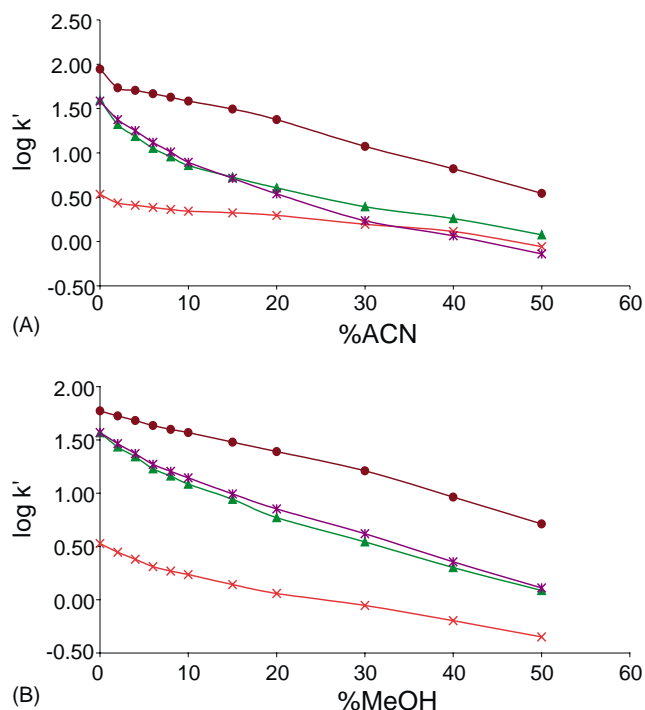


Fig. 4. Chromatographic data for the polar endcapped YMC column. (A) Acetonitrile as the organic modifier and (B) methanol as modifier. Legend: (x) nitroethane; (Δ) diethyl ketone; (X) benzyl alcohol; and (●) benzene.

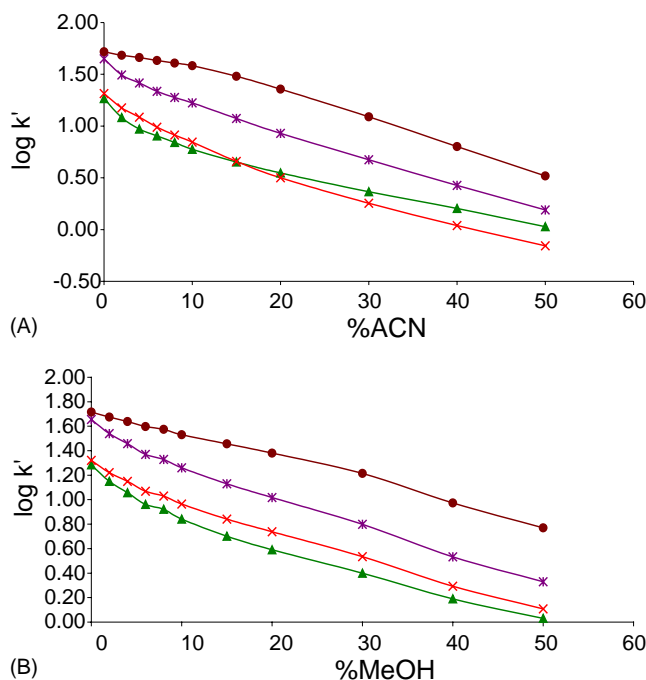


Fig. 5. Chromatographic data for the polar embedded Symmetry Shield column. (A) Acetonitrile as the organic modifier and (B) methanol as modifier. Legend: (x) nitroethane; (Δ) diethyl ketone; (X) benzyl alcohol; and (●) benzene.

using acetonitrile as modifier, since this solute/modifier pair consistently showed the most curvature. The equations are listed according to their decreasing correlations coefficients where  $A\Phi^2 + B\Phi + E\sqrt{\Phi} + \log k'_w$  ( $r^2 = 0.9989$ ),  $B\Phi + E\sqrt{\Phi} + \log k'_w$  ( $r^2 = 0.9986$ ),  $A\Phi^2 + B\Phi + \log k'_w$  ( $r^2 = 0.9793$ ),  $B\Phi + \log k'_w$  ( $r^2 = 0.9321$ ). From this data, we can see that the addition of another term to the linear equation is quite beneficial (from  $r^2 = 0.9321$  to 0.9793 or 0.9986) and that the quadratic term is less efficient at modeling the data than a square root term. We can also see that the addition of both terms to the to the linear equation does not increase the correlation to any significant value when compared to the linear equation with the square root term (from  $r^2 = 0.9986$  to 0.9989). We expect to see some increase in correlation since we are adding another term to the equation and decreasing the degrees of freedom in the modeling system, but the added correlation is inconsequential. For this reason, we have chosen not to show the fitting data for all of these equations, but just the linear (it is the most used and correlated equation) and

linear w/square root equation (it models the curvature very accurately).

### 3.1.1. Conventional $C_{18}$ column

In order to see whether the newer chemistry stationary phases are more accurate in determination of the  $\log k'_w$  value, a basis for comparison is needed. The conventional column used was the Zorbax SB- $C_{18}$  column, and the chromatographic data were as anticipated. Hsieh and Dorsey have shown that more polar solutes have greater upward curvature (concave) in the range of  $0 < \Phi < 0.1$ , and that non-polar solutes have curvature in the opposite fashion (convex) and that there is less curvature with methanol as a modifier than with acetonitrile [28]. As expected, our data (Fig. 3) follows the same general trends. Table 2 gives quantitative regression information about these plots (as well as measured values for  $\log k'_w$ ). There are also slight discrepancies in the measured pure water retention data for the two modifier systems; however, the two retention values should be the same. It has been shown that when changing mobile

Table 3  
Fitting parameters for YMC column

Solute	Modifier	Formula <sup>a</sup>	Equation	$\log k'_w$	Correlation ( $r^2$ )
Diethyl ketone	ACN	N/A	Chromatographically determined	1.60	N/A
		A	$-0.027\Phi + 1.27$	1.27	0.8989
		B	$0.21 [E_T(30)] - 11.902$	1.34	0.9410
		C	$0.0034\Phi - 0.24\Phi^{0.5} + 1.62$	1.62	0.9975
Nitroethane	ACN	N/A	Chromatographically determined	0.53	N/A
		A	$-0.0097\Phi + 0.47$	0.47	0.9614
		B	$0.0743 [E_T(30)] - 4.1986$	0.49	0.9455
		C	$-0.0069\Phi - 0.023\Phi^{0.5} + 0.50$	0.50	0.9682
Benzyl alcohol	ACN	N/A	Chromatographically determined	1.58	N/A
		A	$-0.033\Phi + 1.34$	1.34	0.9450
		B	$0.2569 [E_T(30)] - 14.78$	1.41	0.9787
		C	$-0.0071\Phi - 0.21\Phi^{0.5} + 1.64$	1.64	0.9949
Benzene	ACN	N/A	Chromatographically determined	1.95	N/A
		A	$-0.026\Phi + 1.85$	1.85	0.9897
		B	$0.1975 [E_T(30)] - 10.548$	1.90	0.9767
		C	$-0.023\Phi - 0.024\Phi^{0.5} + 1.89$	1.89	0.9909
Diethyl ketone	MeOH	N/A	Chromatographically determined	1.57	N/A
		A	$-0.029\Phi + 1.43$	1.43	0.9785
		B	$0.2915 [E_T(30)] - 16.946$	1.47	0.9915
		C	$-0.015\Phi - 0.11\Phi^{0.5} + 1.59$	1.59	0.9984
Nitroethane	MeOH	N/A	Chromatographically determined	0.53	N/A
		A	$-0.016\Phi + 0.44$	0.44	0.9727
		B	$0.1666 [E_T(30)] - 10.071$	0.46	0.9851
		C	$-0.0075\Phi - 0.071\Phi^{0.5} + 0.54$	0.54	0.9973
Benzyl alcohol	MeOH	N/A	Chromatographically determined	1.57	N/A
		A	$-0.028\Phi + 1.47$	1.47	0.9882
		B	$0.2864 [E_T(30)] - 16.592$	1.51	0.9956
		C	$-0.018\Phi - 0.080\Phi^{0.5} + 1.58$	1.58	0.9990
Benzene	MeOH	N/A	Chromatographically determined	1.77	N/A
		A	$-0.020\Phi + 1.77$	1.77	0.9953
		B	$0.2062 [E_T(30)] - 11.228$	1.80	0.9835
		C	$-0.022\Phi + 0.014\Phi^{0.5} + 1.75$	1.75	0.9959

<sup>a</sup> Formulas: (A)  $\log k' = S\Phi + \log k'_w$ ; (B)  $\log k' = m[E_T(30)] + b$ ; (C)  $\log k' = B\Phi + E\Phi^{0.5} + \log k'_w$ .

phase compositions to pure water some small amount of modifier can become trapped in the pores of the stationary phase and minor effects from this modifier can be observed [35].

### 3.1.2. Polar endcapped column

The data for the YMC Pro-Pack ODS-AQ can be seen in Fig. 4 and Table 3. From the data, we can see that there is much similarity between this column and the conventional C<sub>18</sub> column. The four test solutes show the same trends in curvature, regression coefficients, and log  $k'_w$  values for the different modeling equations between these two columns. This holds true for both methanol and acetonitrile modifiers. This was not anticipated. Since the surface of this silica was secondarily reacted with the polar endcapping agent, we expected differences in retention behavior—especially in the curvature of the graphs. The purpose of the polar endcapping is to create an environment in the stationary phase solvation layer that is more tolerable to highly aqueous milieus. We anticipated that

there would be less change to the stationary phase solvation layer when using highly aqueous mobile phases, which would result in less curvature in the range 0–0.1 $\Phi$ . Unfortunately it does not exhibit the reduced curvature that we expected. Although we did not expect the retention and trends to be this close, it has been shown that these two column types do not exhibit many differences. Layne [36] studied these types of stationary phases and showed that there were no significant differences in hydrophobicity, hydrogen bonding capacity or methylene selectivity of either the polar/hydrophilic endcapped or conventional RPLC column. Our data are in agreement with his, and others [32,37,38].

### 3.1.3. Polar embedded column

In contrast to the polar endcapped YMC column investigated, the polar embedded Symmetry Shield RP-18 column does exhibit noticeable differences in the data when compared to the conventional (Zorbax) column. These differences are represented in Fig. 5 and Table 4. It is easy

Table 4  
Fitting parameters for Symmetry Shield column

Solute	Modifier	Formula <sup>a</sup>	Equation	log $k'_w$	Correlation ( $r^2$ )
Diethyl ketone	ACN	N/A	Chromatographically determined	1.27	N/A
		A	$-0.024\Phi + 1.07$	1.07	0.9511
		B	$0.1743 [E_T(30)] - 9.8678$	1.30	0.9769
		C	$-0.0052\Phi - 0.14\Phi^{0.5} + 1.27$	1.27	0.9995
Nitroethane	ACN	N/A	Chromatographically determined	1.31	N/A
		A	$-0.029\Phi + 1.17$	1.17	0.9749
		B	$0.223 [E_T(30)] - 12.822$	1.24	0.9952
		C	$-0.014\Phi - 0.118\Phi^{0.5} + 1.35$	1.35	0.9971
Benzyl alcohol	ACN	N/A	Chromatographically determined	1.65	N/A
		A	$-0.028\Phi + 1.53$	1.53	0.9888
		B	$0.2143 [E_T(30)] - 11.924$	1.59	0.9958
		C	$-0.018\Phi - 0.080\Phi^{0.5} + 1.65$	1.65	0.9999
Benzene	ACN	N/A	Chromatographically determined	1.72	N/A
		A	$-0.024\Phi + 1.78$	1.78	0.9871
		B	$0.1827 [E_T(30)] - 9.6956$	1.82	0.9557
		C	$-0.032\Phi + 0.066\Phi^{0.5} + 1.69$	1.69	0.9970
Diethyl ketone	MeOH	N/A	Chromatographically determined	1.28	N/A
		A	$-0.024\Phi + 1.14$	1.14	0.9691
		B	$0.244 [E_T(30)] - 14.246$	1.17	0.9851
		C	$-0.0097\Phi - 0.11\Phi^{0.5} + 1.30$	1.30	0.9984
Nitroethane	MeOH	N/A	Chromatographically determined	1.32	N/A
		A	$-0.023\Phi + 1.24$	1.24	0.9899
		B	$0.2385 [E_T(30)] - 13.804$	1.27	0.9961
		C	$-0.016\Phi - 0.062\Phi^{0.5} + 1.33$	1.33	0.9992
Benzyl alcohol	MeOH	N/A	Chromatographically determined	1.66	N/A
		A	$-0.025\Phi + 1.56$	1.56	0.9891
		B	$0.2589 [E_T(30)] - 14.768$	1.59	0.9951
		C	$-0.017\Phi - 0.070\Phi^{0.5} + 1.66$	1.66	0.9991
Benzene	MeOH	N/A	Chromatographically determined	1.72	N/A
		A	$-0.018\Phi + 1.72$	1.72	0.9953
		B	$0.1861 [E_T(30)] - 10.017$	1.74	0.9835
		C	$-0.020\Phi + 0.016\Phi^{0.5} + 1.70$	1.70	0.9963

<sup>a</sup> Formulas: (A)  $\log k' = S\Phi + \log k'_w$ ; (B)  $\log k' = m[E_T(30)] + b$ ; (C)  $\log k' = B\Phi + E\Phi^{0.5} + \log k'_w$ .

Table 5  
Fitting parameters for Xterra column

Solute	Modifier	Formula <sup>a</sup>	Equation	log $k'_w$	Correlation ( $r^2$ )
Diethyl ketone	ACN	N/A	Chromatographically determined	0.85	N/A
		A	$-0.018\Phi + 0.74$	0.74	0.9721
		B	$0.1353 [E_T(30)] - 7.7594$	0.77	0.9886
		C	$-0.0075\Phi - 0.08\Phi^{0.5} + 0.85$	0.85	0.9996
Nitroethane	ACN	N/A	Chromatographically determined	0.95	N/A
		A	$-0.024\Phi + 0.88$	0.88	0.9861
		B	$0.1867 [E_T(30)] - 10.838$	0.93	0.9993
		C	$-0.016\Phi - 0.068\Phi^{0.5} + 0.98$	0.98	0.9966
Benzyl alcohol	ACN	N/A	Chromatographically determined	1.35	N/A
		A	$-0.024\Phi + 1.25$	1.25	0.9912
		B	$0.1881 [E_T(30)] - 10.556$	1.30	0.9951
		C	$-0.017\Phi - 0.062\Phi^{0.5} + 1.34$	1.34	0.9997
Benzene	ACN	N/A	Chromatographically determined	1.48	N/A
		A	$-0.022\Phi + 1.50$	1.50	0.9866
		B	$0.1639 [E_T(30)] - 8.7975$	1.54	0.9554
		C	$-0.027\Phi + 0.044\Phi^{0.5} + 1.43$	1.43	0.9920
Diethyl ketone	MeOH	N/A	Chromatographically determined	0.85	N/A
		A	$-0.019\Phi + 0.77$	0.77	0.9846
		B	$0.1941 [E_T(30)] - 11.467$	0.80	0.9941
		C	$-0.011\Phi - 0.063\Phi^{0.5} + 0.86$	0.86	0.9989
Nitroethane	MeOH	N/A	Chromatographically determined	0.95	N/A
		A	$-0.020\Phi + 0.91$	0.91	0.9966
		B	$0.2009 [E_T(30)] - 11.759$	0.94	0.9969
		C	$-0.016\Phi - 0.027\Phi^{0.5} + 0.95$	0.95	0.9991
Benzyl alcohol	MeOH	N/A	Chromatographically determined	1.35	N/A
		A	$-0.023\Phi + 1.29$	1.29	0.9950
		B	$0.2286 [E_T(30)] - 13.125$	1.32	0.9958
		C	$-0.018\Phi - 0.038\Phi^{0.5} + 1.34$	1.34	0.9988
Benzene	MeOH	N/A	Chromatographically determined	1.44	N/A
		A	$-0.017\Phi + 1.45$	1.45	0.9880
		B	$0.1664 [E_T(30)] - 9.0407$	1.47	0.9709
		C	$-0.020\Phi + 0.027\Phi^{0.5} + 1.42$	1.42	0.9915

<sup>a</sup> Formulas: (A)  $\log k' = S\Phi + \log k'_w$ ; (B)  $\log k' = m[E_T(30)] + b$ ; (C)  $\log k' = B\Phi + E\Phi^{0.5} + \log k'_w$ .

to notice that diethyl ketone<sup>1</sup> and benzene have reduced intercepts ( $\log k'_w$ ) for both modifiers. We believe the shift to lower retention values is representative of the increased polarity of the stationary phase. This change in polarity of the stationary phase solvation layer makes the stationary and mobile phases thermodynamically closer, that is,  $\Delta\Delta G$  is closer to zero. This belief is further evidenced by the fact that this column has the largest phase ratio (product of surface area and bonding density) so it should have the greatest retention.

The solutes benzyl alcohol and nitroethane have increased intercepts on the polar embedded column, contrary to the other solutes, which we attribute to their hydrogen bonding ability. Benzyl alcohol's hydrogen donating ability creates more interactions with the polar embedded group of

the stationary phase increasing retention compared to the conventional column. As for nitroethane, not only is there the ability to hydrogen bond, but also the polar group embedded in the stationary phase is a carbamate, so there are N–N lone pair interactions that increase the retention of nitrogen-containing solutes.

For acetonitrile-modified systems we also see reduced curvature in the low  $\Phi$  region. This is also true for the methanol systems, but is not as obvious in the figures. The curvature in the system is represented by the coefficient ( $E$ ) in the equation  $\log k' = B\Phi + E\sqrt{\Phi} + \log k'_w$ . The larger absolute value of the coefficient means greater curvature. The sign of the coefficient implies the direction of curvature, where a (+) coefficient implies convex (downward) curvature and a (–) coefficient implies concave (upward) curvature. Looking at the acetonitrile data, benzyl alcohol shows an  $E$  coefficient decrease from 0.20 for the conventional phase to 0.08 for the polar-endcapped phase (Tables 2 and 4), translating into a 60% reduction of curvature. There is also a large decrease in curvature for 3-pentanone (actually all the ketones had this

<sup>1</sup> Data for acetone, methyl ethyl ketone and diethyl ketone were obtained, but since they all have the same retention characteristics, only the highest retained ketone is discussed.



reduction in curvature, but only 3-pentanone is listed). Benzene had seven-fold less curvature than the polar ketones or alcohol (Zorbax column, ACN modifier). When comparing the Zorbax and Symmetry Shield columns benzene shows an  $E$  coefficient increase (from 0.04 to 0.07), respectively. This small change could be due to experimental error. The curvature for nitroethane (in ACN) actually increases by an order of magnitude. This is again attributed to the N–N lone pair interactions between the stationary phase and solute. Overall, the trend seems to be that of increasing linearity of the plots. These observations are reinforced by the correlation coefficient ( $r^2$ ) of the linear equations. The less curvature will produce a better correlation to the linear equation.

### 3.1.4. Hybrid-based polar embedded column

This hybrid Xterra RP-18 column has the same polar embedded ligand as the Symmetry Shield column just discussed, but a different base particle. Investigation of the regression data in Table 5 shows a shift of all solutes to decreased retention values compared to the Symmetry Shield column. The decreased retention is believed to be a result of the lower surface area (175 m<sup>2</sup>/g) of the hybrid phase compared to the Symmetry Shield column (332 m<sup>2</sup>/g). This belief is reinforced when looking at the

similarities in the coefficient of the linear term ( $B\Phi$ ) of the modified Schoenmaker's equation. All the solute/modifier pairs have approximately the same slope as they did when chromatographed using the Symmetry Shield column.

It is also visually evident in Fig. 6 that the curvature in the data seems diminished. Quantitative evidence can again be seen in the  $E$  coefficient of the modified solubility parameter model (Table 5). When comparing the data of the Xterra and Symmetry Shield columns, the deviation from linearity is much less in the Xterra column. For example, the  $E$  coefficient for diethyl ketone is reduced from 0.14 on the Symmetry Shield to 0.08 on the Xterra column. This is a 43% reduction in curvature from Symmetry Shield to the Xterra column (67% from Zorbax to Xterra). Each solute has diminished curvature when using the Xterra column compared to the Symmetry Shield column. Since both columns have the same polar embedded ligand, it would seem logical that the reduced curvature is related to the base particle. There are far less surface silanols on the hybrid particle since the surface contains methyl groups in the place of some surface silanols. The reduction of surface silanols will help in the reduction of curvature. Also, there could be effects from the reduced bonding density because there are not as many polar embedded groups for hydrogen bonding

Table 6  
Extrapolation error for columns and regression equations, in acetonitrile systems

Column	Experimental	Linear extrapolation		E <sub>T</sub> (30) extrapolation		Mod. S.P. <sup>a</sup> extrapolation	
	log $k'_w$	log $k'_w$	Error (%)	log $k'_w$	Error (%)	log $k'_w$	Error (%)
Zorbax							
Diethyl ketone	1.63	0.98	40.2	1.13	30.8	1.57	4.0
Nitroethane	0.49	0.47	3.9	0.56	12.9	0.07	85.4
Benzyl alcohol	1.54	0.99	35.8	1.18	23.1	2.23	45.1
Benzene	1.97	1.87	4.9	2.08	5.5	2.80	42.5
Average			21.2		18.1		44.2
YMC							
Diethyl ketone	1.60	0.98	38.9	1.11	30.3	1.44	9.8
Nitroethane	0.53	0.50	5.6	0.57	7.9	−0.03	105.1
Benzyl alcohol	1.58	1.02	35.3	1.22	23.3	2.11	33.3
Benzene	1.95	1.91	1.9	2.11	8.7	1.94	0.0
Average			20.4		17.5		37.1
Sym. Shield							
Diethyl ketone	1.27	0.91	28.4	1.04	17.9	1.08	14.3
Nitroethane	1.31	0.98	25.8	1.15	12.3	1.60	22.0
Benzyl alcohol	1.65	1.44	12.8	1.63	1.4	1.68	1.7
Benzene	1.72	1.91	10.9	2.11	23.0	1.68	2.1
Average			19.5		13.6		10.0
Xterra							
Diethyl ketone	0.85	0.64	24.6	0.75	11.5	0.73	14.4
Nitroethane	0.95	0.75	21.5	0.91	4.8	1.31	37.3
Benzyl alcohol	1.35	1.19	11.9	1.36	0.7	1.37	1.3
Benzene	1.48	1.62	9.6	1.81	22.1	1.30	12.4
Average			16.9		9.8		16.4

This data shows the extrapolated log  $k'_w$  value (from 15% to the y-axis), and the error in that value. The average number are the average error of all four solutes for each column, and each regression equation.

<sup>a</sup> Mod. S.P. stands for Modified Solubility Parameter model.

interactions with the polar solutes. The hybrid phase has a substantial decrease in the “coefficient of curvature” ( $E$  coefficient) compared to the polar embedded phase, and the polar embedded phase has an overall decrease in curvature compared to the conventional and polar endcapped phases, setting the hybrid phase further apart from the others. In the next section we will see how this translates into extrapolating the  $\log k'_w$  values from isocratic data points.

### 3.2. Estimating $\log k'_w$ by extrapolation

In the previous sections, we showed how accurately the three fitting functions modeled the data, while using all eleven isocratic retention values. This showed that when using all the data points at low percentages of organic modifier, the modified solubility parameter equation was best at fitting the data ( $0.9959 \pm 0.0058$ ), followed by the [E<sub>T</sub>(30)] ( $0.9822 \pm 0.0164$ ) and finally the linear equation ( $0.9767 \pm 0.0238$ ), where  $n = 32$  for each fitting equation. The coefficient of curvature for the modified solubility parameter model allowed non-linear deviations to be more accurately modeled than the one-term equations, resulting in the better fitting, and usually gave very accurate  $\log k'_w$  values. We also

could see that there was reduced curvature in the polar embedded phase and the hybrid-based polar embedded phase when compared to the conventional and endcapped phases.

Most researchers that use these  $\log k'_w$  values do not take the extensive data near pure water as was taken in these experiments, but rather take a few data points in the linear range and extrapolate back to the intercept. Since there are no data at low  $\Phi$  for deducing curvature, there is often great error in the extrapolation, especially when using ACN as the modifier. For this reason we have re-evaluated the data for the purposes of extrapolating a  $\log k'_w$  value. In this exercise, we have discarded all data points lower than 15% organic modifier. This should allow us to see the extrapolation error associated with each column and fitting equation. The five data points in the range of 50–15% modifier are mostly within the linear range of modifiers, so there is a high correlation coefficient for all data and fitting functions ( $r^2 = 0.9950 \pm 0.0082$ ,  $n = 96$ ), but the resulting intercept and its error is the meaningful piece of information. For this reason, the  $r^2$  values will not be further discussed. As Tables 6 and 7 show, there are rational trends in the data. For example, when looking at the three modeling equations, the modified solubility parameter equation is consistently the

Table 7  
Extrapolation error for columns and regression equations, in methanol systems

Column	Experimental $\log k'_w$	Linear extrapolation		E <sub>T</sub> (30) extrapolation		Mod. S.P. <sup>a</sup> extrapolation	
		$\log k'_w$	Error (%)	$\log k'_w$	Error (%)	$\log k'_w$	Error (%)
<b>Zorbax</b>							
Diethyl ketone	1.60	1.33	17.0	1.45	9.3	1.84	14.5
Nitroethane	0.52	0.35	34.1	0.42	20.3	0.27	48.3
Benzyl alcohol	1.56	1.37	12.4	1.50	4.3	1.43	8.5
Benzene	1.85	1.90	3.1	2.01	8.8	1.38	25.2
Average			16.6		10.7		24.1
<b>YMC</b>							
Diethyl ketone	1.57	1.28	18.6	1.39	11.1	1.72	9.8
Nitroethane	0.53	0.35	34.7	0.41	22.8	0.21	59.9
Benzyl alcohol	1.57	1.36	13.1	1.48	5.5	1.38	11.9
Benzene	1.77	1.83	3.3	1.93	9.1	1.24	30.3
Average			17.4		12.1		28.0
<b>Sym. Shield</b>							
Diethyl ketone	1.28	0.98	23.7	1.08	16.3	1.24	3.7
Nitroethane	1.32	1.16	12.0	1.27	4.1	1.18	10.3
Benzyl alcohol	1.66	1.48	10.5	1.59	3.9	1.48	10.5
Benzene	1.72	1.77	3.4	1.87	8.9	1.31	23.4
Average			12.4		8.3		12.0
<b>Xterra</b>							
Diethyl ketone	0.85	0.68	19.4	0.77	9.8	0.70	17.1
Nitroethane	0.95	0.89	6.7	0.97	2.6	0.73	22.8
Benzyl alcohol	1.35	1.26	6.3	1.36	1.3	1.10	18.2
Benzene	1.44	1.55	7.1	1.63	13.0	1.04	27.9
Average			9.9		6.7		21.5

This data shows the extrapolated  $\log k'_w$  value (from 15% to the y-axis), and the error in that value. The average number are the average error of all four solutes for each column, and each regression equation.

<sup>a</sup> Mod. S.P. stands for modified solubility parameter model.

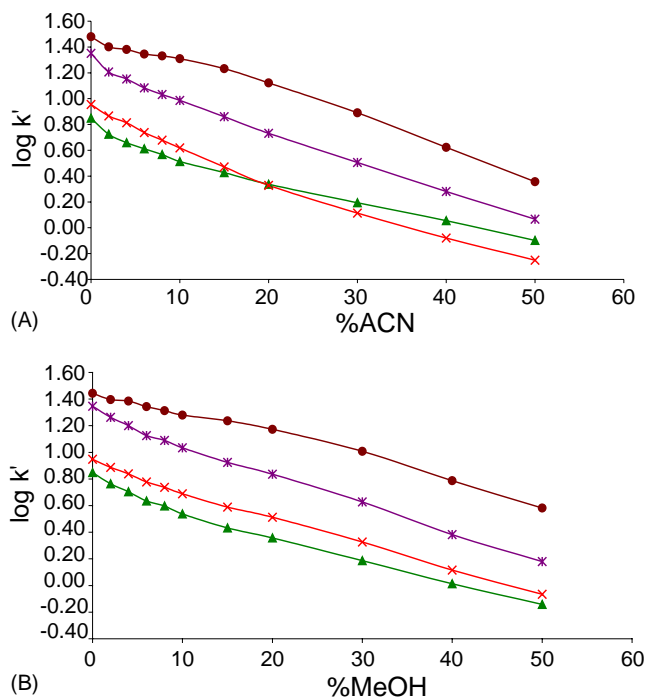


Fig. 6. Chromatographic data for the hybrid-based polar embedded Xterra column. (A) Acetonitrile as the organic modifier and (B) methanol as modifier. Legend: (x) nitroethane; ( $\Delta$ ) diethyl ketone; (X) benzyl alcohol; and ( $\bullet$ ) benzene.

worst in extrapolating a  $\log k'_w$  value. This is because the fitting equation is allowing non-linearity (modeled by the  $E$  term) and with a low number of data points it may be forcing any slight differentiation of the data to be modeled as curvature. Because this is the region that is consistent with linear behavior, any induced curvature at this point has the potential to be grossly unrepresentative of the actual trends at low modifier concentrations. The other trend with the modeling equations is that the  $[E_T(30)]$  model consistently gives lower error in extrapolation compared to the linear equation for all columns. This data is consistent with results published by Dorsey's group [20,21]. So the overall ranking of equation (by percent error,  $n = 32$  each) is:  $[E_T(30)]$  (12.1%) < linear (16.8%) < modified solubility parameter (24.2%). There is also decreased error in extrapolation based on stationary phase type. Noting that the modified solubility parameter model is inducing curvature not necessarily representative of the system, the data for the other two models will be used for the column comparison. The column comparison shows, of decreasing order (in percent error,  $n = 16$  each) is: Zorbax (16.7%)  $\sim$  YMC (16.9%) > Symmetry Shield (13.5%) > Xterra (10.8%). This trend mimics the trend of decreasing curvature in the  $\log k'$  versus  $\Phi$  graphs seen earlier in this paper. This was expected since the non-linearity of the data is what creates the extrapolation error. Also, as expected, the acetonitrile data consistently has a higher error in extrapolation, due to the enhanced non-linearity of these systems.

#### 4. Conclusion

We have attempted to classify reversed-phase packings with respect to their efficacy for the rapid estimation of the hydrophobicity indicator  $\log k'_w$ . The columns studied represent the newer style reversed-phase packings as compared to conventional reversed-phase packings. It was observed that there is almost no difference in retention characteristics for the conventional reversed-phase  $C_{18}$  ligand and the polar endcapped ligand. Their data were almost overlapping on most occasions, showing that the polar endcapping was insufficient at creating a more stable environment for highly aqueous milieu. This resulted in the same error in extrapolation for both columns. The polar embedded column, however, did show a decrease in curvature in the data, which did result in an overall decrease of the extrapolation error. The data for this column did show that the ability of the ligand to hydrogen bond can change the retention characteristics for polar solutes, and that the N–N lone pair interactions can also greatly influence the retention of nitrogen containing compounds. The hybrid base particle trademarked as Xterra, with an attached polar embedded ligand produced results that gave the least curvature in the graphs of  $\log k'$  versus  $\Phi$ , and resulted in the least error in extrapolation.

The three modeling equations revealed that when data points are taken at low concentrations of modifier, the modified solubility parameter equation was the best at modeling the data, and that the coefficient of curvature ( $E$  term) is the quantitative descriptor of curvature in the low  $\Phi$  region. It also showed that the linear equation was the worst at modeling the data. When extrapolating the data to the  $\log k'_w$  value it was observed that the modified solubility parameter equation would model curvature in the few data points that are not representative of the data. This is due to the limited data points and even fewer degrees of freedom. This translated into the worst extrapolation error of the three equations. Conversely, the  $[E_T(30)]$  model was the best at extrapolating to the  $\log k'_w$  value.

The data presented here illustrates the error associated with linear extrapolations using conventional  $C_{18}$  columns. It has been shown that newer generation polar-embedded columns show less curvature in the low  $\Phi$  region. Knowing these polar-embedded phases are adequately solvated at highly aqueous conditions (larger linear range of  $\Phi$  versus retention) is especially useful information. Since data are almost never available in the low  $\Phi$  region, a column that exhibits less change in stationary phase solvation will show less error upon extrapolation to a  $\log k'_w$  value. This allows greater confidence in an extrapolation procedure with no retention data at less than 20% modifier. All else equal, the best reduction in extrapolation error would be to use a polar embedded column (here the hybrid-based polar embedded column) with methanol as the modifier, and extrapolate (from 15% MeOH) using the  $[E_T(30)]$  model, for our data this resulted in an average 7% error in the data ( $n = 4$ ).

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