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RETENTION IN REVERSED-PHASE LIQUID CHROMATOGRAPHY: SOLVATOCHROMIC INVESTIGATION OF HOMOLOGOUS ALCOHOL-WATER BINARY MOBILE PHASES

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SUMMARY

The mechanism of retention in reversed-phase liquid chromatography (RPLC) has been further investigated using the $E_T(30)$ solvatochromic solvent polarity scale. The retention behavior of a variety of solutes was measured using a homologous series of normal alcohols as the organic modifiers in hydroorganic mobile phases. The results imply that a systematic change in the extent of solvation of the stationary phase occurs with respect to the size of the organic modifier. It was also found that a linear extrapolation of the $\log k'$ versus $E_T(30)$ plots for different mobile phases using methanol, ethanol and acetonitrile (but not *n*-propanol) as modifiers, intersected at approximately the $E_T(30)$ value of pure water. This intersection is further evidence that the $E_T(30)$ model of solute retention is a useful tool with which to study the mechanism of retention in RPLC. The extrapolated retention value in water, $\log k'_{w}$, from the $E_T(30)$ plots should then prove to be a more reliable means of estimating solute lipophilicity using RPLC than the percent organic modifier model.

INTRODUCTION

Many approaches have been taken to study the effects of the mobile phase in reversed-phase liquid chromatography (RPLC)^{1–9}. The most commonly used mobile phases in RPLC are binary solutions of water with an organic solvent modifier such as methanol, acetonitrile or tetrahydrofuran. Retention in RPLC is primarily controlled by the chromatographic strength of the mobile phase, with the strength frequently denoted as the percent of the organic modifier in the binary aqueous solution¹⁰. It has been shown both experimentally¹¹ and theoretically⁵ that a quadratic function best describes plots of $\log k'$ versus percent organic modifier.

We have previously developed a method of modeling retention that relates the strength of the mobile phase to a measured polarity of the solvent⁹. An independent examination of the effect of changing mobile phase polarity on chromatographic retention has been performed using $E_T(30)$ solvatochromic solvent polarity measurements. Solvatochromism is a phenomenon that relates changes in the spectral behavior (intensity, position or shape) of a probe molecule to changes in its environment.

The $E_T(30)$ scale relates changes in position of the λ_{\max} for the electronic absorption of the molecule 2,6-diphenyl-4-(2,4,6-triphenyl-N-pyridinio) phenolate (ET-30) to changes in the polarity of the solvent. ET-30 signifies the acronym for the probe molecule itself while $E_T(30)$ stands for the measured polarity value calculated by the equation:

$$E_T(30)(\text{kcal/mole}) = 28\,592/\lambda_{\max}(\text{nm}) \quad (1)$$

It was shown that by plotting $\log k'$ versus the $E_T(30)$ polarity of the mobile phase, a linear relationship was found. The regression analysis of 332 data sets revealed a higher degree of linearity for the $E_T(30)$ model over the percent organic modifier model⁹.

A review of the use of solvatochromism to study retention in RPLC and other forms of chromatography has recently been published¹². There are two solvatochromic scales that have been used to investigate retention processes in reversed-phase chromatography. The $E_T(30)$ scale, the single parameter scale used here, and the multiparameter π^* scale of solvent dipolarity/polarizability developed by Kamlet *et al.*¹³. The π^* scale is intended to represent the solute-solvent interactions in the absence of strong forces such as hydrogen-bonding or ion-dipole interactions. Additionally, both α and β scales of solvent hydrogen-bond donor or acceptor interactions have been derived which independently account for these strong forces. The Kamlet and Taft methods are essentially more rigorous than the single-parameter scales in that they separate the three interactions into individual terms, however, they are also only empirical measures. While the $E_T(30)$ scale is a single parameter scale, it has been shown to be sensitive to both solvent dipolarity/polarizability as well as solvent hydrogen-bond donor ability. This sensitivity to hydrogen bonding effects makes this solvent scale useful for studying aqueous reversed-phase mobile phases.

An interesting perspective on the "meaning" of single and multiparameter solvent scales is found in a paper by Sjöström and Wold¹⁴ and a reply by Kamlet and Taft¹⁵. Sjöström and Wold argue that instead of the classical interpretation of linear free energy relationships expressing a combination of "fundamental" effects that they should be viewed strictly as locally valid linearizations of complicated relationships. It is perhaps best at this point in the solvatochromic studies of chromatographic retention mechanisms, whether with single or multi-parameter scales, to keep this view in mind. While perhaps useful for predictive purposes, it is yet to be determined if they are providing a "fundamental" measure of the retention process: they may merely be providing a convenient linearization of more complicated phenomena.

The most rigorous approach to understanding the retention mechanism of reversed-phase chromatography is that recently developed by Dill⁵ using a lattice statistical thermodynamic approach. He proposed that two driving forces dominate retention; (i) the difference in the chemistry of the contacts of the solute with surrounding molecular neighbors in the stationary and mobile phase, and (ii) the partial ordering of the grafted stationary phase chains which, at sufficiently high bonding density, leads to an entropic expulsion of solute from the stationary phase relative to that which would be expected in a simpler amorphous oil phase-water partitioning process. We have tested (i) against an extensive data base of almost 350 sets of experiments and in agreement with theory have found that the mobile phase contri-

bution to retention can be described by the binary interaction constants of solutes with solvents¹⁶. From this extensive data base it was also found that the $E_T(30)$ solvent polarity appears to provide a direct measure of the binary interaction constants. While this is almost certainly a "convenient linearization of more complicated phenomena", it still provides a useful means of probing mobile phase effects on the retention process. We have also tested (ii) by synthesizing monomeric octadecyl stationary phases over a range of 1.5–4.0 $\mu\text{mol}/\text{m}^2$, and have found, in agreement with theory, that partition coefficients go through a maximum at approximately 3.0 $\mu\text{mol}/\text{m}^2$ (ref. 17). Above this density chain packing constraints become significant, and creation of a solute sized cavity in the stationary phase becomes entropically expensive.

If the measured $E_T(30)$ polarity is truly a good descriptor of mobile phase strength, then by correlating retention *versus* the $E_T(30)$ polarity of the solvent for a given solute on a given column, the retention behavior of the solute in all solvent systems on that particular column should be the same. Retention behavior is characterized by the slope of the $\log k'$ *versus* $E_T(30)$ plots and defined here as the sensitivity of the change in retention of a solute to changes in the mobile phase polarity. This is similar to Snyder's "S" value, which is the slope of $\log k'$ *versus* percent organic modifier¹⁸. Inspection of the regression coefficients for 89 of the 332 data sets revealed that the retention behavior of these systems are not normalized as expected⁹. Data taken from the literature on columns ranging in chain length from C_2 to C_{18} show that, for a given solute and column, the methanol slope is greater than the acetonitrile slope by an average ratio of 1.4⁹.

One interpretation of this 1.4 methanol–acetonitrile slope ratio is the active role of the stationary phase in RPLC. If the stationary phase were truly a passive entity, as is said to be true by the solvophobic theory³, the slopes of the $E_T(30)$ plots should be the same for a solute in any solvent system. The $E_T(30)$ scale has previously been shown to accurately measure solution properties, as evidenced by correlations of $E_T(30)$ polarity with reaction rate constants¹⁹ and heats of solution at infinite dilution²⁰. Retention is a result of the free energy change as a solute transfers between the mobile and stationary phases. As iso- $E_T(30)$ values of two mobile phases suggest that they are energetically equivalent, at least as seen by the ET-30 molecule, then the different slopes suggest that the solute is experiencing a different environment in the stationary phase with methanol–water as compared to acetonitrile–water. While it can be argued that the local environment of the ET-30 molecule is very different between methanol and acetonitrile, similar slope ratio differences are seen between methanol and ethanol, where the local environment of the ET-30 molecule would be more similar. This must primarily result from differences in the extent of solvation of the alkyl chains bonded to the silica as the organic modifier is changed. These differences in extent of solvation have also been shown by others^{21–24}.

To further clarify the meaning of the slope of the $\log k'$ *vs.* $E_T(30)$ plots we attempted to induce systematic changes in the retention behavior for a given system by using a homologous series of organic modifiers. The series chosen was that of the *n*-alcohols (methanol, ethanol and *n*-propanol) because they are readily available, non-toxic, have low wavelength UV cutoffs and their distribution behavior in an RPLC system has been previously characterized²⁵. The extent of solvation of the stationary phase was expected to change in direct proportion to the molecular size of

the modifier, resulting in a systematic change in the slopes of the $E_T(30)$ plots for each solute. Therefore, the aim of this study was to provide further evidence that the change in the $\log k'$ versus $E_T(30)$ slopes is indeed due to changes in the nature of the stationary phase and to also characterize ethanol and *n*-propanol mobile phases for RPLC by the $E_T(30)$ solvatochromic solvent polarity scale.

EXPERIMENTAL

Solvatochromic measurements

All solvatochromic measurements were made using ET-30 (Reichardt's Dye) (Aldrich, Milwaukee, WI, U.S.A.). Binary solvents were prepared by mixing additive volumes of ET-30 in pure organic solvent, pure organic solvent and water to the desired solvent compositions with the final concentration of ET-30 being approximately 200 mg/l. Samples were placed into a 1-cm path length quartz cell and spectra obtained with a Hewlett-Packard (Palo Alto, CA, U.S.A.) Model 8450A diode-array spectrophotometer or an IBM Instruments (Danbury, CT, U.S.A.) Model 9420/9430 UV-VIS spectrophotometer. Maximum absorbance wavelengths were determined using a peak-picking algorithm on each instrument. Three spectra were acquired for each sample and the $E_T(30)$ values for each sample were averaged. The $E_T(30)$ data produced were fit to the appropriate degree polynomial using the Interactive Microwave (State College, PA, U.S.A.) program CURVE FITTER run on an Apple (Cupertino, CA, U.S.A.) II + 48K microcomputer, and any unmeasured $E_T(30)$ values were determined by interpolation.

Retention measurements

All retention measurements were obtained with a Beckman (San Ramon, CA, U.S.A.) Model 100A isocratic LC pump, a Beckman Model 153 fixed wavelength (254 nm) UV detector, a Valco (Houston, TX, U.S.A.) C6W injector with a 10- μ l sample loop, a Fisher (Austin, TX, U.S.A.) Recordall Series 5000 strip chart recorder and a Hamilton (Reno, NV, U.S.A.) 705 SNR LC syringe. A Beckman Ultrasphere ODS (5 μ m), 15 cm \times 4.6 mm I.D. column and a DuPont (Wilmington, DE, U.S.A.) Zorbax TMS (6 μ m), 15 cm \times 4.6 mm I.D. column were used. The columns and solvents were thermostated at 30°C with a Brinkmann Lauda (Westbury, NY, U.S.A.) Model MT heater/circulator. Fisher HPLC-grade methanol and acetonitrile, certified 1-propanol and Florida Distillers (Lake Alfred, FL, U.S.A.) absolute ethyl alcohol (200 proof) were used as received. Water was first purified with a Barnstead (Milford, MA, U.S.A.) Nanopure system, irradiated with UV light in a Photronix (Medway, MA, U.S.A.) Model 816 HPLC reservoir for at least 24 h and then filtered through a Rainin (Woburn, MA, U.S.A.) 0.45- μ m Nylon-66 membrane filter prior to use. Pure solutes were used as received and stock solutions made in HPLC grade methanol: Eastman Kodak (Rochester, NY, U.S.A.) reagent ACS spectro grade toluene, butylbenzene, naphthalene, *p*-nitroanisole and benzylamine, Fisher certified ethylbenzene and nitrobenzene, Mallinckrodt (St. Louis, MO, U.S.A.) organic reagent benzophenone, MCB (Norwood, OH, U.S.A.) *p*-nitrophenol and Alfa (Danvers, MA, U.S.A.) *n*-propylbenzene. Retention times were determined manually and the breakthrough time (t_0) used to calculate capacity factors found by the elution of an injection of HPLC-grade methanol.

Linear regression

Regression calculations were done by using the Interactive Microware program CURVE FITTER run on an Apple II + 48K microcomputer.

RESULTS AND DISCUSSION

Solvatochromic polarity measurements

The results of the $E_T(30)$ solvent polarity measurements for binary aqueous solutions of ethanol and of *n*-propanol are illustrated in Fig. 1. $E_T(30)$ measurements for methanol and acetonitrile aqueous solutions have previously been discussed^{9,26,27}. As previously evidenced with methanol-water and acetonitrile-water solutions, the $E_T(30)$ polarity values for aqueous ethanol and *n*-propanol solutions show non-linear behavior when related to either mole fraction or volume percent of organic modifier. Fig. 1a-d show the change in $E_T(30)$ values for ethanol and *n*-propanol *versus* both volume percent and mole fraction. As has been previously discussed about the organic-rich region of the acetonitrile-water system^{9,28}, the $E_T(30)$ probe molecule may be sensing a breakdown in the hydrogen-bonding network of the solutions in the high organic content ranges. Unlike the acetonitrile-water system, the $E_T(30)$ changes shown here are not as great because ethanol and *n*-propanol are stronger hydrogen-bond donors than acetonitrile. From entropy and enthalpy of mixing data it has been hypothesized for dilute aqueous solutions of a non-electrolyte that a collective stabilization of the hydrogen-bond lattice of water occurs due to an increase in the energy of water-water hydrogen-bonds or in their number²⁹. Therefore, it could be that a change in the hydrogen-bonding network of the solution is being sensed by the $E_T(30)$ probe in the dilute alcohol concentration

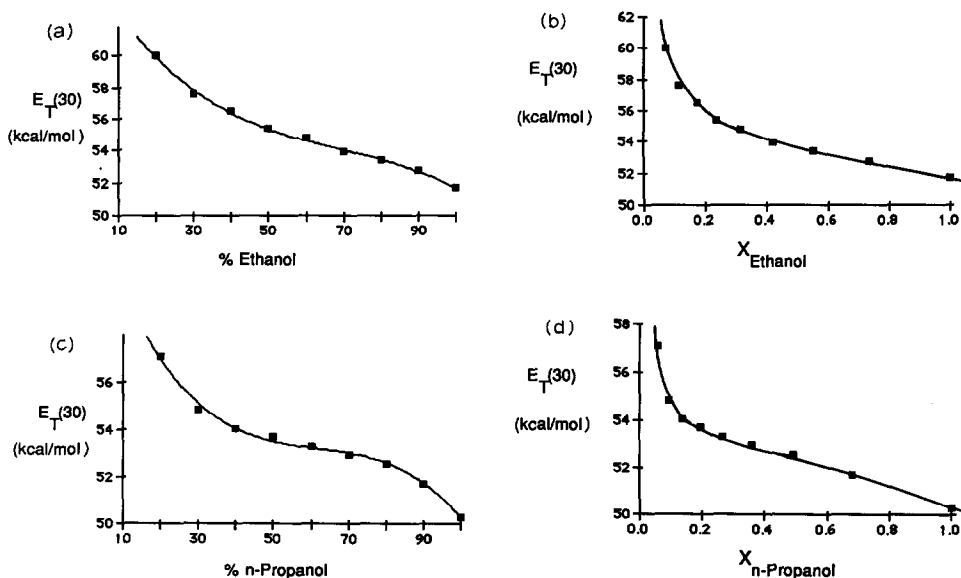


Fig. 1. The $E_T(30)$ polarity of binary hydroorganic mobile phases as it varies with (a) percent ethanol; (b) mole fraction ethanol; (c) percent *n*-propanol and (d) mole fraction *n*-propanol.

region as well. Furthermore, the random mixing approximation is not expected to be generally viable in the limits of extreme composition¹¹, less than a few percent of either mobile phase component. In these regions, the ET-30 probe molecules or the minor component of the binary solvent may associate to form non-random mixtures.

Chromatographic retention measurements

Retention data was gathered for ten solutes on an octadecylsilane (C_{18}) reversed-phase column using aqueous methanol, ethanol, *n*-propanol and acetonitrile solutions as the mobile phases. The test solutes were chosen so that a variety of compound types would be used. Acetonitrile was used as a reference organic modifier from which the homologous alcohols could be compared in the form of $\log k'$ vs. $E_T(30)$ slope ratios. It was expected that upon going from methanol to ethanol to *n*-propanol mobile phases, the slopes of the $\log k'$ vs. $E_T(30)$ plots for any of the test solutes would decrease by an amount linearly related to the carbon number of the alcohol modifier; this is a consequence of the displacement model of reversed-phase retention³⁰. This was believed since it has been shown that for alcohol-water mobile phases on a C_{18} column, the extent of solvation of the surface by the alcohol over the water increases with carbon chain length of the alcohol²⁵.

An example of the data generated in this study is the $\log k'$ versus $E_T(30)$ plots for naphthalene in all four solvent systems shown in Fig. 2. The average correlation coefficient (r) for a total of forty different data sets (similar to Fig. 2 but unreported) is 0.997 ± 0.002 . By observing the positioning of each of the data sets along the $E_T(30)$ axis, it is obvious that not all of the polarities for all the different solvents overlap. The weakest *n*-propanol solvent used (30%) was about 1050 cal/mol less polar than the strongest methanol solvent used (90%). It is also obvious that there are no iso- $E_T(30)$ values in reasonable isocratic retention ranges among the four solvent systems. In other words, there are no points of identical retention at one particular $E_T(30)$ value. If the $E_T(30)$ values are giving a useful measure of mobile phase strength, then one possible explanation is a continued change in the solvation structure of the sta-

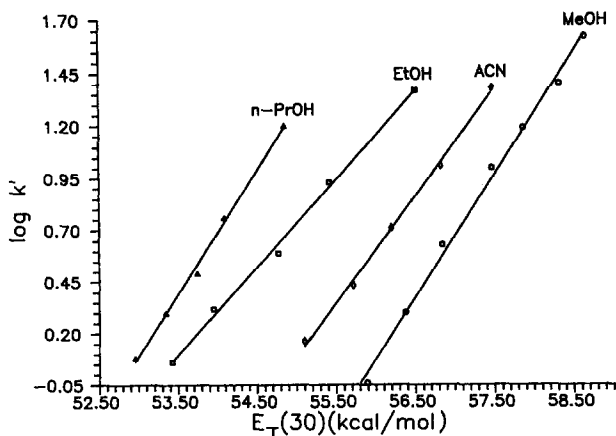


Fig. 2. Retention of naphthalene on an Ultrasphere ODS column at 30°C as a function of $E_T(30)$ polarity with binary hydroorganic mobile phases.

tionary phase even for organic modifiers of the same functionality differing only in size.

From Fig. 2 and the $E_T(30)$ slopes and Snyder S values for all ten solutes in Table I, a few trends can be observed. First, as seen previously with aqueous methanol and acetonitrile mobile phases⁹, the slopes of the $\log k'$ versus $E_T(30)$ plots for the ethanol and *n*-propanol mobile phases increase with increasing solute size. The addition of methylene groups in the alkylbenzene homologous series (toluene to *n*-butylbenzene) produces a linear relationship with respect to the $\log k'$ versus $E_T(30)$ slopes ($r = 0.998$ for ethanol and 0.999 for *n*-propanol). Second, the $\log k'$ versus $E_T(30)$ plots for methanol, ethanol and acetonitrile appear to extend to a common point. In fact, all three extrapolate to an average $E_T(30)$ value of 62.5 ± 2.9 kcal/mol ($n = 27$) which is approximately the $E_T(30)$ value for pure water of 63.1 kcal/mol³¹. The *n*-propanol plots, however, do not approach the other plots near 63.1 kcal/mol at all, which suggests that a different mechanism of retention may be occurring with the aqueous *n*-propanol mobile phases in contrast to the other three. Third, the expected results of a decrease in the $\log k'$ versus $E_T(30)$ slopes with increasing carbon number of the alcohol solvent modifiers did not occur. As seen in Table I, the $E_T(30)$ slopes for each test solute decreased when going from methanol–water to ethanol–water, but upon going from ethanol–water to *n*-propanol–water the slopes increase. None of the solutes, however, showed intersections near 0% modifier when volume-% was used as the organic-concentration descriptor.

If the slope of a $\log k'$ versus $E_T(30)$ plot for a test solute in an alcohol-modified solvent is ratioed to that in acetonitrile-modified solvent for all three alcohols, the retention behavior for each alcohol–water system can be compared to the others for all ten solutes. The average methanol–acetonitrile ratio for all ten solutes came to be 1.13 ± 0.09 , for ethanol–acetonitrile 0.84 ± 0.05 and for *n*-propanol–acetonitrile 1.14 ± 0.06 . The methanol–acetonitrile slope ratio was within experimental error of the previously determined average value of 1.18 ± 0.05 for C_{18} at 40°C ⁹. The larger the slope or slope ratio, the greater the change in retention per unit of polarity change. Methanol slope ratios are greater than those for ethanol due to the fact that in a binary alcohol–water mobile phase, methanol does not selectively solvate the stationary phase to as large a degree as ethanol. When comparing one chromatographic system using methanol to another using ethanol, the stationary phase will be less polar (contain less alcohol) for the methanol system when the mobile phases for both are equal in alcohol composition. If an equivalent increase in mobile phase polarity as measured by $E_T(30)$ is performed in each system, retention will change to a greater extent in the methanol system than the ethanol system due to the greater difference in the polarity of the mobile and stationary phases. For *n*-propanol–water mobile phases, however, the stationary phase may be saturated by *n*-propanol when using solutions of 30% or more. It was calculated that when using a 9% *n*-propanol–water solvent, the C_{18} chains on the surface have already taken up 95% of the maximum uptake by *n*-propanol²⁵. Therefore, for the concentrations of *n*-propanol used (30–70%), increasing the amount of *n*-propanol in the solvent may not produce changes at the stationary phase in the same manner as with methanol and ethanol.

Another consideration is the chemical information obtained from $E_T(30)$ measurements. The ET-30 molecule is sensitive to solvent dipolarity/polarizability and hydrogen bond acidity. According to Kamlet *et al.*³², the $E_T(30)$ polarity of a given

TABLE I
 SLOPES OF THE LOG k' VERSUS $E_T(30)$ PLOTS AND SNYDER S VALUES FOR THE HOMOLOGOUS SOLVENTS AND ACETONITRILE ON
 ALTEX ULTRASPHERE ODS COLUMN AT 30°C

Solute	Methanol-water		Ethanol-water		<i>n</i> -Propanol-water		Acetonitrile-water	
	$E_T(30)$ slope	-S	$E_T(30)$ slope	-S	$E_T(30)$ slope	-S	$E_T(30)$ slope	-S
Toluene	0.504	3.11	0.381	2.92	0.543	2.46	0.464	2.72
Ethylbenzene	0.581	3.59	0.442	3.38	0.598	2.70	0.519	3.04
<i>n</i> -Propylbenzene	0.690	4.12	0.509	3.44	0.667	3.01	0.578	3.38
<i>n</i> -Butylbenzene	0.835	4.63	0.559	3.78	0.728	3.28	0.636	3.72
Naphthalene	0.594	3.66	0.423	3.23	0.598	2.70	0.516	3.02
Benzophenone	0.607	3.73	0.394	2.99	0.546	2.42	0.513	3.00
Nitrobenzene	0.354	2.52	0.287	2.63	0.413	1.83	0.332	2.24
<i>p</i> -Nitroanisole	0.409	2.41	0.347	3.15	0.418	1.85	0.398	2.71
<i>p</i> -Nitrophenol	0.338	2.41	0.260	2.38	0.403	2.22	0.350	2.38
Benzylamine	0.321	2.29	0.240	2.19	0.298	1.66	0.274	1.88

solvent can be related to the dipolarity/polarizability (π^*) and hydrogen bond acidity (α) scales by the regression equation^{33,34}:

$$E_T(30) = 28.21 + 12.40\pi^* + 14.40\alpha \quad (2)$$

For water, methanol, ethanol, *n*-propanol and acetonitrile, respectively, the values of π^* are 1.09, 0.60, 0.54, 0.52 and 0.75 and the values of α are 1.17, 0.93, 0.83, 0.78 and 0.19³³. These π^* and α values show only small differences among the three pure alcohols. If the trends seen in the $\log k'$ versus $E_T(30)$ plots among the various organic modifiers were not due to differences in the solvation structure of the stationary phase, then the trends should be due to the solvent parameters measured by π^* and α . Cheong and Carr have examined the π^* and α properties of aqueous solutions of methanol, 2-propanol and tetrahydrofuran³⁴ and attribute decreases in hydrogen bond acidity for methanol–water mixtures from that of the pure solvents to the formation of less hydrogen bond acidic complexes. These effects between the different mobile phases, however, are normalized by the $E_T(30)$ scale and should not show up as differences in the $\log k'$ versus $E_T(30)$ plots.

It was necessary to see if the hypothesis of *n*-propanol saturation at the C_{18} surface was possibly occurring. Retention measurements were made for benzylamine and *p*-nitrophenol, the two least retained solutes, at *n*-propanol in water compositions below 30% (10–25%). The previously determined *n*-propanol–acetonitrile slope ratio for benzylamine was 1.09 while that for *p*-nitrophenol was 1.15. When the retention measurements were observed from 35 to 10% *n*-propanol, the new slope ratios for *n*-propanol–acetonitrile came to 0.57 for benzylamine and 0.23 for *p*-nitrophenol. It is interesting to note that these slope ratios are less than the average ethanol–acetonitrile value of 0.84 ± 0.05 . The *p*-nitrophenol value is questionable because the $\log k'$ versus $E_T(30)$ plot was not as linear as desired ($r = 0.969$). Fig. 3 shows the $\log k'$ versus $E_T(30)$ plot for benzylamine from 10 to 50% *n*-propanol in water. The linear region of the plot extends from 10 to about 35% with an apparent break occurring between 35 and 40%. It is also interesting to note that when the point of intersection of the 10 to 35% *n*-propanol plots with the other three modifiers was calculated for both benzylamine and *p*-nitrophenol, the average $E_T(30)$ intersection point was 58.70 ± 1.06 kcal/mol ($n = 6$), which is close to the $E_T(30)$ value of pure water. Based on the resulting *n*-propanol–acetonitrile slope ratios and the intersection of the *n*-propanol plots with those of the other three modifiers, it would appear that

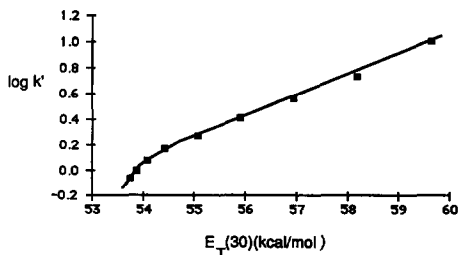


Fig. 3. Retention of benzylamine on an Ultrasphere ODS column at 30°C as a function of $E_T(30)$ polarity with mobile phases ranging from 10 to 50% *n*-propanol in water.

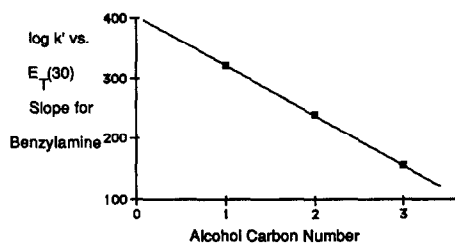


Fig. 4. The relationship between the retention behavior of benzylamine [using the $E_T(30)$ model] on an Ultrasphere ODS column at 30°C and the carbon number of the modifier alcohol in a binary hydroorganic mobile phase.

for *n*-propanol compositions at or below 30% the mechanism of retention is similar to that when using the other modifiers. Above 30% *n*-propanol, the C_{18} stationary phase may be saturated by *n*-propanol and a different mechanism of retention is operative. While only two data sets were measured due to the extremely long retention times and poor peak shapes in this mobile phase range, the implications are encouraging.

If the slopes of the $\log k'$ versus $E_T(30)$ plot for benzylamine are plotted against the carbon number of the alcohol modifier, a linear relationship is found ($r = 0.999$), as illustrated in Fig. 4. The same trend is seen for *p*-nitrophenol but the linearity is slightly worse ($r = 0.979$). When the *n*-propanol slope of the 30 to 50% solvents was used, no linear correlation was found between the $E_T(30)$ slope and alcohol carbon number. Thus, a systematic change in retention may occur when using modifier compositions in a range where no surface saturation occurs.

In order to see if the solvation differences of the stationary phase between solvent systems could be minimized, retention measurements for the ten test solutes were made on a trimethylsilane (C_1) column. By using a C_1 column, it was hoped that specific solvation interactions between the solvent modifier and the bonded phase would be minimized. Normalization of the retention behavior of a solute in various solvent systems should then be reflected in all alcohol-acetonitrile ratios of $\log k'$ versus $E_T(30)$ slopes approaching unity.

No conclusive results could be found in the C_1 retention data, however. One of the problems encountered when using C_1 columns in RPLC is the impreciseness of the chemistry of the surface. The polarity of the reversed-phase surface increases as the bonded carbon chain is shortened since the residual silanol groups are left less shielded from the solute and mobile phase and are freer to interact in the retention process³⁵. Silanol interactions can affect results in the form of irreproducible retention times and tailed peak shapes. Furthermore, a C_1 column likely exhibits an adsorption type mechanism rather than partitioning because of the shallow "depth" of the stationary phase. A representative plot of the data gathered is shown for naphthalene in Fig. 5. The linearity for all data sets deteriorated in contrast to the C_{18} data as expressed in a lower and more scattered average r value of 0.991 ± 0.016 ($n = 40$). A slight degree of curvature was evident with most of the plots. No surprising trends are noticeable from the $E_T(30)$ slopes and S values shown in Table II. When slope ratios were calculated, methanol-acetonitrile remained about the same (1.16 ± 0.15), *n*-

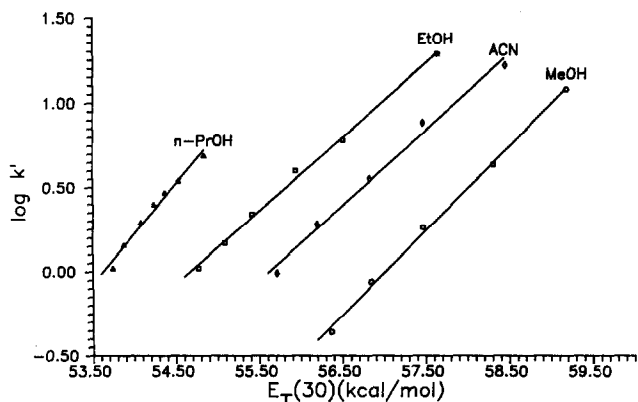


Fig. 5. Retention of naphthalene on a Zorbax TMS column at 30°C as a function of $E_T(30)$ polarity with binary hydroorganic mobile phases.

propanol–acetonitrile increased slightly (1.22 ± 0.20), and ethanol–acetonitrile came to unity (1.00 ± 0.10). The uncertainty in each average slope ratio, though, is too great to make any generalizations with respect to the solvation behavior of each solvent system on the C_{18} surface.

A beneficial aspect of this work is the intersection of the C_{18} $\log k'$ versus $E_T(30)$ plots for different modifiers (except *n*-propanol) at the $E_T(30)$ value of pure water. This intersection is further evidence that $E_T(30)$ plots are monitoring the correct partitioning processes under different equilibrium conditions. A problem often associated with the evaluation of solute lipophilicity by high-performance liquid chromatography (HPLC) is the extrapolation of $\log k'$ versus % organic plots to 0% organic concentration in the mobile phase³⁶. The reproducibility of the retention value in pure water ($\log k'_w$) is affected by the magnitude and direction of curvature in a $\log k'$ versus percentage plot³⁷. Raymond *et al.*³⁸ have recently shown that linear extrapolations of methanol–water retention data yielded practically identical $\log k'_w$ values as parabolic extrapolations of acetonitrile–water mixtures. Schoenmakers *et al.*³⁹, however, reported that the quadratic fit of $\log k'$ versus percent organic modifier does not hold at >90% water, and they recommend adding an empirical (percent modifier)^{1/2} term for that range. Since more than one binary solvent system can be used to determine the $\log k'_w$ value, it would be desirable to have as low of an uncertainty between $\log k'_w$ values as possible. An average and standard deviation of $\log k'_w$ values for each test solute in the different mobile phases was determined. The data sets used were those for methanol, ethanol and acetonitrile. Due to the previously discussed problem of stationary phase saturation, *n*-propanol was not considered. Calculation of $\log k'_w$ for all ten test solutes used in this study at 0% modifier by linearization of percent organic plots for thirty data sets gave an average deviation of 0.32. Subsequent calculation of $\log k'_w$ at 63.1 kcal/mole [the $E_T(30)$ value of pure water] for $E_T(30)$ plots gave an average deviation of 0.19; an improvement of about 40%. In addition, preliminary work has shown that $\log k'$ versus $E_T(30)$ plots for different modifiers converge toward the $E_T(30)$ value of water, as seen in Fig. 6. Plots of $\log k'$ versus percentage for different modifiers do not converge to 0% organic at all

TABLE II
 SLOPES OF THE LOG k' VERSUS $E_T(30)$ PLOTS AND SNYDER S VALUES FOR THE HOMOLOGOUS SOLVENTS AND ACETONITRILE ON ZORBAX TMS COLUMN AT 30°C

Solute	Methanol-water		Ethanol-water		<i>n</i> -Propanol-water		Acetonitrile-water	
	$E_T(30)$ slope	- S	$E_T(30)$ slope	- S	$E_T(30)$ slope	- S	$E_T(30)$ slope	- S
Toluene	0.390	2.78	0.379	3.29	0.530	2.90	0.370	2.54
Ethylbenzene	0.468	3.33	0.444	3.85	0.611	3.33	0.433	2.96
<i>n</i> -Propylbenzene	0.556	3.97	0.515	4.47	0.687	3.73	0.502	3.42
<i>n</i> -Butylbenzene	0.653	4.64	0.597	5.17	0.750	4.07	0.567	3.85
Naphthalene	0.500	3.55	0.435	4.24	0.598	3.25	0.446	3.04
Benzophenone	0.579	4.10	0.474	4.60	0.580	3.25	0.448	3.05
Nitrobenzene	0.357	2.72	0.296	2.89	0.367	2.37	0.331	2.28
<i>p</i> -Nitroanisole	0.412	3.14	0.332	3.24	0.375	2.41	0.361	2.48
<i>p</i> -Nitrophenol	0.329	2.51	0.264	2.76	0.276	2.05	0.323	2.23
Benzylamine	0.321	2.44	0.248	2.59	0.213	1.62	0.208	1.57

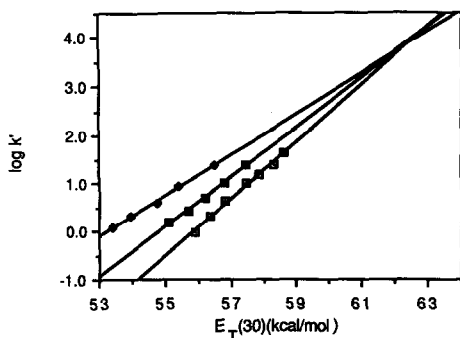


Fig. 6. Convergence of $E_T(30)$ retention plots for naphthalene to the $E_T(30)$ value of pure water (63.1 kcal/mol). (□) Methanol; (■) acetonitrile; (◆) ethanol.

but diverge⁴⁰. The use of $\log k'_w$ values from $E_T(30)$ plots has also been demonstrated for the successful calculation of solvent-solvent contact free energies of binary organic-water mixtures¹⁶. Since the scatter of the values appears to be decreased from those found using the percentage model and the $E_T(30)$ plots for different solvents converge to a common point instead of diverging, the $E_T(30)$ solvatochromic polarity scale should be a reliable means of estimating $\log k'_w$ values for the evaluation of lipophilicity. Further investigations of this possibility are underway.

CONCLUSION

The $E_T(30)$ solvatochromic solvent polarity scale has been used to further study the effects of the mobile phase in reversed-phase liquid chromatography. Retention measurements were made on a variety of test solutes using a homologous series of alcohols (methanol, ethanol and *n*-propanol) as organic modifiers to determine whether a systematic change in the solvation of a C_{18} surface could be observed. We infer from the results and our model that a systematic change in the solvation structure of the stationary phase occurs with respect to the carbon number of the modifying alcohol. This relationship is hypothesized to exist, however, only if the alcohol component in the binary mobile phase is used in a concentration range such that saturation of the stationary phase does not occur. Retention measurements were also made on a C_1 column using the same solutes and mobile phases to determine if solvation effects could be normalized for all solvents, but no conclusive judgements can presently be made. The applicability of using the $\log k'$ versus $E_T(30)$ plots of various solvent systems for the estimation of solute lipophilicity has also been shown to be quite promising.

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