

# Accurate determination of $\log k'_w$ in reversed-phase liquid chromatography

## Implications for quantitative structure–retention relationships

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### ABSTRACT

With increased understanding of the retention mechanisms of reversed-phase LC has come increased usage of the technique for the measurement of physico-chemical data, especially partitioning information that can be used for quantitative structure–activity relationships. However, the use of chromatographic retention requires that a standard set of mobile phase conditions be chosen. The choice of 100% water has theoretical advantages, as an aqueous phase–membrane phase is the most common system being modeled. However, experimental measurement of  $k'$  values with this mobile phase is difficult or impossible for most real solutes. Various retention extrapolation methods to 100% water have been proposed, but when compared, often yield different values for the same solute. Most of the extrapolation methods are based on the retention as a function of the mobile phase only. However, as the retention is controlled by solute partitioning between the mobile phase and stationary phase, stationary phase effects cannot be ignored. In this paper  $\log k'_w$  values extrapolated from different methods are compared to the measured values. Prediction of  $\log k'_w$  is attempted from the retention as a function of both the mobile phase and stationary phase. Solvatochromic analysis is used to deconvolute stationary and mobile phase effects.  $\log k'_w$  values extrapolated from  $E_T(30)$  plots are recommended as the most meaningful representation of retention for quantitative structure–retention relationships.

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### INTRODUCTION

Prediction of solute capacity factors in RPLC with pure aqueous mobile phases ( $\log k'_w$ ) has received a lot of attention because of their application in quantitative structure–retention relationship (QSRR) studies [1–5]. The advantages of using  $\log k'_w$  are that it is independent of any organic modifier effects, it reflects polar–non-polar partitioning in a manner similar to shake-flask measurements, and is dependent on the solute's structure and polar functionalities [6–8].

$\log k'_w$  is most often estimated by extrapolating a plot of  $\log k'$  vs. the volume percent organic modifier through the relationships described in eqns. 1 and 2 to the  $y$ -intercept, representing retention in a 100% aqueous phase:

$$\log k' = A\varphi^2 + B\varphi + C \quad (1)$$

$$\log k' = B\varphi + C \quad (2)$$

where  $A$ ,  $B$ ,  $C$  are fitting coefficients, and  $\varphi$  is the volume fraction of organic modifier in the mobile phase. The accuracy of  $k'_w$  extrapolated from eqns. 1 and 2 based on experimental data using mixed methanol–water mobile phases in various composition ranges was studied by Jandera and Kubat [4]

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for some pesticides. In their work, the worst  $k'_w$  predicted by extrapolation resulted in a relative mean error of 80% versus measured  $k'_w$ , using the linear function of eqn. 2 to fit the experimental data obtained over a mobile phase composition of 30 to 80% methanol in water. The quadratic function of eqn. 2 was also fitted to a wide range of methanol concentrations from 2 to 90%, yielding a relative error in the extrapolated value of  $k'_w$  of about 30%.

Several shortcomings of lattice models or solubility parameter treatments of solute retention used to derive eqns. 1 and 2 may lead to deviations. For example, it is assumed that solute and solvents have equal molar volumes and no change in volume occurs upon mixing. In addition, the solute and mobile phase components are assumed to be randomly mixed. This neglects any preferential orientation or clustering due to strong intermolecular interactions, such as proton donor–acceptor interactions. Katz and co-workers [9,10] noted that there is a strong association between methanol and water and the binary association constant was found to be  $5.22 \cdot 10^{-3}$  at 23°C. This indicates that methanol–water solvent systems constitute “ternary” mixtures of associated water, associated methanol and water associated with methanol. Distribution of a number of different solutes between *n*-hexadecane and a range of methanol–water mixtures was also studied [10] and it was shown that the distribution coefficient is related to the concentration of methanol unassociated with water, not the concentration of methanol originally added.

Association between acetonitrile molecules was also observed by probing the CN stretching frequency of acetonitrile as a function of concentration in water using Raman spectroscopy [11]. Thus, taking into account both solvent–solvent and solvent–solute species, acetonitrile–water mixtures were more thoroughly described by Rowlen and Harris [11] as having at least six general components: (acetonitrile)<sub>n</sub>, acetonitrile, (acetonitrile)<sub>n</sub>–water, and acetonitrile–water, and (water)<sub>n</sub>. As the concentration is varied, the distribution of interactions must also vary. The unique character of aqueous solvent mixtures employed in LC makes it essential to take into account six components in the mobile phase if retention is to be predicted from solvent composition.

Sadek *et al.* [12] first reported the correlation of

HPLC retention properties with fundamental dipolarity–polarizability and hydrogen bonding properties of solutes and mobile phases. As these physical properties were originally measured by the spectroscopic changes of some indicators in solvent, they are also known as solvatochromic parameters. Sadek *et al.* [12] used solvatochromic parameters and the methodology associated with the linear solvation energy relationships (LSER) [13] to evaluate the multiple interactions that influence HPLC capacity factors. Accordingly, the capacity factor may be determined by three principle types of interactions as shown in eqn. 3

$$\log k' = \log k'_0 + \text{cavity term} + \text{dipolar term} + \text{hydrogen bonding term(s)} \quad (3)$$

Here  $k'_0$  includes the volume phase ratio and dipolar interactions between solute and solvents when  $\pi^*$  is equal to zero. The cavity term measures the endoergic process of separating the solvent molecules to provide a suitably sized enclosure for the solute. This term is usually taken as the product of the solute molar volume ( $\bar{V}$ ) and the square of the Hildebrand solubility parameter ( $\delta_H$ ) of the solvent. The dipolar term may be considered to be the product of the solute and the solvent  $\pi^*$  interactions, where  $\pi^*$  is defined as a measure of the dipolarity–polarizability of the species in question. Finally, the hydrogen bonding terms are written as a cross product of the solute  $\alpha$  [an empirical measure of hydrogen bond donating (HBD) acidity] and the solvent  $\beta$  [a similar scale of hydrogen bond acceptor (HBA) basicity] and the product of the solute  $\beta$  and the solvent  $\alpha$ . Thus, in an expanded format, eqn. 3 becomes:

$$\log k' = \log k'_0 + m(\delta_s^2 - \delta_m^2)\bar{V}_2/100 + s(\pi_s^* - \pi_m^*)\pi_2^* + a(\beta_s - \beta_m)\alpha_s + b(\alpha_s - \alpha_m)\beta_s \quad (4)$$

The subscripts s, m and 2 denote the stationary phase, mobile phase and solute, respectively. Coefficients, *m*, *s*, *a* and *b* are fitting parameters that are independent of solutes and phases considered.

In order to simplify eqn. 4, the solvatochromic properties of the stationary phase may be assumed to be a constant over the entire range of mobile phase compositions and eqn. 4 may be changed as represented by eqn. 5:

$$\log k' = \log k'_0 + m'\delta_m^2 + s'\pi_m^* + a'\beta_m + b'\alpha_m \quad (5)$$

where  $m' = m(\bar{V}_2)/100$ ,  $s' = s(\pi^*)$ ,  $a' = a(\alpha_2)$  and  $b' = b(\beta_2)$ . It is difficult to measure the solubility parameter and the solvatochromic properties of solvent mixtures. Even though Cheong and Carr [14] have attempted to measure the physical properties of water and aqueous mixtures of organic solvents, a multiple correlation has not yet been reported.

The  $E_T(30)$  scale, an empirical measure of solvent polarity, may be used to further simplify eqn. 4. The  $E_T(30)$  scale is based on the charge transfer absorption of 2,6-diphenyl-4-(2,4,6-triphenyl-*n*-pyridinio)phenolate (ET-30). This method is also known as a single parameter solvatochromic method. By way of definition,  $E_T(30)$  refers to the value of the polarity measurement and ET-30 is an acronym for the compound's name. The  $E_T(30)$  scale is calculated by eqn. 6:

$$E_T(30) \text{ (kcal/mol)} = 28\,592/\lambda_{\max.} \text{ (nm)} \quad (6)$$

where  $\lambda_{\max.}$  is the maximum absorption wavelength and 28 592 is a product of the speed of light, Planck's constant and Avagadro's number. ET-30 exhibits one of the largest observed solvatochromic dependencies of any known molecule, as the charge transfer absorption maximum shifts from 453 nm in water, a very polar solvent, to 810 nm in diphenyl ether, a very non-polar solvent. ET-30 is thus very sensitive to both solvent dipolarity–polarizability as well as solvent hydrogen bond donor ability. Kamlet *et al.* [13] have further shown that there is a linear relationship between the  $E_T(30)$  value and the  $\pi^*$  and  $\alpha$  scale for a large number of pure polar (both aprotic and protic) and non-polar solvents:

$$E_T(30) = 31.00 + 13.43\pi^* + 15.06\alpha \quad (7)$$

$n = 40, r = 0.984, \text{S.D.} = 1.65$

A linear relationship between  $\pi^*$  and the solubility parameter has also been reported [15]:

$$\delta_H^2 = 44.1 + 95.6\pi^* \quad (8)$$

$n = 17, r = 0.858, \text{S.D.} = 19.7$

Comparing eqns. 7 and 8 to eqn. 5, it may be observed that there is no solvent HBA basicity term,  $\beta$ , in eqns. 7 and 8. Since the contribution of solute HBD acidity and solvent HBA basicity to retention is much smaller than the contributions from other interactions [16,17], the  $a'\beta_m$  term in eqn. 5 can be neglected. Thus eqn. 5 may be simplified to eqn. 9:

$$\log k' = b + m [E_T(30)] \quad (9)$$

The validity of eqn. 9 was empirically proven by Johnson *et al.* [18] Using an extensive base of 332 sets of retention data as a function of mobile phase composition, they compared the  $E_T(30)$  scale with the commonly used volume percent plots and showed that this empirical measure is a better descriptor of mobile phase "strength".

It was also found that for a solute–column pair, extrapolation of  $\log k'$  vs. volume percentage of organic modifier gives significantly different intercepts for different organic modifiers, while the extrapolation of  $\log k'$  vs.  $E_T(30)$  leads to a common intersection point at approximately the  $E_T(30)$  value of pure water [19]. Using over 200 sets of chromatographic retention data, Michels and Dorsey [20] compared the estimation of  $\log k'_w$  by the volume percentage approach and by the  $E_T(30)$  approach and found that the  $E_T(30)$  approach gave a more reliable estimation of this lipophilicity parameter. This evaluation was based on 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.

In this paper two short columns which have very little resistance to mobile phase flow to allow very high volume flow-rates were used to measure actual values of  $\log k'_w$  for 15 compounds. Extrapolated values of  $\log k'_w$  from volume percentage and  $E_T(30)$  scales were compared to the measured  $\log k'_w$ . A relatively high bonding density stationary phase ( $3.41 \mu\text{mol/m}^2$ ) was used to reduce the interaction between the solute and residual silanol groups on the stationary phase.

However, either volume percentage or the  $E_T(30)$  scale is a function of the mobile phase only, while chromatographic retention is a function of both the stationary and mobile phases. Numerous studies have shown that structure and composition of the stationary phase change with changing the mobile phase composition, especially for highly aqueous mobile phases, and retention can also be affected [21–31]. Estimation of retention from only the properties of the mobile phase may cause errors. Mobile phase and stationary phase effects on retention can be derived by measuring the retention times for a wide range of solute types with a single mobile phase composition using eqn. 10

$$\log k' = \log k'_0 + m''(\bar{V}_2)/100 + s''\pi_s^* + a''\alpha_2 + b''\beta_2 \quad (10)$$

where  $m'' = m(\delta_s^2 - \delta_m^2)$ ,  $s'' = s(\pi_s^* - \pi_m^*)$ ,  $a'' = a(\beta_s - \beta_m)$  and  $b'' = b(\alpha_s - \alpha_m)$ . Limited ranges of mobile phase compositions (30–90% methanol in water and 40–80% acetonitrile in water) have in fact been studied using eqn. 10 [16,32–34]. In this paper eqn. 10 is solved, for the first time, over the entire range of mobile phase compositions. Predictions of  $\log k'_w$  are attempted from the retention as a function of both the stationary phase and the mobile phase.

## EXPERIMENTAL

### Materials

HPLC-grade methanol and acetonitrile (Fisher Scientific, Fair Lawn, NJ, USA) were used without further purification. Water was obtained from a Barnstead Nanopure II water purification system (Barnstead, Boston, MA, USA) fitted with a 0.45- $\mu\text{m}$  filter. Pure solutes were used as received and stock solutions made in HPLC-grade methanol. Naphthalene, dimethyl phthalate and *p*-chlorophenol were from Eastman Kodak (Rochester, NY, USA). Benzene, toluene, ethylbenzene, *p*-chlorotoluene, acetophenone, pyridine and benzyl alcohol were from Fisher Scientific. Fluorobenzene, 1-nitrobutane, 1-nitrohexane, anisole, methyl paraben and ethyl paraben were from Sigma (St. Louis, MO, USA). ET-30 (Reichardt's Dye) was purchased from Aldrich (Milwaukee, WI, USA).

### Retention measurements

All retention measurements were made using an Applied Biosystems (Ramsey, NJ, USA) Spectroflow 400 pump, with UV detection at 254 nm using a Kratos Spectroflow 757 variable-wavelength absorbance detector. Sample injection was performed using a Valco (Houston, TX, USA) C6W injector fitted with a 10- $\mu\text{l}$  sample loop, and detector output was recorded on a Scientific Products Quantigraph chart recorder (Houston Instruments, Austin, TX, USA). Two "homemade"  $\text{C}_{18}$  columns, 7 cm  $\times$  4.6 mm I.D. and 2 cm  $\times$  4.6 mm I.D., with an alkyl chain bonding density of 3.41  $\mu\text{mol}/\text{m}^2$  were used. The base silica material was from a single lot of 10  $\mu\text{m}$  particle diameter Davisil (W. R. Grace, Balti-

more, MD, USA) with a surface area ( $S_{\text{BET}}$ ) of 300  $\text{m}^2/\text{g}$  and a pore size of 150  $\text{\AA}$ . The synthesis and bonding density determination for this packing material may be found elsewhere [35]. All solutes were dissolved in methanol–water (50:50) or weaker mobile phase. For the short 2-cm columns, a 10- $\mu\text{l}$  injection volume may represent up to 5% of the void volume, and the injection solvent may slightly affect retention time measurements made in very weak mobile phases. The columns were packed by use of a Shandon high-pressure HPLC column packer with a 33-ml slurry reservoir (Shandon Southern Instruments, Sewickey, PA, USA). Each column was thermostatted at 30°C using a water jacket and a Brinkmann Lauda (Westbury, NY, USA) Model MT heater/circulator. The eluent flow-rate was varied from 1.0 to 4.5 ml/min depending on the mobile phase composition. Retention times were determined manually based on the peak maximum position. The breakthrough time ( $t_0$ ) used to calculate capacity factors was determined at each composition by the elution of an injection of  $^2\text{H}_2\text{O}$  (Sigma). Multi-variable regression calculations of eqn. 4 were done by using a self written program run on a personal computer.

## RESULTS AND DISCUSSION

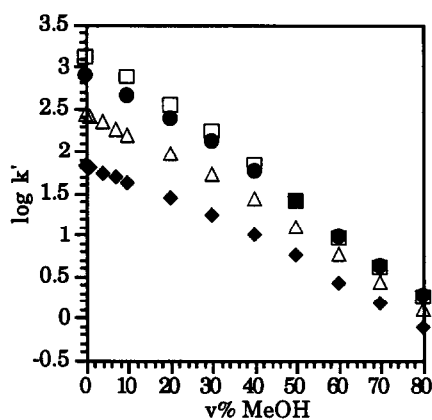
### Comparison of calculated $\log k'_w$ from volume percentage of organic modifier and experimentally measured $\log k'_w$

$\log k'$  values for all compounds are plotted vs. volume percentage of methanol or acetonitrile in Fig. 1a to h. Correlation of determination,  $r^2$ , for these plots is shown in Table I. Obviously, the second polynomial term cannot be neglected for the relationship between  $\log k'$  and volume percentage of acetonitrile. Even though the  $r^2$  values are high for the plots of  $\log k'$  vs. volume percentage of methanol, a distinct curvature can be observed for virtually all the compounds, especially from 0–10% methanol. Because of the curvature, the extrapolated value of  $\log k'_w$  as measured using capacity factors in the range of 10% to 80% methanol in water is different from the experimentally measured  $\log k'_w$ . Measured  $\log k'_w$ , extrapolated  $\log k'_w$  and the percentage errors for various compounds are listed in Table II. It is shown in Table II that the extrapolated value of  $\log k'_w$  from a linear plot of  $\log k'$

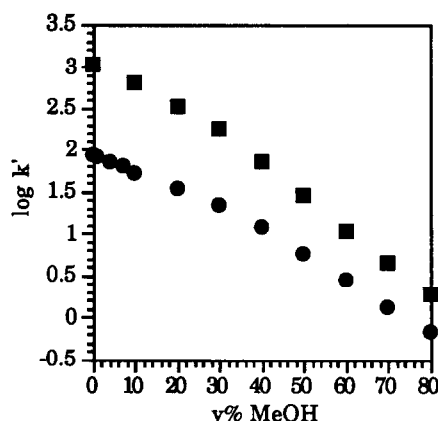
versus volume percentage of methanol can be larger or smaller than the measured  $\log k'_w$  and the sign of the error depends on direction of curvature between 10% and 0% methanol in water. It seems that this direction of curvature for the plot of  $\log k'$  vs. volume percentage of methanol between 10% and 0% methanol in water is related to the inherent polarity nature of a compound. Retention curves for non-polar compounds appear to plateau at volume percentages greater than about 90% water in the mobile phase, and the extrapolated  $\log k'_w$  becomes larger than the actual  $\log k'_w$ . For polar compounds, because of the dramatic increasing  $\log k'$  from 10% to 0% methanol in water, the extrapolated  $\log k'_w$  is smaller than the measured value. To verify the re-

tention behavior in the high percentage water region, retention times for eight compounds were measured using 7%, 4% and 1% methanol in water. A similar trend in sign of the error between the extrapolated and the measured  $\log k'_w$  is also found for polar and non-polar compounds using acetonitrile-water mixtures. Calculation of extrapolated  $\log k'_w$  using the acetonitrile-water mobile phase is based on the quadratic relationship between  $\log k'$  and volume percentage of organic modifier. The different retention behaviors of compounds in the high water content mobile phase (>90%) and lower water content mobile phase (<90%) were also found by Schoenmakers *et al.* [36], Gilpin and Gangoda [37] and Scott and Simpson [27].

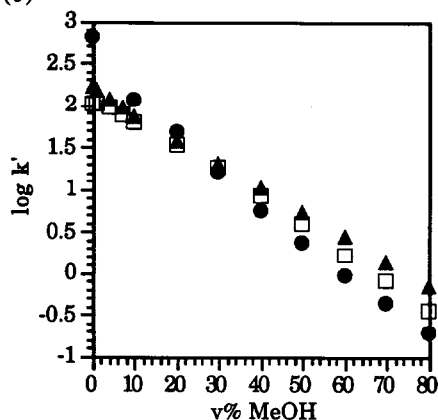
(a)



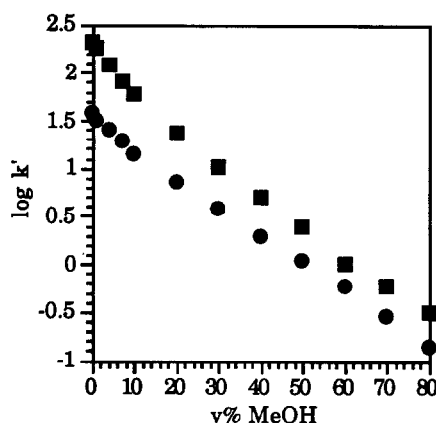
(b)



(c)



(d)



(Continued on p. 68)

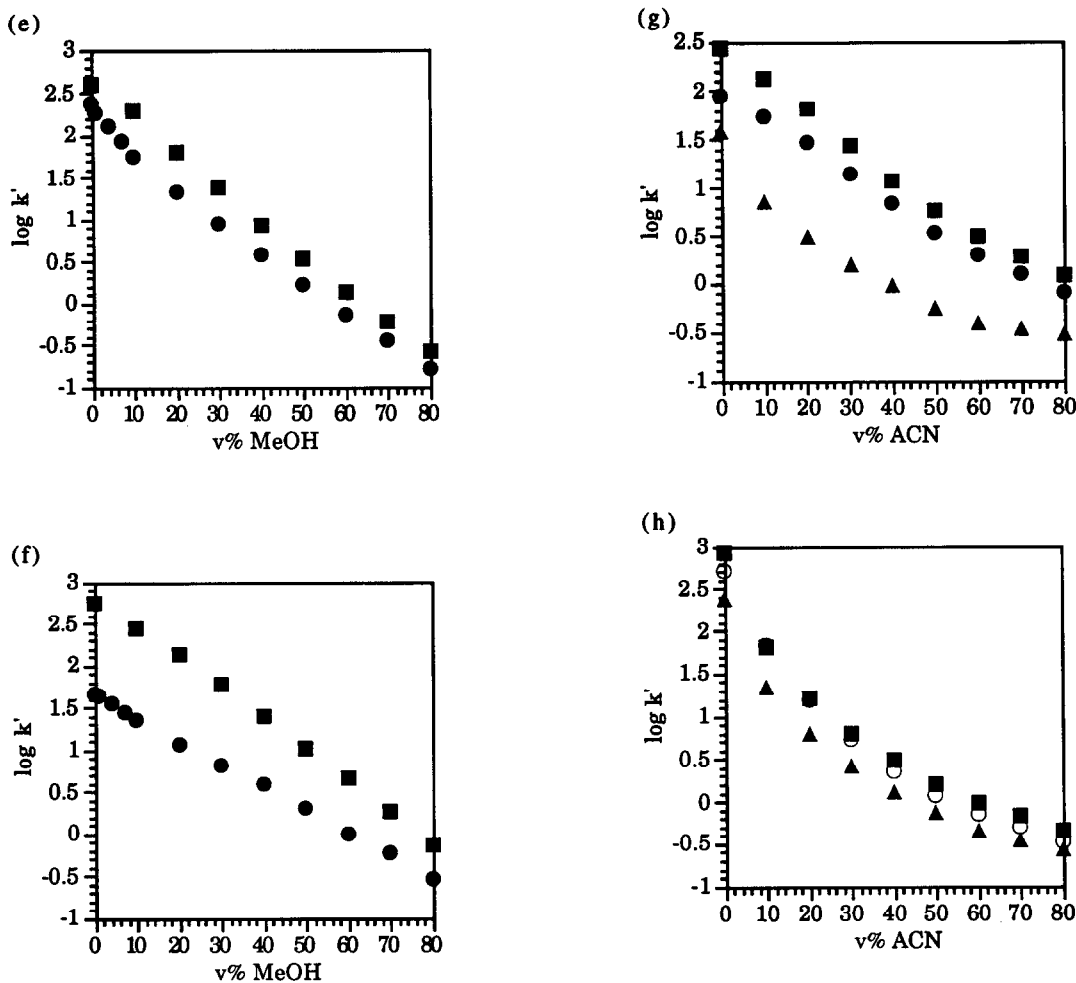


Fig. 1. Log  $k'$  vs. volume percentage (v%) of organic modifier on a column with a bonding density of  $3.41 \mu\text{mol}/\text{m}^2$ . Symbols: (a)  $\square$  = naphthalene,  $\bullet$  = ethylbenzene,  $\triangle$  = toluene,  $\blacklozenge$  = benzene; (b)  $\blacksquare$  = *p*-chlorotoluene,  $\bullet$  = fluorobenzene; (c)  $\square$  = *p*-chlorophenol,  $\bullet$  = dimethyl phthalate,  $\blacktriangle$  = anisole; (d)  $\blacksquare$  = acetophenone,  $\bullet$  = benzyl alcohol; (e)  $\blacksquare$  = ethylparaben,  $\bullet$  = methylparaben; (f)  $\blacksquare$  = nitrohexane,  $\bullet$  = nitrobutane; (g)  $\blacksquare$  = toluene,  $\bullet$  = fluorobenzene,  $\blacktriangle$  = benzyl alcohol; (h)  $\blacksquare$  = dimethyl phthalate,  $\circ$  = ethylparaben,  $\blacktriangle$  = methylparaben.

According to Schoenmakers *et al.* [36] and Gilpin and Gangoda [37], the abnormal retention behaviour observed using  $\leq 10\%$  methanol mobile phase is caused by sorption of organic modifier. Schoenmakers *et al.* [36] believe that eqn. 1 is only valid for mobile phases containing less than 90% water. In order to include the influence of stationary phase modification on retention, one extra term proportional to the square root of  $\varphi$  is necessary to

describe the retention over the full range of composition  $0 < \varphi < 1$ :

$$\ln k' = A\varphi^2 + B\varphi + C + E\sqrt{\varphi} \quad (11)$$

Based on their study, the curvature of the  $\ln k'$  vs.  $\varphi$  plot for the mobile phase composition between 0 and 0.1 changes with  $E$  term and the value of  $E$  is determined by the organic modifier and solute. Ten solutes and three organic modifiers were used to de-

TABLE I

CORRELATION OF DETERMINATION ( $r^2$ ) FOR THE PLOTS BETWEEN  $\log k'$  AND VOLUME PERCENTAGE OF METHANOL OR ACETONITRILE USING MOBILE PHASES FROM (0:100) TO (80:20) ORGANIC-WATER MIXTURES

Compound	$r^2$	
	Methanol	Acetonitrile
Benzene	0.9930	
Toluene	0.9947	0.9907
Ethylbenzene	0.9960	
Fluorobenzene	0.9908	0.9931
Naphthalene	0.9945	
<i>p</i> -Chlorotoluene	0.9944	
Acetophenone	0.9919	
Dimethyl phthalate	0.9846	0.8963
Anisole	0.9996	
Benzyl alcohol	0.9978	0.8918
<i>p</i> -Chlorophenol	0.9962	
Methylparaben	0.9932	0.8859
Ethylparaben	0.9976	0.9204
Nitrobutane	0.9991	
Nitrohexane	0.9939	

termine the validity of eqn. 11 in that paper. Some deviations were observed between the calculated curve (eqn. 11) and the individual data points and no relationship was found between the solute polarity and the value of  $E$ .

Retention for only one solute, ethanol, was studied by Scott and Simpson [27] using methanol-water mixtures with high water content on several different columns. In their study, retention volume of ethanol on a monomeric  $C_{18}$  column was found to reach a maximum at 5% methanol in water and then decreases as the volume percentage of water is increased. The explanation provided for this behavior is that at high water content, the  $C_{18}$  chains tend to interact more strongly with themselves than with the surrounding mobile phase. This dispersive interaction of the hydrocarbon chains results in a reduction in effective chromatographic surface area, and consequently anomalously low retentive characteristics. This explanation is supported by the observation that retention volume on a  $C_2$  column is higher than that on the  $C_{18}$  column using a 100% aqueous mobile phase. In our study, a similar trend is found

TABLE II

MEASURED  $\log k'_w$ , EXTRAPOLATED  $\log k'_w$  FROM VOLUME PERCENTAGE OF ORGANIC MODIFIER AND THE PERCENTAGE ERROR BETWEEN THE EXTRAPOLATED AND MEASURED  $\log k'_w$  USING METHANOL OR ACETONITRILE AS THE ORGANIC MODIFIER

$$\text{Error \%} = \frac{\log k'_w (\text{extrapolated}) - \log k'_w (\text{measured})}{\log k'_w (\text{measured})} \cdot 100$$

Compound	$\log k'_w$ <sup>a</sup> (measured)	$\log k'_w$ <sup>b</sup> methanol	$\log k'_w$ <sup>c</sup> methanol	$\log k'_w$ <sup>d</sup> acetonitrile	Error % <sup>b</sup> methanol	Error % <sup>c</sup> methanol	Error % <sup>d</sup> acetonitrile
Benzene	1.82	1.96	1.82		7.69	0.00	
Toluene	2.43	2.57	2.42	2.59	5.76	-0.41	6.58
Ethylbenzene	2.88	3.08	2.93		6.94	1.74	
Fluorobenzene	1.94	2.10	1.92	2.14	8.25	-1.03	10.31
Naphthalene	3.12	3.31	3.24		6.09	3.85	
<i>p</i> -Chlorotoluene	3.01	3.27	3.13		8.64	3.99	
Acetophenone	2.31	2.02	2.16		-12.55	-6.49	
Dimethyl phthalate	2.81	2.43	2.57	2.31	-13.52	-8.54	-17.79
Anisole	2.22	2.17	2.14		-2.25	-3.60	
Benzyl alcohol	1.58	1.43	1.40	1.24	-9.49	-11.39	-21.52
<i>p</i> -Chlorophenol	2.01	2.18	2.08		8.46	3.48	
Methylparaben	2.37	2.05	2.17	1.84	-13.50	-8.44	-22.36
Ethylparaben	2.58	2.60	2.76	2.41	0.78	6.98	-6.59
Nitrobutane	1.67	1.62	1.58		-2.99	-5.39	
Nitrohexane	2.75	2.86	2.79		4.00	1.45	

<sup>a</sup> Measured  $\log k'_w$ .

<sup>b</sup> Calculation of the extrapolated  $\log k'_w$  is based on the linear function between  $\log k'$  and volume percentage using mobile phases containing 10-80% methanol.

<sup>c</sup> Calculation of the extrapolated  $\log k'_w$  is based on the second polynomial function between  $\log k'$  and volume percentage using mobile phases containing 10-80% methanol.

<sup>d</sup> Calculation of the extrapolated  $\log k'_w$  is based on the second polynomial function between  $\log k'$  and volume percentage using mobile phases containing 10-80% acetonitrile.

for non-polar compounds and *p*-chlorophenol. Different curves under a highly aqueous mobile phase for polar compounds were also observed in our study and cannot be explained by this dispersive interaction between hydrocarbon chains of the stationary phase.

*Comparison of calculated  $\log k'_w$  from  $E_T(30)$  scale and experimentally measured  $\log k'_w$*

$\log k'$  values are plotted against  $E_T(30)$  values in Fig. 2a to h. Correlation of determination,  $r^2$ , for these plots is shown in Table III. Measured  $\log k'_w$ , extrapolated  $\log k'_w$  from the  $E_T(30)$  scale, and the percentage errors for various compounds are listed

in Table IV. Comparison of the correlation of determination of the plots between  $\log k'$  and either  $E_T(30)$  scale or volume percentage of organic modifier in Tables I and III shows that for volume percentage plots,  $r^2$  values are greater than 0.99 using methanol-water mixtures for all compounds and using acetonitrile-water mixtures for non-polar compounds, and  $r^2$  values are between 0.88 and 0.92 for polar compounds using acetonitrile-water mixtures. For  $E_T(30)$  plots using methanol-water or acetonitrile-water mixtures,  $r^2$  values are greater than 0.98 for polar compounds, while  $r^2$  values are between 0.95 and 0.97 for non-polar compounds and *p*-chlorophenol.

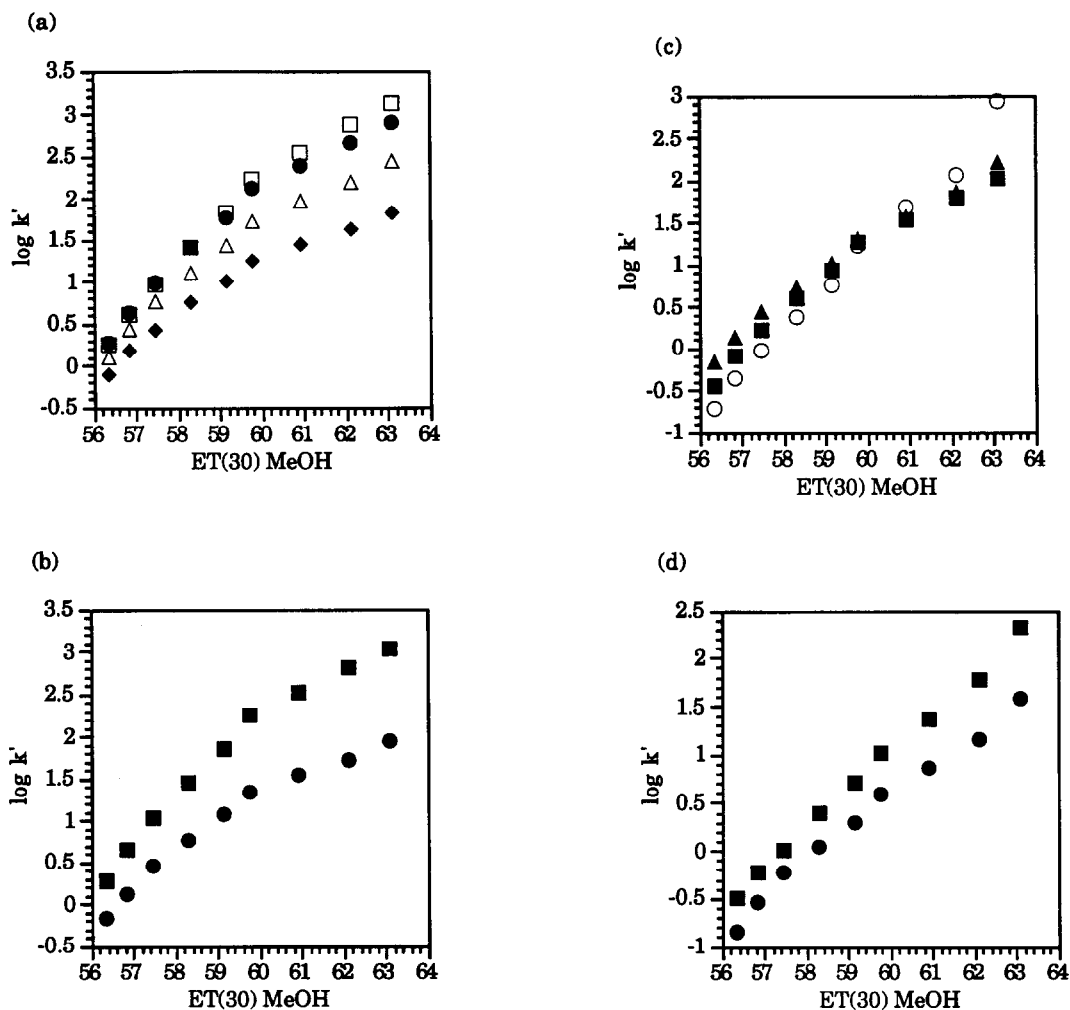


Fig. 2.



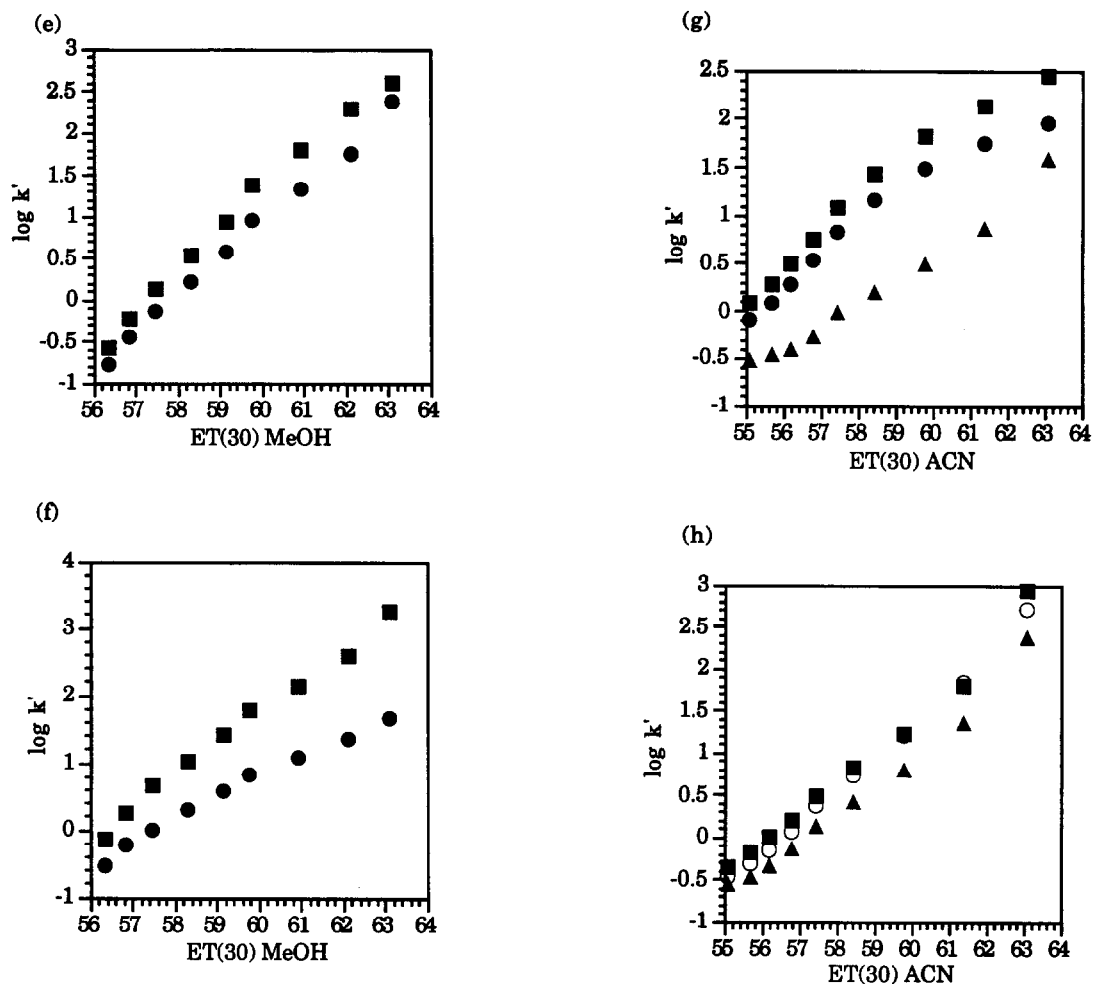


Fig. 2.  $\log k'$  vs.  $E_T(30)$  scale using methanol (or acetonitrile)–water mixtures on a column with a bonding density of  $3.41 \mu\text{mol}/\text{m}^2$ . Symbols as in Fig. 1.

Comparison between the extrapolated  $\log k'_w$  from either  $E_T(30)$  scale or volume percentage of organic modifier and the measured  $\log k'_w$  in Tables II and IV shows that the errors are within a similar range from both extrapolation methods. The errors can be as high as 10% using methanol–water and 20% using acetonitrile–water. Extrapolated  $\log k'_w$  can be obtained from linear plots between  $\log k'$  and  $E_T(30)$  values using both methanol–water and acetonitrile–water mixtures, yet a second polynomial fit between  $\log k'$  and volume percent of organic modifier is needed to predict  $\log k'_w$  when acetonitrile is used as an organic modifier. For non-polar compounds the errors are larger using  $E_T(30)$

plots than using the volume percentage plots. The errors are relatively smaller for polar compounds using  $E_T(30)$  plots than using volume percentage plots. The different curvature in high water (or high polarity) region relative to the low water (or low polarity) region in plots of  $\log k'$  versus  $E_T(30)$  scale shown in Fig. 2 is similar to the volume percentage plots. For non-polar compounds, the retention increases with increasing the polarity of the mobile phase, however, the retention did not increase in high polarity region of mobile phase as expected according to the slope in low polarity region of mobile phase. Thus the retention curve appears to plateau at the polarity of mobile phase above the

TABLE III  
CORRELATION OF DETERMINATION ( $r^2$ ) FOR THE PLOTS BETWEEN  $\log k'$  AND  $E_T(30)$  SCALE USING MOBILE PHASES FROM 0% TO 80% METHANOL (OR ACETONITRILE)-WATER MIXTURES

Compound	$r^2$	
	Methanol	Acetonitrile
Benzene	0.9577	
Toluene	0.9621	0.9697
Ethylbenzene	0.9710	
Fluorobenzene	0.9524	0.9569
Naphthalene	0.9709	
<i>p</i> -Chlorotoluene	0.9672	
Acetophenone	0.9958	
Dimethyl phthalate	0.9907	0.9887
Anisole	0.9843	
Benzyl alcohol	0.9860	0.9818
<i>p</i> -Chlorophenol	0.9663	
Methylparaben	0.9943	0.9803
Ethylparaben	0.9937	0.9980
Nitrobutane	0.9840	
Nitrohexane	0.9896	

$E_T(30)$  value of 60 (ca. 30% organic modifier in water) for non-polar compounds. For polar compounds, there is an abrupt increase in retention from the  $E_T(30)$  value of mobile phase about 62 to 63 (ca. 10% to 0% organic modifier in water). It seems that modification of the stationary phase is very important for solute retention under highly aqueous mobile phase and cannot be neglected in prediction of  $\log k'_w$  values.

*Retention studies by the solvatochromic comparison method*

The solvatochromic parameters ( $\pi^*$ ,  $\beta$ ,  $\alpha$ ,  $\delta$ ) of the solutes listed in Table V were cited from the values reported by Kamlet *et al.* [34]. A polarizability correction factor,  $\delta$ , is defined as zero for aliphatic compounds and 1 for aromatic compounds. These values were chosen to bring the aromatic and aliphatic compounds into line with the select compounds in a wide variety of correlations. There are many ways to determine solute molar volume and it was proved that Leahy's computer-calculated in-

TABLE IV  
MEASURED  $\log k'_w$ , EXTRAPOLATED  $\log k'_w$  FROM THE  $E_T(30)$  SCALE AND PERCENTAGE ERROR BETWEEN THE EXTRAPOLATED AND MEASURED  $\log k'_w$  USING METHANOL OR ACETONITRILE AS THE ORGANIC MODIFIER

Compound	$\log k'_w$ <sup>a</sup> (measured)	$\log k'_w$ <sup>b</sup> methanol	$\log k'_w$ <sup>b</sup> acetonitrile	Error % methanol	Error % acetonitrile
Benzene	1.82	2.10		15.38	
Toluene	2.43	2.73	2.86	12.35	17.70
Ethylbenzene	2.88	3.27		13.54	
Fluorobenzene	1.94	2.25	2.41	15.98	24.23
Naphthalene	3.12	3.52		12.82	
<i>p</i> -Chlorotoluene	3.01	3.47		15.28	
Acetophenone	2.31	2.22		-3.90	
Dimethyl phthalate	2.81	2.65	2.38	-5.69	-15.30
Anisole	2.22	2.34		5.41	
Benzyl alcohol	1.58	1.56	1.23	1.27	-22.15
<i>p</i> -Chlorophenol	2.01	2.36		17.41	
Methylparaben	2.37	2.27	1.84	-4.22	-22.36
Ethylparaben	2.58	2.85	2.43	10.47	-5.81
Nitrobutane	1.67	1.78		6.59	
Nitrohexane	2.75	3.07		11.64	

<sup>a</sup> Measured  $\log k'_w$ .

<sup>b</sup> Calculation of the extrapolated  $\log k'_w$  is based on the linear function between  $\log k'$ , and  $E_T(30)$  scale using mobile phases containing 10-80% methanol or acetonitrile.

TABLE V  
SOLUTE  $V, \pi^*, \beta, \alpha$  AND  $\delta$  VALUES

	V(1/100)	$\pi^*$	$\beta$	$\alpha$	$\delta$
<i>Non-polar compounds</i>					
Benzene	0.491	0.59	0.10	0.00	1
Toluene	0.592	0.55	0.11	0.00	1
Ethylbenzene	0.668	0.53	0.12	0.00	1
Fluorobenzene	0.520	0.62	0.07	0.00	1
Naphthalene	0.753	0.70	0.15	0.00	1
p-Chlorotoluene	0.679	0.67	0.08	0.00	1
<i>Compounds with hydrogen bond acceptor groups</i>					
Acetophenone	0.69	0.90	0.49	0.04	1
Dimethyl phthalate	0.98	0.85	0.80	0.00	1
Anisole	0.639	0.73	0.32	0.00	1
Nitrobutane	0.641	0.76	0.25	0.00	0
Nitrohexane	0.837	0.72	0.25	0.00	0
<i>Compounds with hydrogen bond donor groups</i>					
Benzyl alcohol	0.634	0.99	0.52	0.39	1
p-Chlorophenol	0.626	0.72	0.23	0.67	1
<i>Compounds with hydrogen bond acceptor and donor groups</i>					
Methylparaben	0.778	1.34	0.70	0.61	1
Ethylparaben	0.874	1.33	0.69	0.61	1

TABLE VI

MULTIPLE LINEAR REGRESSION BETWEEN  $\log k'$  AND  $V_2/100, \pi_2, \beta_2, \alpha_2,$  AND  $\delta_2$  USING VARIOUS METHANOL-WATER AND ACETONITRILE-WATER COMPOSITIONS

Methanol values were based on a data set of 15 compounds. Acetonitrile values were based on a data set of 6 compounds.  
 $\log k' = \log k'_0 + m''\bar{V}_2/100 + s''\pi_2^* + b''\beta_2 + a''\alpha_2 + d''\delta_2$

Organic modifier (%, v/v)	$m''$	$s''$	$b''$	$a''$	$d''$	$\log k'_0$	$r^2$
<i>Methanol</i>							
80	1.98	0.54	-2.30	-0.44	0.42	-1.63	0.9553
70	2.38	0.52	-2.48	-0.37	0.43	-1.51	0.9578
60	2.85	0.56	-2.72	-0.34	0.47	-1.51	0.9559
50	3.37	0.61	-3.00	-0.33	0.53	-1.52	0.9655
40	3.98	0.69	-3.28	-0.30	0.59	-1.64	0.9688
30	4.66	0.76	-3.47	-0.28	0.68	-1.84	0.9717
20	5.17	0.78	-3.42	-0.29	0.70	-1.94	0.9728
10	5.51	1.08	-3.49	-0.34	0.72	-2.09	0.9400
0	5.35	0.87	-2.59	-0.52	0.72	-1.74	0.9637
<i>Acetonitrile</i>							
80	1.57	-0.65	-1.17	0.41		-0.41	0.9812
70	2.25	-0.46	-1.68	0.29		-0.65	0.9865
60	2.28	-0.20	-2.14	0.09		-0.86	0.9856
50	3.24	0.03	-2.54	-0.05		-0.96	0.9806
40	3.62	-0.07	-2.78	0.08		-0.78	0.9815
30	4.47	0.09	-3.37	0.07		-0.95	0.9810
20	5.43	0.10	-3.87	0.23		-1.10	0.9808
10	6.39	0.23	-4.07	0.35		-1.40	0.9778
0	6.15	0.40	-2.76	0.03		-1.25	0.9482

intrinsic volumes ( $V_i$ ) leads to more precise correlations as well as to a “cleaner” dissection of the multiple solute–solvent interactions [32].  $V_i$  can be approximated by using McGowan's characteristic volume ( $V_x$ ) through the relationship between the two sets of volumes [38]:

$$V_i = 0.597 + 0.6823 V_x \quad (12)$$

A comprehensive list of atomic  $V_x$  values can be found in Abraham and McGowan's paper [38]. The unit for solute molar volume is in  $\text{cm}^3 \text{mol}^{-1}$ . Results from this study are shown in Table VI using methanol–water mixtures and acetonitrile–water mixtures. The linear equations in Table VI include aromatic and aliphatic compounds.

From the magnitude of the coefficients in Table VI,  $m''$  and  $b''$  are much larger than  $s''$  and  $a''$  and this result is in accord with other related studies. The most important solute parameters which influence retention are solute size and HBA basicity, but not HBD acidity. The solute dipolarity is a minor but still significant factor. The positive and negative signs of the solute size term and hydrogen bond basicity term show that capacity factor increases with increasing the solute size, and decreases with increasing solute HBA ability at certain mobile phase compositions.

A solvent property complementary to solute intrinsic molar volume is solvent cohesiveness, which is measured by the square of the solubility parameter ( $\delta$ ) difference between the stationary phase and mobile phase. This is the  $m''$  term of eqn. 10. If  $\delta^2$  of the stationary phase does not change with changing the mobile phase composition, the  $m''$  coefficient should be proportional to  $\delta^2$  of the mobile phase. Because water is more cohesive ( $\delta = 23.4 \text{ cal}^{1/2} \text{ cm}^{-3/2}$ ) (1 cal = 4.184 J) than methanol ( $\delta = 14.3 \text{ cal}^{1/2} \text{ cm}^{-3/2}$ ), acetonitrile ( $\delta = 11.75 \text{ cal}^{1/2} \text{ cm}^{-3/2}$ ) and alkanes ( $\delta = 11.75 \text{ cal}^{1/2} \text{ cm}^{-3/2}$ ), the process of forming the cavity in the solvent becomes increasingly endoergic with increasing water content. Accordingly, the  $m''$  increases as the water content is increased from (80:20) to (10:90) organic–water in Fig. 3. However, the  $m''$  coefficient decreases from organic–water (10:90) to pure water. This means that  $\delta^2$  of the stationary phase is not a constant when the composition of mobile phase is changed and it increases significantly from organic–water (10:90) to pure water.

Because stationary phase can be selectively solvated by organic modifier in the mobile phase,  $\delta_s$  may change over the entire range of mobile phase compositions. Abrupt change of  $\delta_s$  from (10:90) to (0:100) methanol–water may be due to the structure change of the stationary phase within this mobile phase region. Numerous studies have shown that alkyl chains of the stationary phase are in a more extended “bristle” state in an organic containing mobile phase, while they are “collapsed” to the surface in a pure water mobile phase [21–27]. This con-

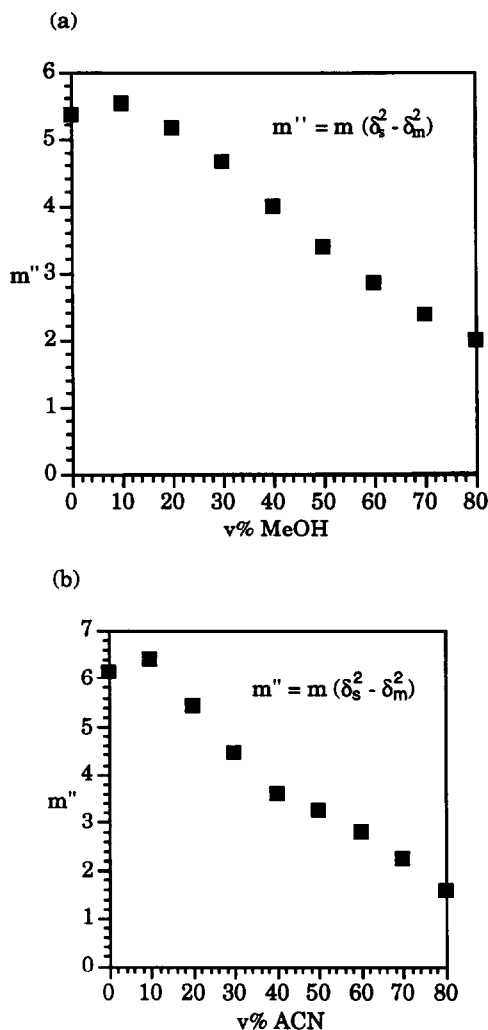


Fig. 3. Plot of the contribution of  $\log k'$  from the cavity formation difference between the stationary phase and mobile phase,  $m''$ , versus volume percentage (v%) of (a) methanol, (b) acetonitrile.

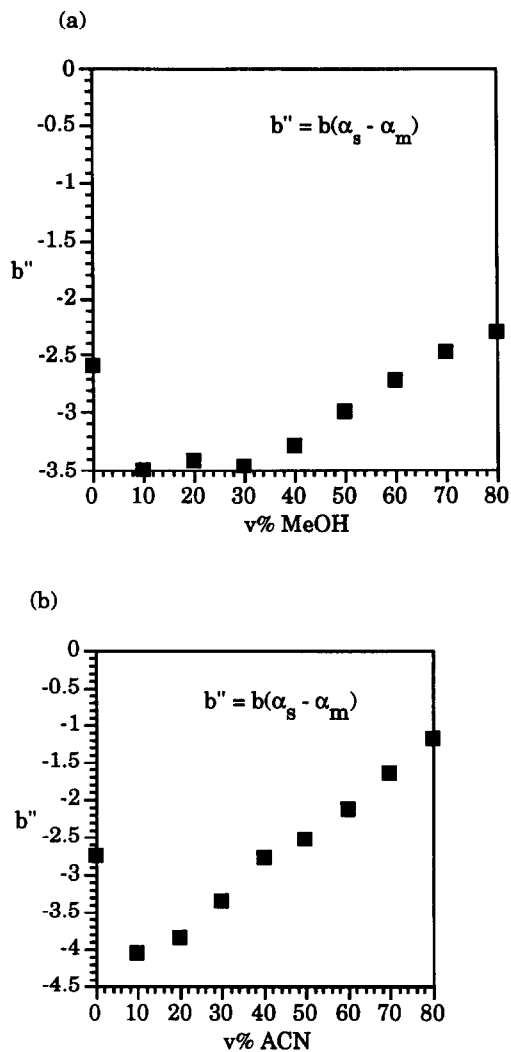


Fig. 4. Plot of the hydrogen bonding term contributed from the hydrogen bond acidity difference between the stationary phase and mobile phase,  $b''$ , versus volume percentage (v%) of (a) methanol, (b) acetonitrile.

formational change reduces the surface area of the stationary phase and the contact interactions between solutes and the stationary phase. Thus,  $m''$  did not increase with increasing the water concentration as expected at highly aqueous region, instead it decreased.

However, the idea of collapsed vs. extended chain configurations was recently rejected by measuring

the orientation and reorientation of a fluorescent probe, 1,4-bis(*o*-methylstyryl)benzene assuming that any changes in the orientational distribution of the chains will cause related changes in the orientational distribution of the probe solute [39]. Montgomery *et al.* [39] found that the  $C_{18}$  chains lie flat on the surface when water is the mobile phase and change little in the presence of small amount of alcohol. Another possible reason for abnormal behavior of  $m''$  is the pore effects. As the mass transfer of solute molecules into and out of the stationary phase zone is controlled mainly by their diffusion within the porous particles, the pore structure of the packing is also important with respect to the retention mechanism. Because of the non-polar alkyl chains on the surface, solute diffusion into the pores is dependent on the organic modifier in the mobile phase. Solutes may diffuse into the pores of silica less effectively in highly polar aqueous mobile phases than in the organic rich mobile phases. As the alkyl chains in the pores are less accessible in a highly aqueous mobile phase compared to the organic rich mobile phase, the effective volume of the stationary phase is reduced, and  $m''$  is reduced also.

The solvent property complementary to solute HBA basicity is the HBD acidity difference between the stationary phase and mobile phase. This is the  $b''$  term of eqn. 4. Since the stationary phase is highly solvated at higher organic content mobile phases, hydrogen bonding interactions are dominated by the interaction between solutes and the mobile phase. As shown in Fig. 4,  $b''$  coefficients become increasingly negative in going from organic-water (80:20) to (10:90) and this is because water is a stronger hydrogen bond donor acid ( $\alpha = 1.17$ ) than methanol ( $\alpha = 0.93$ ) and acetonitrile ( $\alpha = 0.19$ ). However, the  $b''$  coefficient increases significantly from organic-water (10:90) to (0:100). Again, like  $\delta_s$ ,  $\alpha_s$  changes with changing the mobile phase composition and there is a significant increase of  $\alpha_s$  from organic-water (10:90) to (0:100). The possible explanation is that the stationary phase is less solvated at lower organic content mobile phases, so residual silanol groups on the stationary phase might be more exposed to solutes and the hydrogen bonding interactions between solutes and the stationary phase become more significant.

### Correction of predicted $\log k'_w$ by a multivariable equation

Extrapolating  $\log k'_w$  from either volume percentage or  $E_T(30)$  scale from data obtained above organic–water (10:90) assumes that either conformation and composition of the stationary phase do not change with changing the mobile phase composition or solute diffusion within the porous silica particles is independent of mobile phase conditions. It may also assume that solute retention is not affected by the structural change or diffusion process. However, structure of the stationary phase and solute diffusion in pure water may not be the same as in organic–water mixtures. If the extended alkyl chains in organic rich mobile phase collapse to the surface of silica in highly aqueous mobile phase, surface area of the reversed-phase will be reduced. If solute diffusion within the pores of the silica is less efficient in highly aqueous mobile phases than in organic rich mobile phases, accessible volume of the stationary phase will be reduced. For both cases, retention time for non-polar compounds will not increase with increasing the water concentration as expected at highly aqueous region because of the reduction of surface area or volume of the reversed-phase, and the deviation is proportional to

solute size. Because the stationary phase is less solvated at lower organic content mobile phases, hydrogen bonding interactions between polar compounds and hydroxyl groups on the silica surface may increase the retention time.

This suggests that the difference between extrapolated and measured  $\log k'_w$  values is a function of solute properties:

$$\log k'_w (\text{extrapolated}) - \log k'_w (\text{measured}) = a(V/100) + b(\pi_2^*) + c(\beta_2) + d(\alpha_2) + e(\delta_2) + k \quad (13)$$

Deviations as functions of properties of solutes are listed in Table VII. From Table VII, correlation of determination is high for  $\log k'_w$  extrapolated from the  $E_T(30)$  scale using either methanol–water ( $r^2 = 0.9178$ ) or acetonitrile–water ( $r^2 = 0.9844$ ), and it is relatively lower for  $\log k'_w$  extrapolated from the volume percentage of organic modifier using either linear plot ( $r^2 = 0.7065$ ) or second polynomial plot ( $r^2 = 0.7874$ ) in methanol–water. This suggests that  $\log k'_w$  extrapolated from the  $E_T(30)$  plot gives more reproducible, meaningful values for correlation with bioavailability. The actual  $\log k'_w$  can be predicted from the  $E_T(30)$  scale and then corrected by eqn. 13.

TABLE VII

MULTIVARIABLE EQUATION OF DIFFERENCE BETWEEN THE EXTRAPOLATED AND MEASURED  $\log k'_w$  AS FUNCTION OF SOLUTE'S PROPERTIES

$\log k'_w$  values are extrapolated from either the volume percentage or  $E_T(30)$  scale using acetonitrile–water or methanol–water mobile phases.

$$\Delta = \log k'_w (\text{extrapolated}) - \log k'_w (\text{measured}) = a(V/100) + b(\pi_2^*) + c(\beta_2) + d(\alpha_2) + e(\delta_2) + k$$

	<i>a</i>	<i>b</i>	<i>c</i>	<i>d</i>	<i>e</i>	<i>k</i>	$r^2$	$n^a$
<i>Methanol–water</i>								
$\Delta$ (% , v/v, lin) <sup>b</sup>	0.41	0.28	−1.03	0.01	0.10	−0.21	0.7065	15
$\Delta$ (% , v/v, pol) <sup>c</sup>	0.84	0.32	−0.95	−0.15	0.07	−0.64	0.7874	15
$\Delta$ (ET) <sup>d</sup>	1.07	0.21	−1.37	0.26	0.09	−0.39	0.9178	15
<i>Acetonitrile–water</i>								
$\Delta$ (% , v/v, pol)	1.79	−0.34	−1.91	0.67		−0.44	0.9574	6
$\Delta$ (ET)	2.29	0.05	−2.64	0.27		−0.61	0.9844	6

<sup>a</sup> Number of compounds.

<sup>b</sup> Calculation of the extrapolated  $\log k'_w$  is based on the linear function between  $\log k'$  and volume percentage using mobile phases containing 10–80% organic.

<sup>c</sup> Calculation of the extrapolated  $\log k'_w$  is based on the second polynomial function between  $\log k'$  and volume percentage using mobile phases containing 10–80% organic.

<sup>d</sup> Calculation of the extrapolated  $\log k'_w$  is based on the linear function between  $\log k'$  and  $E_T(30)$  scale using mobile phases containing 10–80% organic.

## CONCLUSIONS

Based on the correlation of determination,  $\log k'$  is linearly in proportion to the  $E_T(30)$  scale using either methanol–water or acetonitrile–water mobile phase and is linearly related to volume percentage of methanol, yet it is a second polynomial function of the volume percentage of acetonitrile. A distinct curvature was observed for all compounds from 0 to 10% organic modifier in water. The direction of curvature was found to correlate with solute polarity. Retention curves for non-polar compounds appear to plateau at volume percentages greater than about 90% water in the mobile phase. An upward curve toward pure water mobile phase was observed for compounds with strong hydrogen-bond acceptor ability. The different retention behaviors of compounds in high water content mobile phases (>90%) and lower water content mobile phases (<90%) causes the errors of prediction of  $\log k'_w$  from extrapolation. Errors of predicted  $\log k'_w$  from either the  $E_T(30)$  scale or volume percentage of organic modifier can be as much as about 10% using methanol–water and 20% using acetonitrile–water. The reason is that either volume percentage or  $E_T(30)$  scale is a function of mobile phase only but chromatographic retention is a function of both the stationary and mobile phases.

Mobile phase and stationary phase effects on retention were partially deconvoluted by the linear solvation energy relationship. Eqn. 10 was solved by retention of 15 compounds over the entire range of mobile phase compositions. It was found that characteristics of the stationary phase in eqn. 10 ( $\delta_s$ ,  $\pi_s$ ,  $\beta_s$ ,  $\alpha_s$ ) are smaller than those of the mobile phase, and changed little using mobile phase from methanol–water (10:90) to (80:20). However, these values changed significantly when 100% water was used as the mobile phase, presumably due to the change of the structure of the stationary phase or the diffusion process of solutes with respect to the mobile phase conditions. Conformational change of the stationary phase from a bristle structure (high organic content mobile phases) to a collapsed structure (high water content mobile phases) reduces the active surface area of the stationary phase. The loss of the efficiency for solute diffusion within the pores of the silica in highly aqueous mobile phases relative to the organic rich mobile phases reduces the volume

of the stationary phase. This provides explanation as to why retention time does not increase in the manner expected with increasing volume percentage of water for non-polar compounds.

As stationary phase is less solvated at highly aqueous mobile phases relative to organic rich mobile phases, the hydrogen bonding interactions between polar compounds and hydroxyl groups on the stationary phase may be stronger at highly aqueous mobile phases. Thus retention of polar compounds at highly aqueous mobile phases is greater than expected from retention at highly organic content mobile phases.

Extrapolating  $\log k'_w$  values from data obtained above methanol–water (10:90) does not represent the actual  $\log k'_w$ . Rather, it represents what the capacity factor would be, if the conformation and composition of stationary phase and solute diffusion process within porous silica particles in pure water were the same as in organic–aqueous mixtures. Thus this extrapolated  $\log k'_w$  value is still valid as an estimator of compound's lipophilicity, which is used heavily in QSRR studies. This is because the extended bristle structure of the RPLC stationary phase using high organic content mobile phase more closely mimics the structure of a bio-membrane–water interphase.

Without information on the physical properties of aqueous mixtures of organic solvents, eqn. 5 cannot be solved. However, eqn. 5 can be simplified using the  $E_T(30)$  scale, because  $E_T(30)$  is sensitive to solvent dipolarity–polarizability as well as solvent hydrogen bond donor ability. Thus the  $\log k'_w$  extrapolated from the  $E_T(30)$  plot is more meaningful for correlation with bioavailability. The difference between the calculated and measured  $\log k'_w$  is related to the characteristics of stationary phase and the interaction between solutes and stationary phase, and can be estimated from solute properties according to eqn. 13.

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