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Evaluation of the retention dependence on the physicochemical properties of solutes in reversed-phase liquid chromatographic linear gradient elution based on linear solvation energy relationships

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Abstract

This paper describes the results of the evaluation of retention dependence on the physicochemical properties of solutes in linear gradient elution by reversed-phase liquid chromatography (RPLC) based on linear solvation energy relationships (LSERs). Retention time data on Inertsil ODS(3) column by linear gradient elution were collected for both acetonitrile–water and methanol–water binary mobile phases under various gradient steepness. Based on the LSERs, the retention times were linearly correlated with the physicochemical properties (size, dipolarity, and hydrogen bond donor–acceptor acidity and basicity) of solutes. As predicted by LSERs, very acceptable linear relationships are observed for both mobile phases. While the magnitudes of the coefficients are modified by the gradient steepness, their signs are consistent with those obtained by isocratic elution. As obtained for isocratic elution, the dominant factors to retention in linear gradient elution of RPLC are the solutes' size and hydrogen bond acceptor basicity. The conclusions of the study allow us to predict retention in chromatographic method development by gradient elution. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Linear solvation energy relationships; Gradient elution; Mobile phase composition; Retention prediction; Column characterization

1. Introduction

There is no doubt that the majority of high-performance liquid chromatographic (HPLC) applications today are based on the reversed-phase separation mode due to its robustness and versatility. In developing HPLC methods based on RPLC, ODS columns are usually the first choice, and the optimization of the retention and selectivity is then performed by varying the compositions of the mobile

phases. This process can be repeated with different columns and refined by varying different separation variables (such as temperature and mobile phase additives) to provide sufficient resolutions within a reasonable run time. For most chromatographers, a stepwise change in mobile phase compositions is usually preferred for retention and selectivity optimization.

Although the isocratic elution mode is commonly used, gradient elution provides a means for the separation of very complex samples with quite different polarities. Furthermore, the use of the initial gradient elution for method development is being increasingly emphasized [1–4], and the initial scout-

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ing gradient run provides rich information for the method conditions. However, our understanding of retention in gradient elution is still rather limited. In fact, there is almost no study on the relationship between retention and the physicochemical properties of solutes [5–7]. The existence and determination of such a relationship are obviously very important because it offers us the ability to estimate or predict the retention and selectivity of solutes during method development. In particular, if a quantitative relationship can be established between retention and the physicochemical properties of solutes, it will allow us to predict the retention of solutes with known properties in gradient elution or to search solutes with desired retention. For example, if we want to insert an internal standard in the desired position for a gradient separation, the quantitative relationship between retention and the physicochemical properties of solutes can be established first using selected reference solutes. Once we have the relationship, the retention of solutes in a database can be calculated, and the solutes that match the desired retention can be selected as the internal standard. Although there are certain errors in the prediction, the benefits of the approach are obviously extremely significant.

The retention in isocratic elution chromatography has been successfully correlated with the physicochemical properties of solutes using linear solvation energy relationships (LSERs) [8–16]. Therefore, the retention in isocratic elution can be easily predicted if the physicochemical properties of solutes are known. In fact, the LSER approach has been found very applicable in RPLC [8–22], NPLC [23–26], and many other types of chromatography [27–32]. Although LSERs have been mainly used to characterize the properties of the stationary phases, their ability to predict solutes' retention is extremely useful in chromatographic method development. However, despite the success of LSERs for isocratic elution, their use in gradient elution is rather limited [5,7]. Valko et al. correlated gradient time with solutes' properties via linear regression, and the coefficients were used to compare different columns [5]. A similar study via principal component analysis was also reported [7]. Although gradient time was used to linearly correlate with solutes' properties, the origin was not fundamentally established.

The principle of a generalized LSER is based on a simple and conceptually explicit model of solute–solvent interactions, in which the solute solvation processes are identified and dissected into four types of solute–solvent interactions [12,33–35]. They include the cavity formation–dispersive interactions, dipolarity–polarizability interactions, and hydrogen bonding interactions. These interactions occur in both mobile and stationary phases in chromatography, and retention is the result of difference in the solute's interactions with both mobile and stationary phases. Because each solute possesses a unique set of physicochemical properties, it shows a different retention for a chromatographic system. If we measure the retention for solutes of known physicochemical properties, LSERs allow us to correlate retention with their properties. The outcome of a LSER analysis is a set of regression coefficients that relate retention with the physicochemical properties of solutes. Accordingly, if we know the properties of different solutes, their retention can be predicted.

In this study, LSERs will be applied to gradient elution to correlate retention times with the physicochemical properties of solutes. In particular, we will evaluate the retention dependence in linear gradient elution with the physicochemical properties of solutes based on LSERs and linear gradient elution theories using binary mobile phases of acetonitrile (ACN)–water and methanol (MeOH)–water. Although the results of the study can be used to quickly characterize chromatographic stationary phases, the focus of the study is to evaluate the existence of the relationship. Finally, the difference between isocratic and gradient LSERs will be examined.

2. Theoretical

In most method development involving the use of gradient elution, a linear gradient (or linear-solvent-strength, LSS, model) is usually used [1–4]. The retention time in LSS model is given by:

$$t_R = \frac{t_0}{b} \cdot \log(2.3bk'_i + 1) + t_0 + t_d \quad (1)$$

where t_R is the retention time, t_0 is the column (or system) dead time, k'_i is the isocratic value of k' at the start of the gradient, and t_d is the equipment

hold-up or “dwell” time. The parameter b is called the gradient steepness:

$$b = \frac{t_0(\Delta\varphi)S}{t_G} = \frac{V_m(\Delta\varphi)S}{t_GF} \quad (2)$$

where V_m is the column (or system) dead volume, $\Delta\varphi$ is the change in the mobile phase composition (in percent organic/100), t_G is the gradient time, F is the flow-rate, and S is the slope of $\log k'$ vs. φ (the mobile phase composition), and reported to be approximately a constant of 4.2 (SD=0.8) for small molecules [1]. In this study, S is regarded as a constant. The possible impact of the assumption on LSER coefficients will be discussed at the end (the error of the assumption in Eq. (1) is shown in the Appendix). It is noted that Eq. (1) is less reliable if the solute is not strongly retained at the beginning of the gradient (i.e. k'_i not large) [1]. Eq. (1) can be rearranged as follows:

$$\begin{aligned} t_R &= \frac{t_0}{b} \cdot \log(2.3bk'_i + 1) + t_0 + t_d \\ &= \frac{t_0}{b} \cdot \log(2.3bk'_i) + t_0 + t_d \quad (2.3bk'_i \gg 1) \\ &= \frac{t_0}{b} \cdot \log(2.3b) + t_0 + t_d + \frac{t_0}{b} \cdot \log k'_i \\ &\approx C + \frac{t_0}{b} \cdot \log k'_i \end{aligned} \quad (3)$$

where C is considered as a constant. The assumption in Eq. (3) is $k'_i \gg 1/(2.3b)$, which is usually true in most cases because the organic modifier composition at the beginning of the gradient is usually low, resulting in a very large k'_i . The error of the approximation is shown in the Appendix.

The retention of neutral solutes in isocratic elution can be well correlated by LSERs [8–32]:

$$\log k'_i = \log k'_0 + m \cdot \frac{V_2}{100} + s\pi_2 + a\Sigma\alpha_2^H + b\Sigma\beta_2^H \quad (4)$$

where the subscript 2 denotes a solute, $\log k'_0$ is the regression constant, V_2 is the solute McGowan characteristic molar volume, π_2 is the solute dipolarity/polarizability, $\Sigma\alpha_2^H$ and $\Sigma\beta_2^H$ are the solute

“overall” or “effective” hydrogen bond acidity and basicity, respectively, and m , s , a and b are the coefficients determined by multiple linear regression analysis. V_2 , π_2 , $\Sigma\alpha_2^H$ and $\Sigma\beta_2^H$ are called solutes’ descriptors, and they represent the physicochemical properties of solutes. It is noted that the coefficients in Eq. (4) depend on the type of column and the mobile phase compositions.

When Eq. (4) is substituted into Eq. (3), the following is obtained:

$$\begin{aligned} t_R &= C + \frac{t_0}{b} \cdot \log k'_i \\ &= C + \frac{t_0}{b} \\ &\quad \cdot \left(\log k'_0 + m \cdot \frac{V_2}{100} + s\pi_2 + a\Sigma\alpha_2^H + b\Sigma\beta_2^H \right) \\ &= \left(C + \frac{t_0}{b} \cdot \log k'_0 \right) + \frac{t_0}{b} \\ &\quad \cdot \left(m \cdot \frac{V_2}{100} + s\pi_2 + a\Sigma\alpha_2^H + b\Sigma\beta_2^H \right) \\ &= I + \frac{t_0}{b} \cdot \left(m \cdot \frac{V_2}{100} + s\pi_2 + a\Sigma\alpha_2^H + b\Sigma\beta_2^H \right) \\ &= I + m' \cdot \frac{V_2}{100} + s'\pi_2 + a'\Sigma\alpha_2^H + b'\Sigma\beta_2^H \end{aligned} \quad (5)$$

where I is the intercept, $m' = m(t_0/b)$, etc. Because t_0/b is regarded as a constant, Eq. (5) indicates that the retention time in linear gradient elution should be linear vs. the four solutes’ descriptors. It is noted that Eq. (5) is different from Eq. (3) because each coefficient (m , s , a , and b) is modified by t_0/b . Furthermore, the retention time is strongly determined by the initial mobile phase composition, and as a result, the final LSER coefficients will be determined by both the initial mobile phase composition and t_0/b . However, the focus of the study is on the evaluation of the correlation of the linear gradient elution time with the physicochemical properties of solutes. It should also be emphasized that Eq. (5) predicts a linear correlation of retention time (response) with the properties of solutes. However, in isocratic chromatography, the response is the logarithm of the capacity factor ($\log k'$).

We will use solutes with well-characterized physicochemical properties to evaluate the relationships shown in Eq. (5) in binary RPLC.

3. Experimental

3.1. Chromatographic system, column, and mobile phases

All experiments were performed on a Waters chromatograph (LC module 1) with a gradient pump, autosampler, and UV detector (Millipore, Waters Chromatography Division, Milford, MA, USA). Chromatographic data were acquired on a computer-based workstation interfaced to the chromatograph via TURBOCHROM software (PE Nelson, Cupertino, CA, USA). An Inertsil ODS(3) column (100×4.6 mm I.D., particle size 5 μm, pore size 100 Å, and surface area 450 m²/g, carbon load, 15%) (MetaChem Technologies, Torrance, CA, USA) was used for all the experiments. The mobile phase solvents were ACN, MeOH (EM Sciences, Gibbstown, NJ, USA), and Milli-Q water. The system dwell volume is about 4 ml.

3.2. Solutes and gradient conditions

The solutes used in the study are tabulated in Table 1 with their physicochemical properties. They are also called solvatochromic parameters of solutes obtained by gas chromatographic measurements developed by Abraham and co-workers [36–41].

The solutes are dissolved in ACN, and their concentrations are approximately 2 mg/ml. The chromatographic conditions are indicated in Table 2. The binary mobile phases are ACN–water and MeOH–water. The detection wavelength was 250 nm and the injection volume was always 10 μl. It is noted in Table 2 that the gradient steepness is widely changed from 0.051 to 0.371 (t_0/b from 4.7 to 18.8), and $1/(2.3b)$ is varied from 8.7 to 1.2. Because the initial mobile phase compositions are low, it is expected that $k'_i \gg 1/(2.3b)$, and the assumption of Eq. (5) are valid.

The retention times are shown in Table 3 for ACN–water mobile phases and in Table 4 for MeOH–water mobile phases. The system dead volume (and time) is determined by the ACN system peak. The retention times in Tables 3 and 4 are linearly regressed with the parameters of solutes based on Eq. (5). The coefficients and statistical results are shown in Table 5.

Table 1

Solute used in the study and their physicochemical properties^a

Solute ^b	$V/100^c$	π^{*d}	$\Sigma\alpha^{He}$	$\Sigma\beta^{Hf}$
Acetophenone (1)	1.014	1	0	0.48
Aniline (2)	0.816	0.96	0.26	0.41
Anisole (3)	0.916	0.75	0	0.29
Benzaldehyde (4)	0.873	1	0	0.39
Benzene (5)	0.716	0.52	0	0.14
Benzonitrile (6)	0.871	1.11	0	0.33
Benzyl acetate (7)	1.214	1.06	0	0.65
Benzyl cyanide (8)	1.012	1.15	0	0.45
Bromobenzene (9)	0.891	0.73	0	0.09
Butylbenzene (10)	1.28	0.51	0	0.15
<i>p</i> -Chloroanisole (11)	1.038	0.86	0	0.24
4-Chlorophenol (12)	0.898	1.08	0.67	0.2
<i>p</i> -Cresol (13)	0.916	0.87	0.57	0.31
Fluorobenzene (14)	0.734	0.57	0	0.1
Methyl benzoate (15)	1.073	0.85	0	0.46
2-Nitrotoluene (16)	1.032	1.11	0	0.27
4-Nitrotoluene (17)	1.032	1.11	0	0.28
Phenol (18)	0.775	0.89	0.6	0.3
3-Phenyl-1-propanol (19)	1.198	0.9	0.3	0.67
Propiophenone (20)	1.1548	0.95	0	0.51

^a This table summarizes the physicochemical properties of solutes used in the study. They are also called solvatochromic parameters [36–41].

^b The number in the parenthesis indicates the assigned solute number for convenience.

^c Solute's McGowan characteristic molar volume.

^d Solute's dipolarity/polarizability.

^e Solute's effective acidity.

^f Solute's effective basicity.

4. Results and discussion

4.1. Dependence of retention time on the physicochemical properties of solutes

Although it is expected from the theoretical consideration (Eq. (5)) that the retention time in linear gradient elution should be linearly proportional to the physicochemical properties, almost no experimental data have been reported to confirm this expectation. Part of the reason is due possibly to the unawareness of the significance of the relationships.

It can be seen from Table 5 that the linear relationship between retention time and the physicochemical properties of solutes is very acceptable for different gradient steepness of both mobile phases, as indicated by the correlation coefficients and relative standard errors. The correlation coefficients are in most cases greater than 0.97, and the relative stan-

Table 2
Linear gradient conditions used in the study^a

Binary mobile phase	ACN–MeOH composition change (%)	Flow-rate (ml/min)	Gradient time (min)	Gradient steepness (100 <i>b</i>) ^b	<i>t</i> ₀ / <i>b</i> ^c
ACN and water	15–75	2	15	13.6	6.3
	5–100	2	30	11.9	7.9
	15–75	2	30	7.0	12.5(a)
	15–45	2	15	6.9	12.5(b)
	5–70	2	30	7.8	11.5
	15–75	1	30	13.8	12.5(c)
MeOH and water	20–100	2	15	19.0	4.7(a)
	20–100	1	15	37.1	4.7(b)
	5–100	2	30	12.7	7.9
	20–100	1	30	18.6	9.4
	20–60	2	30	5.1	18.8(a)
	20–60	1	30	9.8	18.8(b)

^a This table shows all the experimental conditions. The range of *b* is from 0.05 to 0.37. $1/(2.3b)$ is from 8.7 to 1.2. The initial k'_i is assumed to be much greater than 8.7.

^b Gradient steepness calculated by Eq. (2). The column dead time is estimated by the ACN system peak. The parameter *S* is assumed to be 4.

^c Calculated t_0/b — the letter in the parenthesis is used to differentiate the same t_0/b .

dard errors on average are usually no more than 10%. This level of linearity is about the same as those observed for the correlation under the isocratic conditions [8–32]. It is further noted that the correlation coefficient is not observed to relate to the gradient steepness for both mobile phases. We have assumed in deriving Eq. (3) that the *S* value is a constant. Although this is not totally true, this assumption does not seem to reduce the quality of the fits.

At this point, the relative standard errors in Table 5 deserve more discussion because they are good indications of prediction errors in gradient retention by LSERs. The relative standard errors in retention time are no more than 10% in all cases. Thus, we can conclude that, if we use the LSER approach to correlate gradient retention time with solutes' descriptors, the prediction error in retention is on average less than 10%. This level of error is sometimes very acceptable, such as in the selection of an internal standard. We can also estimate the prediction error in Eq. (5) using Eqs. (3 and 4), and the data in Tables 2–4. It is commonly observed that the predicted error in $\log k'_i$ in Eq. (4) (isocratic) is within 0.04–0.06 log units. Based on Eq. (3), the prediction error in t_R should be $\delta(t_R) = (t_0/b)\delta(\log$

k'_i). The range in (t_0/b) in Table 2 is approximately 5–20, and the average retention times in Tables 3–4 are 9–23 min. Thus, the estimated relative prediction error in t_R is on average less than 5%. This estimation is reasonable and consistent with the results in Table 5 because we have neglected the change in *b* (see Appendix).

To further show the linear relationships, Fig. 1 (ACN–water) and Fig. 2 (MeOH–water) show the predicted vs. measured retention times at three different gradient steepness. The predicted retention times based on Eq. (5) are linearly correlated very well with the measured times. The slopes of the correlation are essentially unity [close to 45° line with a slope +1 through (0, 0)]. Thus, the estimated regression functions give accurate predictions of the values actually observed, and both figures provide a visual assessment of model effectiveness in making predictions. Although a change (increase) in retention time is observed when the gradient steepness decreases (t_0/b increases, see Tables 3 and 4), the correlation slopes remain unchanged. This conclusion can be drawn for both mobile phases.

The qualities of the fits in Figs. 1 and 2 can be further examined by their residual plots. If the model (Eq. (5)) is correct, the residual plots should not

Table 3
Retention times obtained for ACN–water binary mobile phases^a

Solute ^b	t_0/b^c					
	6.3 (15%)	7.9 (5%)	12.5[a] (15%)	12.5[b] (15%)	11.5 (5%)	12.5[c] (15%)
1	7.28	10.1	8.75	8.95	12.78	14.66
2	3.98	6.05	2.95	4.14	6.58	8.16
3	9.98	13.92	13.38	13.55	17.26	20.1
4	6.68	9.98	7.67	7.85	11.38	13.55
5	10.1	14.23	13.42	13.59	17.14	20.48
6	7.73	11.3	9.38	9.51	13.12	15.73
7	9.72	13.95	13.26	13.36	17.29	19.62
8	8.19	12	10.35	10.47	14.29	16.64
9	12.84	17.81	19.04	NA	22.71	25.93
10	NA	17.34	18.38	NA	NA	NA
11	12.43	23.2	27.3	NA	22.13	25.06
12	8.35	12.27	10.94	10.98	15	16.9
13	6.98	10.54	8.41	8.47	12.41	14.13
14	10.48	14.71	14.34	14.36	18.1	21.18
15	9.61	13.79	12.91	12.97	16.95	19.41
16	10.91	15.41	15.48	15.49	19.37	22.04
17	11.09	15.63	15.84	15.82	19.72	22.38
18	4.89	7.74	5.13	5.18	8.43	9.94
19	7.31	11.13	9.19	9.27	13.43	14.66
20	9.88	14.14	13.39	13.49	17.46	19.91
Average ^d	8.86	13.26	12.48	11.03	15.56	17.92

^a This table summarizes the retention times (min) obtained by linear gradient elution of binary ACN–water mobile phases. NA denotes that the retention times are not available (obtained) because they exceed the gradient times.

^b Solute number as indicated in Table 1.

^c Retention times are arranged by increasing t_0/b , as indicated in Table 2. The percentage under each t_0/b value indicates the initial ACN concentration in the mobile phases.

^d Average retention times. It is used for the calculation of average relative standard errors in Table 5.

exhibit distinct patterns. The residuals should be randomly distributed about zero according to a normal distribution. If standardized residual plots are used, most of the standardized residuals should lie between -3 and $+3$. Figs. 3 and 4 show the standardized residuals of the fits in Figs. 1 and 2. They are obtained by dividing the residual over the standard error of the fit. It can be observed from the two figures that the residual distributions are all nearly symmetrical around the center lines (zero), and the standardized residuals are no more than 3. Accordingly, we can conclude that the residual distributions of all the data are essentially the same. Furthermore, there is not any given residual that is unusually large in the data.

Overall, the linear dependence model of Eq. (5) accurately and correctly correlates the retention time

in linear gradient elution of RPLC with the physico-chemical properties of solutes. The statistics are consistent with those obtained by isocratic elution.

4.2. Dependence of LSER coefficients on gradient steepness (t_0/b)

It has been indicated in Section 2 that the coefficients in Eq. (5) are determined by both the initial mobile phase compositions and gradient steepness (t_0/b). It is expected that a decrease in the initial organic composition of the mobile phase and an increase in t_0/b will enhance the magnitudes of the LSER coefficients. This is clearly confirmed by the data in Table 5. It can be generally observed from Table 5 that as t_0/b increases, the magnitudes of the

Table 4
Retention times obtained for MeOH–water binary mobile phases^a

Solute ^b	t_0/b^c					
	4.7(a) (20%)	4.7(b) (20%)	7.9 (5%)	9.4 (20%)	18.8(a) (20%)	18.8(b) (20%)
1	7.72	11.71	14.36	15.07	12.5	20.36
2	3.69	6.9	7.1	7.17	3.79	7.64
3	10.02	14.25	17.89	19.66	18.93	27.87
4	6.77	10.75	12.54	13.2	9.43	16.24
5	10	14.36	17.48	19.67	17.86	27.22
6	7.3	11.32	13.43	14.22	10.79	17.97
7	9.86	13.89	17.96	19.39	20.07	28.07
8	7.39	11.33	13.8	14.44	11.46	18.61
9	12.5	NA	22.33	24.68	29.86	NA
10	NA	NA	27.45	NA	NA	NA
11	12.31	NA	22.06	24.29	29.33	NA
12	9.03	13.09	16.33	17.71	16.24	24.49
13	7.65	11.71	13.96	15	11.71	19.23
14	10.33	14.55	18.26	20.37	19.84	28.99
15	10.01	14.08	18.17	19.71	20.33	28.49
16	10.45	14.53	18.88	20.6	21.91	30.17
17	10.61	14.69	19.16	20.9	22.38	30.79
18	5.14	9.01	9.34	10.08	5.85	11.1
19	9.01	13.01	16.54	17.69	16.87	24.77
20	9.82	13.93	17.89	19.5	20.17	28.21
Average ^d	8.93	12.54	16.75	17.55	16.81	22.95

^a This table summarizes the retention times (min) obtained by linear gradient elution of binary MeOH–water mobile phases. All symbols as in Table 3.

^b Solute number as indicated in Table 1.

^c Retention times are arranged by increasing t_0/b , as indicated in Table 2. The percentage under each t_0/b value indicates the initial MeOH concentration in the mobile phases.

^d Average retention times. It is used for the calculation of average relative standard errors in Table 5.

LSER coefficients show an upward change for both mobile phases. A decrease in the initial ACN composition from 15% (rows 3 and 4) to 5% (row 5) in the upper portion of Table 5 does increase the LSER coefficients although t_0/b decreases from 12.5(a and b) to 11.5.

However, even though t_0/b and the initial mobile phase composition remain the same (for example, $t_0/b = 12.5$ and % ACN=15 in rows 3, 4 and 6), an obvious change is observed on the magnitudes of the LSER coefficients. For example, the m' coefficient increases from 29.15 to 36.36. This increase seems to relate to the increase in the gradient steepness (b). It is noted that Eq. (3) is derived by assuming that k'_i is much greater than $1/(2.3b)$. However, if b is varied, the contribution of $1/(2.3b)$ to k'_i will be somewhat different. Mathematical examination of

Eq. (3) indicates that, if gradient steepness (b) is becoming smaller, the validity of the approximation in Eq. (3) will be increasingly weakened, resulting in an increasingly significant contribution to k'_i (see Appendix). This increasing contribution is compensated by the larger LSER coefficients, as observed by the results in Table 5. However, the larger contribution of $1/(2.3b)$ to k'_i has not altered the quality of the fits.

Because it is not intended to use the coefficients to characterize the properties of the stationary phases, the effect of b on the magnitudes of the coefficients is not important to us. The focus of the study is on the evaluation of the dependence of retention time on the physicochemical properties of solutes in linear gradient elution. If LSERs are used to characterize the properties of the stationary phases via linear

Table 5
Multivariable linear regression coefficients and statistical results on retention data^a

t_0/b^b	Initial Org. (%)	LSER coefficients ^c					R	$\left(\frac{\text{S.E.}}{t_{\text{Av.}}}\right) \cdot 100^d$
		Int. (I)	m'	s'	a'	b'		
ACN–water								
6.3	15	0.41 (1.03)	18.26 (1.32)	−3.18 (0.78)	−3.42 (1.54)	−16.34 (1.11)	0.982	5.6
7.9	5	2.81 (1.77)	20.18 (1.66)	−2.59 (1.31)	−4.37 (0.99)	−18.85 (1.79)	0.976	7.1
12.5(a)	15	−2.59 (1.97)	29.15 (1.85)	−3.83 (1.46)	−5.90 (1.10)	−27.20 (1.99)	0.985	8.3
12.5(b)	15	−3.37 (1.82)	31.94 (2.61)	−5.29 (1.37)	−5.50 (0.92)	−28.37 (2.33)	0.977	7.7
11.5	5	−1.70 (1.77)	35.09 (2.26)	−5.72 (1.35)	−5.29 (0.92)	−29.99 (1.91)	0.984	5.5
12.5(c)	15	1.12 (2.10)	36.36 (2.69)	−6.23 (1.60)	−6.93 (1.10)	−32.87 (2.28)	0.982	5.7
MeOH–water								
4.7(a)	20	−0.04 (1.58)	19.83 (1.48)	−5.03 (0.88)	−1.74 (0.60)	−14.92 (1.26)	0.975	6.3
4.7(b)	20	3.27 (1.54)	22.23 (2.21)	−5.81 (1.16)	−1.90 (0.78)	−17.11 (1.98)	0.958	5.7
7.9	5	3.63 (3.01)	25.03 (2.83)	−4.54 (2.23)	−4.14 (1.68)	−19.79 (3.05)	0.950	9.6
9.4	20	−0.34 (2.23)	39.65 (2.86)	−10.13 (1.70)	−3.42 (1.16)	−29.76 (2.42)	0.977	6.2
18.8(a)	20	−13.90 (2.98)	63.91 (3.82)	−14.50 (2.27)	−5.18 (1.55)	−47.53 (3.22)	0.983	8.6
18.8(b)	20	−9.15 (3.74)	72.26 (5.36)	−17.58 (2.81)	−6.20 (1.88)	−53.52 (4.79)	0.976	7.6

^a This table summarizes the LSER coefficients of Eq. (5) and related statistics obtained from the data in Tables 3 and 4.

^b Gradient steepness value in terms of t_0/b from Table 2.

^c LSER coefficients (Eq. (5)). The values in the parenthesis indicate the standard deviations of the coefficients.

^d Average relative standard error in percent. It is computed by dividing the standard error of the fit over the average retention time shown in Tables 3 and 4.

gradient elution, the gradient steepness (b) should remain unchanged.

Finally, the signs and magnitudes of the coefficients are all consistent with those obtained by isocratic elution after t_0/b is accounted for (see also Table 6) [8–22]. Moreover, as obtained for isocratic elution, the dominant factors to retention in linear gradient elution of RPLC are the solutes' size and hydrogen bond (HB) acceptor basicity.

4.3. Characterization of the stationary phases by linear gradient elution chromatography

When LSERs are used to characterize the prop-

erties of the stationary phases, the retention in terms of capacity factor is usually obtained by isocratic elution of binary mobile phases [8–32]. Obviously, from the conclusion of the study, linear gradient elution may offer an alternative approach to obtaining the retention for the characterization. In fact, this approach may be better because the retention times of solutes are not excessively long, and the experiments can be done in a short time frame. Because the same experimental conditions are used, the effect of b on the coefficients can be avoided. The linear gradient, initial and final mobile phase compositions should be selected appropriately to allow the retention within the range of the gradient time.

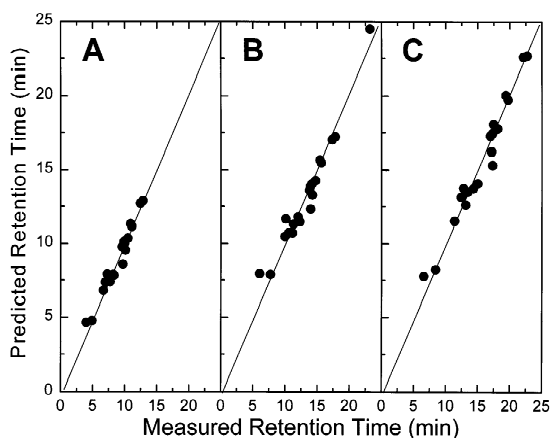


Fig. 1. Illustration of predicted vs. measured retention times for ACN–water mobile phases. The symbols in each plot represent the experimental data, and the solid line is the diagonal with a slope of unity through point (0,0). (A) $t_0/b=6.3$; (B) $t_0/b=7.9$; (C) $t_0/b=11.5$.

4.4. Comparison of LSER coefficients obtained by isocratic and gradient elution

Although it is not intended to use the LSER coefficients from this study for the characterization of the stationary phases (columns), it is interesting to compare those obtained from two different elution modes. Table 6 shows the LSER coefficients ob-

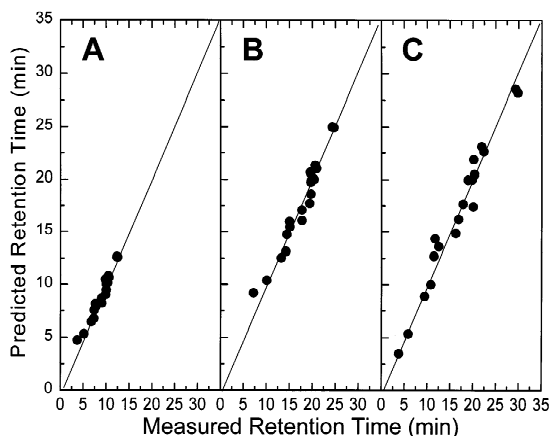


Fig. 2. Illustration of predicted vs. measured retention times for MeOH–water mobile phases. The symbols in each plot represent the experimental data, and the solid line is the diagonal with a slope of unity through point (0,0). (A) $t_0/b=4.7(a)$; (B) $t_0/b=9.4$; (C) $t_0/b=18.8(a)$.

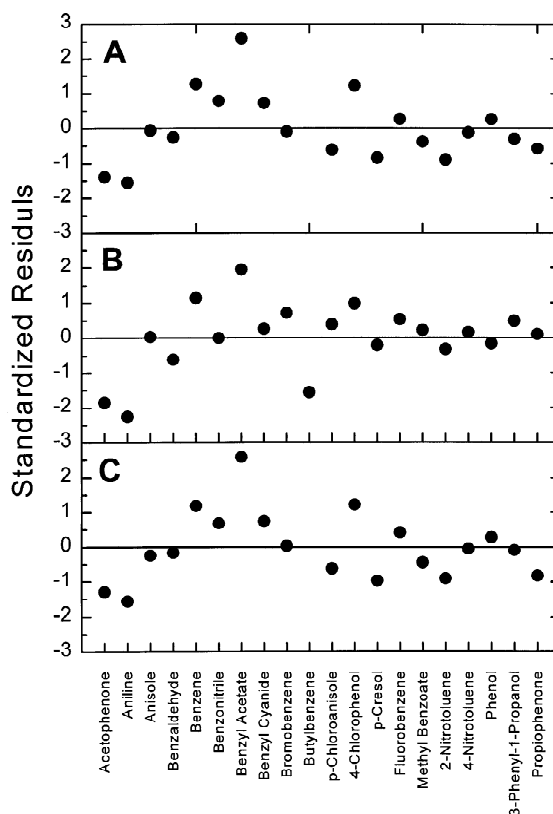


Fig. 3. Plot of standardized residual vs. solute for ACN–water mobile phases for the data shown in Fig. 1. The standardized residuals are obtained by dividing the residuals by the standard error of the fit.

tained for ODS phases. The data for isocratic elution are calculated based on Ref. [42]. The coefficients for gradient elution are obtained from the current data (Table 5) corrected by t_0/b . It is generally observed from Table 6 that, for the four mobile phase compositions, the two dominant LSER coefficients (m and b) obtained by the linear gradient elution are smaller in amplitude, while the signs of the coefficients from the two elution modes are consistent. The consistency of the signs is more important because they indicate that the chromatographic retention in both isocratic and gradient elution are governed by the same molecular interaction processes. Conversely, LSERs can be applied to both isocratic and gradient elution to evaluate the dominant molecular interactions that contribute to chromatographic retention.

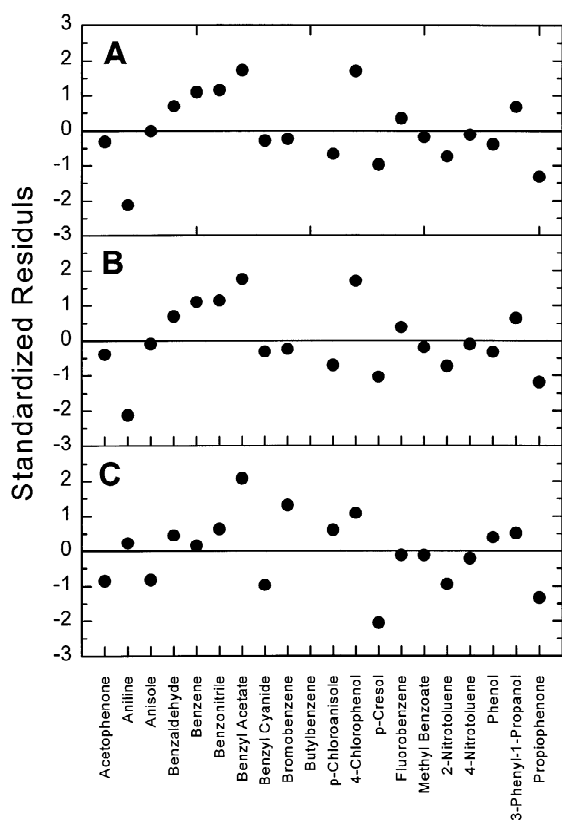


Fig. 4. Plot of standardized residual vs. solute for MeOH–water mobile phases for the data shown in Fig. 2. Calculations as in Fig. 3.

Table 6 shows that some differences exist in the magnitudes of the LSER coefficients. Although it appears very difficult to evaluate the causes, several reasons may contribute to the differences. First, the gradient steepness in Eq. (2) is computed based on an S parameter of 4, which may not be the correct value to use. In fact, each compound has a different S value [1]. As indicated in the Appendix, there is a model error due to inconsistent S values, and the error is on average about 15%. Although this model error has not deteriorated the regression statistics (relative to isocratic elution), the magnitude of LSER coefficients may be affected due to the adjustment of coefficients for best fits. Second, the calculation of the LSER coefficient by Ref. [42] is another approximation. The data used to generate the results in the reference are not complete in the mobile phase compositions that contain very low organic com-

Table 6
Comparison of LSER parameters obtained by isocratic and linear gradient elutions^a

Elution mode	Initial mobile phase	LSER coefficients ^b			
		m	s	a	b
Isocratic ^c	5% ACN	4.08	-0.53	-0.37	-3.03
	15% ACN	3.36	-0.41	-0.44	-2.69
	5% MeOH	4.23	-0.59	-0.33	-3.08
	20% MeOH	3.51	-0.54	-0.32	-2.70
Linear gradient ^d	5% ACN ^e	3.05	-0.50	-0.46	-2.61
	15% ACN ^f	2.33	-0.31	-0.47	-2.18
	5% MeOH ^g	3.17	-0.57	-0.52	-2.51
	20% MeOH ^h	3.40	-0.77	-0.28	-2.53

^a This table compares the LSER coefficients obtained by isocratic and gradient elution.

^b LSER coefficients in Eq. (4).

^c Calculated based on Ref. [42].

^d Obtained by the data in Table 5 corrected by t_0/b .

^e Obtained from the data in row 5 in Table 5 (upper portion).

^f Obtained from the data in row 3 in Table 5 (upper portion).

^g Obtained from the data in row 3 in Table 5 (lower portion).

^h Obtained from the data in row 5 in Table 5 (lower portion).

ponents. Therefore, the LSER coefficients in the corresponding mobile phase compositions may not be accurate for the isocratic elution. It is certain that other factors may also contribute the differences. However, the focus of the study is on the evaluation of the relationship between gradient retention time and the physicochemical properties of solutes.

5. Conclusions

This paper confirms the expectation, based on LSERs, that the retention time in linear gradient elution by RPLC is linearly correlated with the physicochemical properties of solutes. This linear relationship is confirmed by both ACN–water and MeOH–water mobile phases with different gradient steepness. The statistics for the linear relationships are essentially the same as those obtained by isocratic elution. The magnitudes and signs of the coefficients are also consistent with those obtained for isocratic elution once the gradient steepness (t_0/b) is corrected. It is also expected that the conclusions drawn from this study should also apply to ternary gradient elution. Finally, the linear gradient

elution can be a faster means for the characterization of the stationary phases.

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Appendix A

Dependence of retention on solutes' parameters without approximation

If k'_i is not significantly greater than $1/(2.3b)$, the correlation relationship may be further modified. This can be done by rewriting Eq. (1) as follows:

$$\log(2.3bk'_i + 1) = \frac{b}{t_0} \cdot (t_R - t_0 + t_d) \quad (\text{A.1})$$

and rearrangement of Eq. (A.1) yields:

$$(2.3bk'_i + 1) = \exp\left[\frac{2.3b}{t_0} \cdot (t_R - t_0 + t_d)\right] \quad (\text{A.2})$$

Now k_0 can be solved from Eq. (A.2) to obtain:

$$k'_i = \frac{\exp\left[\frac{2.3b}{t_0} \cdot (t_R - t_0 + t_d)\right] - 1}{2.3b} \quad (\text{A.3})$$

and finally:

$$\log k'_i = \log\left\{\frac{\exp\left[\frac{2.3b}{t_0} \cdot (t_R - t_0 + t_d)\right] - 1}{2.3b}\right\} \quad (\text{A.4})$$

Substitution of Eq. (3) into Eq. (A.4) yields:

$$\begin{aligned} \log k'_i &= \log\left\{\frac{\exp\left[\frac{2.3b}{t_0} \cdot (t_R - t_0 + t_d)\right] - 1}{2.3b}\right\} \\ &= \log k'_0 + mV_2 \cdot 100 + s\pi_2 + a\Sigma\alpha_2^H + b\Sigma\beta_2^H \end{aligned} \quad (\text{A.5})$$

Eq. (A.5) correlates the retention time and other

parameters (b , t_0 , and t_d) with the physicochemical properties of solutes.

Errors of the approximation ($k'_i \gg 1/2.3b$)

The error due to the approximation can be estimated below:

$$\begin{aligned} t_R &= \frac{t_0}{b} \cdot \log(2.3bk'_i + 1) + t_0 + t_d \\ &= \frac{t_0}{b} \cdot \log\left[2.3b \cdot \left(k'_i + \frac{1}{2.3b}\right)\right] + t_0 + t_d \\ &= \left[\frac{t_0}{b} \cdot \log(2.3b) + t_0 + t_d\right] + \frac{t_0}{b} \\ &\quad \cdot \log\left(k'_i + \frac{1}{2.3b}\right) \\ &= C + \frac{t_0}{b} \cdot \log\left[k'_i \cdot \left(\frac{1}{2.3bk'_i}\right)\right] \\ &= C + \frac{t_0}{b} \cdot \log k'_i + \frac{t_0}{b} \cdot \log\left(1 + \frac{1}{2.3bk'_i}\right) \\ &= C + \frac{t_0}{b} \cdot \log k'_i + \frac{t_0}{b} \cdot \frac{\ln\left(1 + \frac{1}{2.3bk'_i}\right)}{2.3} \\ &\approx C + \frac{t_0}{b} \cdot \log k'_i + \frac{t_0}{b} \\ &\quad \cdot \left(\frac{1}{2.3^2bk'_i}\right) \quad (\text{series expansion}) \\ &\approx C + \frac{t_0}{b} \cdot \log k'_i \end{aligned} \quad (\text{A.6})$$

The series expansion is obtained by $\ln(1+x) \approx x$. It can be seen from Eq. (A.6) that the error of the approximation is approximately $(t_0/b) \cdot (1/2.3^2bk'_i)$ and that a decrease in the gradient steepness will increase the error of the approximation.

Model error in retention time due to different S values

In Eqs. (1–3), we have assumed that S is a constant for all solutes. However, the S value (and hence the gradient steepness, b) can be somewhat different for different solutes [1]. If the average S value is used in Eqs. (1–3) to model retention time, there will be a model error for solutes that have different S values. This error (Δt_R) can be estimated by differentiating Eq. (1) with respect to b because b is proportional to S . The result is shown below:

$$\Delta t_R = \frac{t_0}{b} \cdot \left[\frac{k'_i}{2.3bk'_i + 1} - \frac{\log(2.3bk'_i + 1)}{b} \right] \Delta b \quad (\text{A.7})$$

where Δb is the change in b due to a change in S . The S value is approximately 4.2 with a standard deviation of 0.8 for small molecules [1], computer simulations indicate that the model error in retention time is on average about 15%.

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