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Comparison of methods for extracting linear solvent strength gradient parameters from gradient chromatographic data

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Abstract

The linear solvent strength (LSS) theory of gradient elution is useful in the optimization of separations in high-performance liquid chromatography. While the fundamental parameters of this theory are defined in terms of isocratic behavior, gradient operation has been used previously to estimate those parameters to allow rapid optimization of the separation. In this study, various methods of extracting the LSS parameters from gradient retention data were examined. Sets of synthetic retention data were calculated directly from the equations of the LSS theory. When realistic experimental uncertainties were incorporated into these data sets, the LSS parameters used to generate the synthetic data were not recovered accurately unless special precautions were taken. For large molecules, an approximate LSS expression could be used to determine the solvent strength parameter with an error of less than 13%, which is comparable to or better than those for the other methods evaluated.

Keywords: Linear solvent strength theory; Solvent strength parameters; Gradient elution; Retention models

1. Introduction

The semi-empirical linear solvent strength (LSS) theory of gradient elution is useful in the design and optimization of separations. LSS theory is fully developed elsewhere [1–4] and its success in predicting gradient and isocratic elution behavior has been amply demonstrated (e.g., [2,5,6]). According to LSS theory, the gradient retention time of a solute, t_g , is

$$t_g = \frac{t_G}{S \Delta\Phi} \log \left(\frac{2.303S \Delta\Phi t_M k'_0}{t_G} + 1 \right) + t_M + t_d \quad (1)$$

where t_G is the gradient duration time, $\Delta\Phi$ is the fractional change in solvent composition, t_M is the column void time and t_d is the delay time between the chromatographic injection and the time when the gradient reaches the head of the column, due to the finite volume between the injector and the gradient mixer. S , the solvent strength parameter, is the slope of the plot of the logarithm of the capacity factor versus the volume fraction of organic modifier in the mobile phase and k'_0 is the capacity factor in the initial mobile phase composition of the gradient. The parameters S and k'_0 are characteristic of a particular solute–solvent–stationary phase combination; the other parameters on the right-hand side of Eq. 1 are directly controlled by the experimenter.

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Refinements to Eq. 1 have been made to incorporate exclusion effects [7] and non-ideal instrument behavior [8]. However, these refinements have no effect on the discussion that follows.

Since the LSS parameters S and k'_0 are defined in terms of isocratic behavior, the preferred method of determination is to perform the isocratic measurements. However, for large molecules, the extreme sensitivity of retention to mobile phase composition (e.g., [7,9]) and the length of the retention time render the application of the isocratic method difficult, and an iterative method using gradient elution has been applied [7]. In this method, two gradient runs of different t_G s are performed, and the resultant t_g s and corresponding t_G s are substituted into a modified form of Eq. 1, generating two non-linear equations in the two unknowns, S and k'_0 . Iterative solution yields values for the LSS parameters. A similar procedure in this study will be referred to as the pairwise gradient determination method. This type of method, although practical for optimization [6–8], is statistically unsatisfactory for accurate determination of the LSS parameters since the procedure propagates all of the experimental uncertainties directly into the calculated parameters. Thus, experimental S values so determined will be unsuitable for comparison with S values calculated from an a priori theory of gradient elution.

The statistically preferable alternative is to perform a regression fit of Eq. 1 to a set of experimental data, adjusting the values of S and k'_0 to give the best fit to the entire data set. Lundell [10] has shown that such an approach can be used to optimize the gradient separation of peptides fairly successfully, where success is judged by the agreement between the calculated and experimentally observed t_g s.

These studies were focused on the use of LSS theory to optimize the gradient separation of a mixture and amply demonstrated the utility of the LSS theory in that application. Thus, any successful a priori theory of gradient elution must be able to predict the parameters of the LSS theory. Consequently accurate determination of the LSS parameters becomes an impor-

tant issue. Here we concern ourselves with the determination of these parameters. In other words, the following discussion addresses the ability of a researcher to extract the true S and k'_0 from experimental data.

Synthetic gradient retention times ($t_{g,s}$) were calculated using Eq. 1 and realistic values of S , k'_0 and the necessary experimental parameters. Appropriate amounts of noise (errors) were added to the gradient retention times in some of the synthetic data sets, which allowed us to evaluate the parameter extraction methods in a realistic manner. The abilities of the pairwise gradient determination and of two non-linear regression algorithms to extract the true S and k'_0 values from the synthetic gradient data were evaluated.

Except for the unrealistic case of noise-free data, the pairwise gradient method was unreliable, as the extracted parameters varied significantly with the choice of specific t_G pairs. The tested non-linear regression techniques were more reliable for data incorporating realistic noise. In the case of large-molecule data, typical fitting criteria (e.g., a relative change in reduced χ^2 of less than 0.01) did not reliably recover the original S and k'_0 values that were used to generate the synthetic gradient data sets according to Eq. 1. A more stringent fitting criterion (a change in relative reduced χ^2 of less than 0.001) yielded acceptable results, and a grid-search algorithm gave better results than the Marquardt algorithm. A linear regression to a modified form of Eq. 1 gave comparable results to non-linear regression for large molecules.

2. Procedure

2.1. Synthetic data sets

Eq. 1 was used to generate synthetic gradient data used in all fitting procedures. Three pairs of values for S and k'_0 were used, corresponding to small, moderate and large molecules: $S = 3.4$, $k'_0 = 4.0$; $S = 14.3$, $k'_0 = 646$; and $S = 22.5$, $k'_0 = 75\,858$. These values were taken directly from the literature or calculated from published data

{Refs. [2], [7] and [7], respectively, for the small (benzene), moderate (M_r 2000 polystyrene) and large (M_r 9000 polystyrene) molecule parameters}. The other “experimental” parameters used were $\Delta\Phi = 0.30$, $t_d = 0$ min (i.e., no delay time) and $t_M = 2.20$ min.

The omission of size-exclusion and non-ideal instrumental effects makes this simulation a “best-case” situation. Analysis of real data incorporating these effects will be more seriously in error than is shown here.

For each particular S and k'_0 pair, t_g values were calculated for ten t_G s (15–150 min at 15-min intervals) using Eq. 1. A single set of ten t_g s was calculated and used as the noise-free data group.

Three hundred such sets of ten t_g s each were generated for each S and k'_0 pair at each of two noise levels (0.1% and 1%). These will be referred to as the 0.1% and 1% noise groups, respectively. Normally distributed random (NDR) noise was added to the individual gradient retention times by means of the algorithm of Rubinstein [11]. The noise generator was randomly seeded so that the noise in each set of gradient data was independent of the preceding set.

In summary, the three noise-free groups correspond to the experimentally impossible noise-free data and each group consists of one set of ten t_G , t_g data pairs. The 0.1 and 1% noise values were chosen following the flow-rate precisions suggested by Jandera and Churacek [12], who indicated that 0.5% R.S.D. is a realistic flow variation. Each of the three 0.1% noise groups contains 300 sets of ten t_G , t_g data pairs and collectively represent an exceptionally reproducible experimental system. Each of the three 1% noise groups also contains 300 sets of data pairs and collectively represent a more poorly functioning gradient system.

2.2. Approximate LSS retention equation

Although Eq. 1 is the fundamental retention relationship in LSS theory, for cases where S and k'_0 are large, t_g is, to a good approximation, given by

$$t_g \approx \frac{t_G}{S \Delta\Phi} \log\left(\frac{2.303S \Delta\Phi t_M k'_0}{t_G}\right) + t_M \quad (2)$$

(This approximation has been used frequently [1,13].) Eq. 2 can be rearranged to give

$$\gamma = \frac{1}{S} \ln \Gamma + \frac{1}{S} \ln(Sk'_0) + \Gamma \quad (3)$$

where $\gamma = 2.303\Delta\Phi t_g/t_G$ and $\Gamma = 2.303\Delta\Phi t_M/t_G$. Further manipulation yields

$$\gamma - \Gamma = \frac{1}{S} \ln \Gamma + \frac{1}{S} \ln(Sk'_0) \quad (4)$$

which yields $1/S$ as the slope of the linear fit of $\gamma - \Gamma$ versus $\ln \Gamma$.

2.3. Parameter extraction

Previously, LSS parameters have been extracted from genuine experimental gradient data by an iterative numerical technique [2,7,13]. In our study, iterative calculation of S and k'_0 for the pairwise gradient determination method was performed by the Newton–Raphson method. An initial estimate of S was required. When, for a particular data pair, the method failed to converge within 500 iterations, that pair of data were not included in error calculations.

Non-linear regression fitting to Eqs. 1 and 3 was performed using the grid-search (GRIDLS) and Marquardt (CURFIT) algorithms using programs given by Bevington [14]. In both cases, initial estimates of S and k'_0 were also required. The search was stopped when the goodness-of-fit criterion was met. The goodness-of-fit estimator was the relative change in reduced χ^2 , i.e.,

$$\frac{\chi^2_{\nu}(\text{previous iteration}) - \chi^2_{\nu}(\text{new iteration})}{\chi^2_{\nu}(\text{previous iteration})}$$

where

$$\chi^2_{\nu} = \frac{\sum_{i=1}^{10} [t_{g_i} - t_{g_i}(\text{fit})]^2}{8}$$

or

$$\chi^2_{\nu} = \frac{\sum_{i=1}^{10} [\gamma_i - \gamma_i(\text{fit})]^2}{8}$$

where the 8 in the denominator is the number of degrees of freedom.

Linear regression fitting to Eq. 4 was performed using standard programs given by Bevington [14].

2.4. Residual surfaces

Residual surfaces (error contours) were calculated by varying both S and k'_0 from about 30% of the true value to about 200% of the true value. The residual, R , is the reduced sum of the square of the differences between these calculated $t_{g,s}$ and the $t_{g,s}$ for the noise-free data set, i.e., after Eq. 1, with $t_d = 0$,

$$R = \frac{\sum_{i=1}^{10} [t_{g_i}(t_{G_i}, S_{\text{true}}, k'_{0,\text{true}}) - t_{g_i}(t_{G_i}, S_{\text{trial}}, k'_{0,\text{trial}})]^2}{8}$$

where X_{true} refers to the true LSS parameter value used to generate the data, X_{trial} refers to the abscissa or ordinate values in Fig. 1a or b and the summation is made for each of the ten t_{G_i} values used to generate the data.

All programs were written in Microsoft FORTRAN 5.0 (Microsoft, Redmond, WA, USA) and run on a Ulta 486 computer (Ulta Computers, Weirton, WV, USA). Plotting was performed using the Axum graphics package (TriMetrix, Seattle, WA, USA).

3. Results and discussion

In the following, results from each single-set, noise-free group are reported as percentage error. Results from each 300-set 0.1% or 1% noise group are reported as the average percentage error plus or minus the standard deviation of the percentage error. This corresponds to a ca. 70% confidence interval.

3.1. Residual surfaces

The shape of the residual surface is critical in determining the success of any regression tech-

nique. A well defined minimum allows accurate extraction.

For the LSS model, the shape of the residual surface depended strongly on the magnitude of the LSS parameters (Fig. 1). The residual surface for the small-molecule data contained a single minimum at the bottom of a "crater" (Fig. 1a). For the moderate-molecule data, the residual surface was a long, elliptically shaped valley with steep sides and a shallow "floor" (Fig. 1b). For large-molecule data, this elliptically shaped valley became even more elongated (not shown).

Residual surfaces were generated for S ranging from 1.5 to 20 and for k'_0 ranging from 5 to 1000. The general shapes of these surfaces were similar to those shown and are omitted here for brevity. In all cases, small S s (i.e., <5) gave relatively well defined minima, while larger S s resulted in elliptically shaped valleys. The ellipticity and shallowness of the valley increased with increasing S and with increasing k'_0 (data not shown).

The implication of these differences is that extraction of the LSS parameters from small-molecule data will be accurate and the extracted values will be independent of the initial estimates used in the extraction procedure. The shallow and long, elliptically shaped residual surfaces of the moderate- and large-molecule data will cause the extracted values to be strongly dependent on the initial estimates. These difficulties in fitting parameters on long, elliptically shaped residual surfaces are well documented [15].

3.2. Pairwise gradient determination

For each of the three noise-free groups, every possible combination (45 pairs) of the gradient retention data was used in the pairwise gradient determination of the LSS parameters. The program converged to the correct value of S regardless of the initial estimate of the S value, provided that these initial estimates were larger than the true S values. If the initial estimates were smaller than the true S value, the program frequently failed to converge. The program also converged to the correct value of k'_0 (data not shown).

This procedure was repeated for each set of

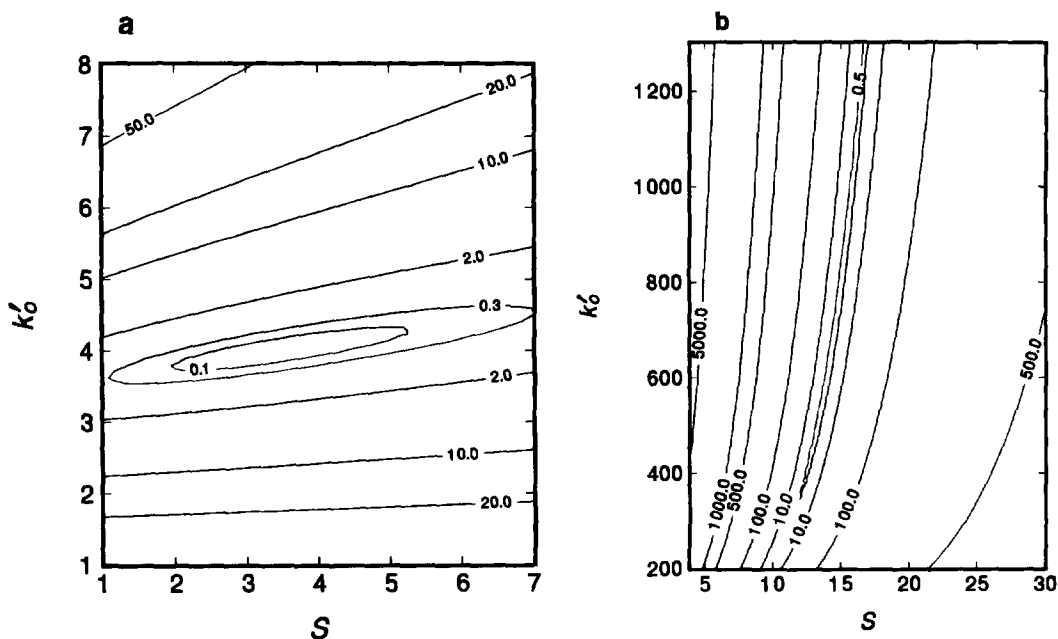


Fig. 1. Residual surfaces for noise-free gradient retention times as functions of S and k'_0 . Labels on the contour lines represent the R value. (a) True $S = 3.4$, true $k'_0 = 4.0$ (small molecule); (b) true $S = 14.3$, true $k'_0 = 646$ (moderate-size molecule).

each 0.1% noise group and again for each set of each 1% noise group. From each data set in each group, 45 pairs were constructed. Thus, 13 500 pairwise combinations were tested at each noise level. A detailed presentation of these results is not feasible. The best- and worst-case errors at each noise level are presented in Table 1. Although the average best-case errors shown (particularly for S) are fairly small, it should be remembered that these averages represent 300 distinct trials, or 600 separate chromatographic runs. The error ranges shown indicate that, in the best case, a single pair of chromatographic trials has only a 70% chance of giving extracted parameter values within about 6% of the true value when the noise level is 0.1%, or within about 16% of the true value when the noise level is 1%. Clearly, if the gradient times are not optimally chosen, the situation is much worse.

In general, the best cases occurred when the gradient times were different; the worst cases occurred when they were similar. A similar tendency in the error analysis of S values using experimental gradient retention data was previ-

ously noted by Quarry et al. [13]. Also, regardless of whether the initial estimate of S was larger or smaller than the true S value, not all 300 trials for a given pairwise combination of t_G s converged. For initial estimates of S which were smaller than the true S value, the program rarely converged. For all values of S and k'_0 , the failure to converge was more frequent as the noise in the data increased. The worst case results improved significantly as the initial estimate of S was closer to the true value; the best case results improved only slightly under these circumstances. The results were generally poorer for the larger molecules, as found by Larmann et al. [7] and Quarry et al. [13].

3.3. Regression analysis of noise-free data

Since the shape of the residual surfaces indicated that non-linear regression for this model would be difficult, a determination of an appropriate goodness-of-fit criterion was made by evaluating the errors in the extracted parameters

Table 1
Pairwise gradient determination of linear solvent strength parameters

Noise			Best case		Worst case	
Parameter	Level (%)	True value	Error (% $\pm \sigma$)	t_{G_1}, t_{G_2} (min)	Error (% $\pm \sigma$)	t_{G_1}, t_{G_2} (min)
S	0.1	3.4	0.05 ± 7.8	15, 75	190 ± 250	135, 150
		14.3	0.04 ± 4.1	45, 135	5.8 ± 36	135, 150
		22.5	-0.06 ± 5.5	15, 120	17 ± 76	135, 150
	1	3.4	-0.10 ± 20	15, 135	690 ± 780	135, 150
		14.3	0.34 ± 11	15, 120	25 ± 82	15, 30
		22.5	1.93 ± 18	15, 120	13 ± 81	30, 45
k'_0	0.1	4.0	0.06 ± 2.1	15, 75	17 ± 25	135, 150
		646	1.7 ± 17	45, 135	$31\,000 \pm 520\,000$	135, 150
		75858	14 ± 60	15, 120	$>10^{14} \pm 10^{15}$	135, 150
	1	4.0	0.22 ± 5.1	15, 135	140 ± 308	135, 150
		646	13 ± 53	15, 120	$>10^{14} \pm 10^{15}$	15, 30
		75858	550 ± 1900	15, 120	$>10^{14} \pm 10^{15}$	30, 45

A Newton-Raphson method was used, with an initial estimate of S of 100. Convergence was considered complete when the absolute difference in S values of successive iterations was less than 0.001.

using the noise-free groups. GRIDLS and CURFIT were tested using Eq. 1, setting the initial estimates for both S and k'_0 at 1.00 for all three S and k'_0 pairs.

The results are shown in Table 2 and indicate that the appropriate goodness-of-fit criterion is 0.001 relative change in the estimator. CURFIT did not successfully extract the parameter values for the large molecule at this criterion level. Further reduction of the goodness-of-fit criterion did not work with the large-molecule data using CURFIT and these initial estimates. This is because the initial estimates were too far from the true values and the long, elliptically shaped error surface then made convergence to the true parameter values impossible.

The results of varying the initial estimates and fitting the noise-free groups to Eq. 1 using a goodness-of-fit criterion of 0.001 are shown in Table 3. The initial estimates were important in determining the success of parameter extraction using Eq. 1. For these noise-free data, GRIDLS was superior for small- and moderate-molecule data, while CURFIT was generally unreliable. For large-molecule data, GRIDLS gave large errors when the initial estimate of k'_0 was larger

than the true value. In this case, CURFIT gave superior results to GRIDLS, provided that the initial parameter estimates were larger than the true values.

Corresponding results for fitting to Eq. 3 are shown in Table 4 (Eq. 3 results from an approximation which is inappropriate for small-molecule data). For both methods, the results were essentially independent of the initial estimates, and the percentage errors were small particularly for the large-molecule data.

Linear regression of the noise-free data groups on Eq. 4 gave exactly the same errors as those of CURFIT fitting to Eq. 3. The smaller errors observed for the large-molecule data are consistent with the assumption used in Eqs. 3 and 4.

3.4. Regression analysis of realistic data

Three hundred sets of ten gradient retention times were generated for each S and k'_0 pair at each of two noise levels (0.1% and 1%). Eq. 1 was fitted to each set using both GRIDLS and CURFIT. The goodness-of-fit criterion was set at 0.001. The initial estimates for both parameters were 1.0 for GRIDLS and 100 and 1 000 000 for

Table 2

Effect of goodness-of-fit criterion on error in extracted linear solvent strength parameters for non-linear regression methods GRIDLS and CURFIT

Goodness-of-fit criterion	True values		Error in extracted parameter (%)			
			GRIDLS		CURFIT	
	S	k'_0	S	k'_0	S	k'_0
0.01	3.4	4.0	0.01	0.00	0.00	0.00
	14.3	646	0.01	0.05	0.00	0.00
	22.5	75 858	-43.01	-97.67	-98.68	-100.00
0.001	3.4	4.0	0.01	0.00	0.00	0.00
	14.3	646	0.01	0.05	0.00	0.00
	22.5	75 858	0.01	0.10	-98.68	-100.00
0.0001	3.4	4.0	0.01	0.00	0.00	0.00
	14.3	646	0.01	0.05	0.00	0.00
	22.5	75 858	0.01	0.10	-99.89	-100.00
0.00001	22.5	75 858	0.01	0.10	-99.98	-100.00
0.0000001	22.5	75 858	0.01	0.10	-99.89	-100.00

The fits were performed on noise-free data groups calculated directly from Eq. 1, as described in the text. The initial estimates of the values of S and k'_0 were both 1.0.

S and k'_0 , respectively, for CURFIT. (As mentioned above, CURFIT worked better when the initial estimates were larger than the true values.)

The results in Table 5 indicate that the two methods gave similar errors fitting to Eq. 1, although GRIDLS was better than CURFIT in fitting the large-molecule group. However, GRIDLS tended to underestimate the parameter values. This trend became more pronounced as the parameter values increased.

Fitting in the same fashion to Eq. 3, with initial estimates for both parameters of 1.1 for both GRIDLS and CURFIT, gave the results shown in Table 6. GRIDLS gave smaller ranges of error than CURFIT for the extraction of S ; otherwise, the performance was essentially equivalent for the two methods. Moreover, the errors fitting to Eq. 3 were similar to the errors fitting to Eq. 1.

Table 7 shows the results of performing linear regression of the 0.1% and 1% noise-level groups (generated by Eq. 1) on Eq. 4. Again, these results were similar to those of either of the

non-linear regression methods. The range of error for the extraction of S by linear regression was similar to that of GRIDLS.

Since the non-linear regression methods require initial estimates of the parameters, and the results were found to depend on those estimates, the linear regression results were used as initial estimates for both non-linear regression methods. The errors in the extracted parameters using this approach were as large as those resulting from simply guessing the initial estimates, provided that those "guesses" were reasonable, i.e., the prior use of linear regression did not significantly improve the end result. This is probably again due to the nature of the residual surface; starting the non-linear regression in a region near the true values simply results in rapidly satisfying the convergence criterion without improving the results because of the shallow "floor" of the residual surface. While this may appear to contradict the findings in Table 1, where the results depended on the starting val-

Table 3

Effect of initial parameter estimates on error in extracted linear solvent strength parameters for non-linear regression methods GRIDLS and CURFIT using Eq. 1

Parameters				Error in extracted parameter (%) using non-linear regression method			
True value		Initial estimate		GRIDLS		CURFIT	
S	k'_0	S	k'_0	S	k'_0	S	k'_0
3.4	4.0	1.0	1.0	0.01	0.00	0.00	0.00
		100	1.0	0.01	0.00	-100	-50
		1.0	1×10^6	0.03	0.00	-49	$\geq 1 \times 10^6$
		100	1×10^6	0.03	0.00	-130	$\geq 1 \times 10^6$
14.3	646	1.0	1.0	0.01	0.05	0.00	0.00
		100	1.0	0.01	0.05	-100	-100
		1.0	1×10^6	1.6	6.6	-87	1.0×10^5
		100	1×10^6	1.6	6.6	0.00	0.00
22.5	75 858	1.0	1.0	0.01	0.10	-99	-100
		100	1.0	0.01	0.07	-100	-100
		1.0	1×10^6	28	1200	0.00	0.00
		100	1×10^6	28	1200	0.00	0.00

The fits were performed using noise-free data sets calculated from Eq. 1, as described in the text. The goodness-of-fit criterion was 0.001.

Table 4

Effect of initial parameter estimates on error in extracted linear solvent strength parameters for non-linear regression methods GRIDLS and CURFIT using Eq. 3

Parameters				Error in extracted parameter (%) using non-linear regression method			
True value		Initial estimate		GRIDLS		CURFIT	
S	k'_0	S	k'_0	S	k'_0	S	k'_0
14.3	646	1.1	1.1	0.38	2.4	0.40	2.4
		100	1.0	0.42	2.5	0.40	2.4
		1.0	1×10^6	0.38	2.4	0.40	2.4
		100	1×10^6	0.42	2.5	0.40	2.4
22.5	75 858	1.1	1.1	-0.02	0.03	-0.02	0.04
		100	1.0	-0.01	0.05	-0.02	0.04
		1.0	1×10^6	-0.02	0.03	-0.02	0.04
		100	1×10^6	-0.01	0.05	-0.02	0.04

The fits were performed using noise-free data sets calculated from Eq. 1, as described in the text. The goodness-of-fit criterion was 0.001.

Table 5

Extraction of linear solvent strength parameters from realistic data by non-linear regression methods GRIDLS and CURFIT using Eq. 1

True parameter value		Noise level (%)	Average \pm S.D. of error (%) using non-linear regression method			
S	k'_0		GRIDLS		CURFIT	
			S	k'_0	S	k'_0
3.4	4.0	0.1	0.17 ± 4.8	0.09 ± 0.78	0.52 ± 4.8	0.13 ± 0.78
14.3	646	0.1	-0.78 ± 2.1	-2.5 ± 8.3	1.8 ± 7.7	15 ± 63
22.5	75 858	0.1	-3.1 ± 3.7	-20 ± 29	0.62 ± 3.8	13 ± 43
3.4	4.0	1.0	0.96 ± 15	0.34 ± 2.5	2.2 ± 16	0.50 ± 2.5
14.3	646	1.0	-3.2 ± 6.3	-8.0 ± 25	6.4 ± 16	77 ± 350
22.5	75 858	1.0	-12 ± 8.5	-45 ± 180	3.2 ± 11	230 ± 1900

The fits were performed on synthetic data calculated directly from Eq. 1, as described in the text. The goodness-of-fit criterion was 0.001. The initial estimates in GRIDLS were 1.0 and 1.0, and in CURFIT were 100 and 1 000 000 for S and k'_0 , respectively.

ues, those results involved starting the fitting procedures relatively far from the true values.

It is significant that the success of the LSS theory has been evaluated in primarily two fashions: (i) the isocratically determined parameters have been used to predict gradient retention behavior, which has been compared to experimental results (e.g., [2]); and (ii) the LSS parameters have been extracted from gradient retention data and compared with the isocratically determined values (e.g., [13]). Case (i) is more common. The shape of the residual surface indicates that, for $S > 14$, fairly accurate values of

t_g can be obtained with erroneous S and k'_0 values. In other words, the LSS mode is extremely "robust" in its ability to predict t_g .

However, this "robustness" also implies that the accurate extraction of the true LSS parameter values from gradient data is difficult. Only in a study of the second type would this problem be detectable. For small molecules, the values of LSS parameters extracted from gradient data are in good agreement with their isocratically determined values (e.g., [13]). This is because there is a relatively well defined minimum in the residual surface. For large molecules, there is a

Table 6

Extraction of linear solvent strength parameters from realistic data by non-linear regressions methods GRIDLS and CURFIT using Eq. 3

True parameter value		Noise level (%)	Average \pm S.D. of error (%) using non-linear regression method			
S	k'_0		GRIDLS		CURFIT	
			S	k'_0	S	k'_0
14.3	646	0.1	0.09 ± 2.3	1.6 ± 9.6	0.57 ± 2.3	3.7 ± 9.9
22.5	75 858	0.1	-0.49 ± 3.8	2.2 ± 40	-0.67 ± 10	9.2 ± 45
14.3	646	1.0	-0.23 ± 7.4	5.1 ± 35	-0.31 ± 14	10 ± 41
22.5	75 858	1.0	-0.50 ± 12	180 ± 1300	-0.47 ± 21	330 ± 2400

The fits were performed on synthetic data calculated directly from Eq. 1, as described in the text. The goodness-of-fit criterion was 0.001. The initial estimates for both parameters were 1.1 in all cases.

Table 7

Extraction of linear solvent strength parameters from realistic data by linear regression to Eq. 4

True parameter value		Noise level (%)	Average \pm S.D. of error (%)	
<i>S</i>	k'_0		<i>S</i>	k'_0
14.3	646	0.1	0.57 \pm 2.3	3.7 \pm 9.9
22.5	75 858	0.1	0.33 \pm 3.9	11 \pm 44
14.3	646	1.0	1.3 \pm 7.6	13 \pm 40
22.5	75 858	1.0	2.2 \pm 13	330 \pm 2400

The fits were performed on synthetic data calculated directly from Eq. 1, as described in the text.

larger variance in the extracted large-molecule LSS parameters [7,13]. This larger variance is consistent with the discussion above; extraction of the LSS parameters is sensitive to experimental noise.

4. Conclusions

Pragmatically, the need for accurate determination of the LSS parameters from gradient data is limited. Large molecules are typically separated by gradient elution; the gradients run to determine the LSS parameters serve to allow gradient optimization directly. The LSS parameter values for small molecules can be determined directly from isocratic measurements or with acceptable accuracy following the suggestions given here. However, the general success of LSS theory in predicting gradient elution behavior indicates that any a priori theory of gradient elution should be able to predict the LSS parameter values and that the accuracy of such a prediction can be used to judge the success of that theory. Consequently, the accurate determination of the LSS parameters for this purpose is an important issue.

The pairwise gradient determination method is easy to perform since it requires the fewest chromatographic experiments and is suitable for use in optimizing gradient elution [7,13]. However, it should be considered unreliable for the

accurate extraction of the true LSS parameter values, as shown in this study.

Although the computation time for CURFIT is much shorter than than for GRIDLS, these results indicate that CURFIT was unreliable for extracting LSS parameters from experimental gradient retention data using Eq. 1. When appropriate, the use of Eq. 3 is preferred for either CURFIT or GRIDLS, since there was virtually no dependence on the initial estimates for Eq. 3.

For small molecules, GRIDLS and CURFIT give essentially equivalent, reliable results.

For moderate-sized and large molecules (i.e., $S \geq 10$), the linear regression method on Eq. 4 is recommended. It is the simplest method and provided comparable accuracy in the extraction of *S*. Although *S* values considerably larger than 22 have been reported, our results suggest that the accurate extraction of the true LSS parameter values will be increasingly difficult as the value of *S* increases. None of the tested methods yielded reliable extractions of k'_0 from realistically noisy data.

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