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PREDICTING REVERSED-PHASE GRADIENT ELUTION SEPARATIONS BY COMPUTER SIMULATION

A COMPARISON OF TWO DIFFERENT PROGRAMS

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SUMMARY

Two computer programs for developing and improving high-performance liquid chromatographic methods, DryLab G and LCSIM, have recently been described. The accuracies of these two programs were examined using experimental (*o*-phthalaldehyde-derivatized amino acids) and synthetic data. DryLab G, which uses gradient data for input, correctly predicted retention times for various gradient and isocratic separations. Predicted retention times for the simulation of certain isocratic conditions are susceptible to errors in the measured dwell volume, but the prediction of resolution is not seriously affected. LCSIM uses isocratic data for input, and predicted gradient retention times are affected by the accuracy of the measured dwell volume. The resolution of closely eluting analytes was usually predicted within a small fraction of the peak width, *i.e.*, with negligible errors.

Examples are given of some unique features of the LCSIM program: solvent switching and mixing, optional descending gradients, graphics display of the separation process and an iterative fit of the separation parameters to the retention characteristics of a new column (as measured from a single run).

INTRODUCTION

Within the past few years, computer programs for simulating gradient elution separations have been reported by several groups[1–5]. In the work described here, the accuracies of two such programs for reversed-phase gradient elution (LCSIM and DryLab G) were compared. The LCSIM program[1] uses an equation for retention *vs.* φ (volume fraction of solvent B in the mobile phase) with parameters fitted to experimental data. During the simulation, this equation is accessed repeatedly to calculate the motion of both the solvent front and the solute across the column. DryLab G is a commercially available program based on several years of research by Dolan *et al.*[2]. In the latter prgram, retention-time data from two experimental gradient runs are used as input; this substantially reduces the time required for data acquisition.

The intended use of these programs for developing a gradient method requires an accuracy in predicted retention times that is better than ± 1 min (*ca.* 5%) for

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planning gradient timing. Much better accuracy (equal to a small fraction of the peak width) is needed for optimizing resolution. Experimental and idealized synthetic data discussed here indicate that both programs satisfy these criteria.

EXPERIMENTAL

Equipment, materials and procedures

A Waters binary-gradient high-performance liquid chromatograph with a WISP 710B automatic injector was used with a Waters 840–DEC Pro-350 control/data acquisition system (Waters Assoc., Milford, MA, U.S.A.). Pre-column *o*-phthalaldehyde (OPA)–mercaptoethanol derivatization of amino acids was carried out using the Waters Autotag method[6]; for this purpose, a reaction coil for mixing reagent and analytes was positioned between the outlet of the WISP and the guard column. Separations were carried out with a Waters 10 × 0.8 cm I.D. Resolve Radial-PAK analytical cartridge at 24°C and a flow-rate of 1.2 ml/min. The mobile phase compositions, 4% (v/v) methanol–0.05 *M* phosphate buffer (pH 6.9) (A) and methanol–water (65:35, v/v) (B), and other experimental details, have been described previously[1].

For this study of the accuracy of the LCSIM and DryLab G programs, all experimental Autotag runs were carried out during a single 24-h period. Flushing with 100% B (65% methanol) for 5 min was always followed by pre-equilibration for 7 min with the mobile phase for the following run. With Autotag, injection of OPA–mercaptoethanol reagent (with no flow) is followed by injection of the analyte. There is slow flow (0.1 ml/min for 2 min, for mixing reagent and analyte in the coil) before the 1-min ramp (Waters curve 5) to 1.2 ml/min, at 3 min. Short (24 min), intermediate (50 min) and long (78.8 min) gradient runs with $\varphi = 0.4$ (28% methanol) to 1.0 (65% methanol) and isocratic runs with $\varphi = 0.4$, 0.5, 0.6, 0.7 and 0.8 were included.

Computer simulations

Isocratic retention data were fitted by means of Bevington's CURFIT[7] to the function

$$t_R = A3 + A1 \cdot \exp(A2 \cdot \varphi) \tag{1}$$

with $A3 = t_0 + 2.25$ min and $t_0 = 2.58$ min, where t_0 is the retention time of the solvent front, and the 2.25 min is added only for Autotag runs. Allowing $A3$ to vary did not materially improve the fits for these data; with $A3$ defined as above ($t_0 + 2.25$), the more common fitting equation[2]

$$\log k' = \log k_w - S\varphi \tag{2}$$

is identical with eqn. 1 and could be used, except for Autotag. Here, k' is the capacity factor, k_w is the value of k' for buffer A as mobile phase, S is a constant for solvent dependence and φ is the strong mobile phase fraction. The parameters $A1$, $A2$ and $A3$ provided input to LCSIM for gradient simulations. The gradient dwell volume (V_D, the volume of solvent between the inlet to the gradient mixer and the column inlet) is required for simulations by both LCSIM and DryLab G; V_D was measured as 3.5 ml [(2.9 ± 0.1 min) · (1.2 ml/min)][a].

TABLE I

RETENTION FUNCTIONS FOR SYNTHETIC DATA[a]

A1 (min)	*A2*	*Isocratic* t_R *(φ)*		*Setup gradients used*[b]
787.305	−10	17.00	(0.40)	A, B, C
237.132	−7			
71.423	−4			
2043.063	−10	40.00	(0.40)	D
615.3586	−7			
185.3425	−4			
15096.30	−10	40.00	(0.60)	D
8285.028	−9			
4546.92	−8			
412.4873	−4			

[a] Eqn. 1 format, obeys eqn. 2; $A3 = t_0 = 2.58$ min.

[b] See Experimental.

Retention times from the short and long gradient runs provided inputs for DryLab G. An average plate number $N = 7250$ was determined from isocratic values of retention time t_R and bandwidth W, where $W = 1.6$ (band area/peak height) from the Waters 840[8].

The Autotag procedure requires adjustments when entering experimental retention times and dwell volume into DryLab G. Previous experiments[1] have shown that the precolumn Autotag derivatization (with slow initial flow for mixing of reactants) causes an effective 2.25-min delay in an isocratic run. Therefore, for DryLab G simulations 2.25 min were subtracted from the original data and entries in the tables presented here have the 2.25 min. added back to the DryLab G output. Gradients which start at 3 min in the experimental Autotag sequence were started at zero time in the simulation, but the extra delay (3.00 − 2.25 = 0.75 min) requires that the dwell volume for DryLab G be set at 0.75 min · 1.2 ml/min = 0.9 ml greater than the value measured for the system (3.5 + 0.9 = 4.4 ml). LCSIM has a provision for Autotag derivatization which makes it unnecessary for the operator to rewrite the gradient events for simulations. The *A3* retention parameter (eqn. 1) for real Autotag data input to LCSIM is truncated by 2.25 min, but no solute movement is allowed before 2.25 min during the simulation.

Computer simulations with synthetic data

Retention parameters (eqns. 1 and 2) were defined (no Autotag), covering a wide range of values of *A2* (solvent strength dependence) and retention; parameters are listed in Table I. Using these functions, LCSIM calculated retention time values that were suitable as inputs for different DryLab G simulations A–D: A, 5–100% B in 20 and 60 min; B, 5–100% B in 30 and 90 min; C, 25–100% B in 20 and 60 min; and D,

[a] The gradient dwell volume was measured with the column disconnected, using a ramped change over one minute to a second mobile phase which contained a fluorophore.

40–100%B in 25 and 75 min. The retention times for a series of gradient and isocratic runs were then predicted by DryLab G, and compared with the corresponding predictions by LCSIM.

Abbreviations

The following abbreviations are used: OPA = *o*-phthalaldehyde; THF = tetrahydrofuran; ASP = aspartate; CSA = cysteinesulfonic acid; GLU = glutamate; ASN = asparagine; SER = serine; GLN, glutamine; HIS = histidine; CIT = citrulline; GLY = glycine; THR = threonine; MEH = 3-methylhistidine; ARG = arginine; TAU = taurine; ALA = alanine; TYR = tyrosine; AMB = α-aminobutyrate; TRP = tryptophan; MET = methionine; VAL = valine; PHE = phenylalanine; ILE = isoleucine; LEU = leucine; ORN = ornithine; and LYS = lysine.

RESULTS AND DISCUSSION

Accuracy of simulations

Experimental gradient data. Tables II and III summarize experimental retention times for the 24-, 50- and 79-min gradient runs (linear gradients from 40 to 100% B). Also shown (Table IV) are predicted isocratic retention times from LCSIM (isocratic data input, shown in Table IV), and corresponding predictions for the 50-min run from DryLab G (24- and 79-min gradient data used as input). The accuracy of the predicted separations is excellent in all instances, and the predicted retention times vary by only a small fraction of W from those measured from the real data.

Dwell volume estimates. The simulations of gradients by both LCSIM and DryLab G rely on accurate estimates of the dwell volume V_D. For the prediction of gradient retention on the basis of gradient input data (*e.g.*, DryLab G), errors in V_D usually have a minor effect on the prediction of gradient retention, but a larger effect on the prediction of isocratic retention. Similarly, for the prediction of gradient retention on the basis of isocratic input data (*e.g.*, LCSIM), errors in V_D can lead to significant errors.

Several comparisons of experimental *vs.* DryLab G-predicted data for isocratic separation showed systematic errors that varied linearly with dwell volume. The value of V_D giving the best fit was 4.4 ml, in agreement with the calculations described under Experimental. When the 0.9-ml dwell-volume correction is ignored, *i.e.*, V_D = 3.5 ml, small errors arise, as reported in Tables II–IV.

Isocratic data. Results based on these two different values of V_D (4.4 ml, correct; 3.5 ml, incorrect) are shown in Table IV. When the gradient data (Table III) from the LCSIM simulation, which used the Bevington fits for parameters, were input to DryLab G, qualitatively similar results were obtained for both values of V_D; however, with a 4.4-ml dwell volume the isocratic simulations matched the real data without systematic errors. Internal parameters from DryLab G also indicate good agreement with the Bevington fits when V_d = 4.4 ml (Table V).

Synthetic data. In order to avoid errors associated with "real" data, simulations with synthetic data (obeying eqn. 2) were carried out via LCSIM. The LCSIM output was then used for input to DryLab G (no autotag delays, and the dwell volume was set at 3.5 ml for both programs). Now nearly all gradient and isocratic simulations matched within a few hundredths of a minute, showing that the programs are performing as expected.

TABLE II

EXPERIMENTAL RETENTION TIMES AND BAND WIDTHS *vs.* VALUES PREDICTED BY DRYLAB G AND LCSIM

Conditions as described under Experimental; gradient time = 50 min.

Solute	*Retention time, t_R (min)*					*Band width W, (min)*[d]
	Exptl.	*LCSIM*		*DryLab G*[a]		
		A[b]	*B*[c]	*V_d=4.4 (correct)*	*V_d=3.5 (error)*	
TAU	23.06	23.02	23.04	23.05	23.10	0.55
ALA	25.21	25.17	25.22	25.21	25.27	0.66
TYR	25.49	25.42	25.50	25.51	25.58	0.69
TRP	36.93	36.87	36.91	36.92	37.03	0.55
MET	37.53	37.49	37.45	37.46	37.56	0.60
Errors in t_R: C.V. (%)		±0.2	±0.1	±0.1	±0.3	
Errors in separation, as % of *W*		2	5	4	6	

[a] Using experimental data from 24- and 78.8-min runs as input.
[b] Using values of *A*1, *A*2 and *A*3 from isocratic data.
[c] Using parameters (S, k_w) from DryLab G.
[d] Using LCSIM calculations based on plate counts from experimental isocratic data.

TABLE III

EXPERIMENTAL RETENTION TIMES AND BAND WIDTHS *vs.* VALUES PREDICTED BY LCSIM

Conditions as described under Experimental.

Gradient time (min)	*Solute*	*Retention time, t_R (min)*				*Band width, W (min)*[c]
		Exptl.	*LCSIM*			
			A[a]	*V_d=4.4*[b]	*V_d=3.5*[b]	
24	TAU	18.57	18.56	18.56	19.20	0.37
	ALA	19.85	19.86	19.85	20.50	0.38
	TYR	19.50	19.50	19.50	20.20	0.34
	TRP	24.93	25.01	24.93	25.68	0.37
	MET	25.57	25.69	25.56	26.29	0.37
	Errors in t_R: C.V. (%)		0.3	0.03	3.6	
	Errors in separation, as % of *W*		6	2	9	
78.75	TAU	25.99	25.95	26.00	26.45	0.72
	ALA	28.86	28.79	28.89	29.36	0.78
	TYR	29.98	29.80	29.99	30.58	0.74
	TRP	47.65	47.41	47.67	48.39	0.80
	MET	47.81	47.70	47.83	48.51	0.83
	Errors in t_R: C.V. (%)		0.4	0.07	1.9	
	Errors in separation, as % of *W*		11	2	10	

[a] Using values of *A*1, *A*2 and *A*3 from isocratic data.
[b] Using parameters (S, k_w) from DryLab G.
[c] Using LCSIM calculations based on plate counts from experimental isocratic data.

TABLE IV

EXPERIMENTAL *vs.* PREDICTED ISOCRATIC DATA

Solute	*φ (%)*	*Retention times, t_R (min)*					*Band width, W (min)*[c]
		Exptl.	*Fitted*[a]	*LCSIM, V_d=4.4*[b]	*DryLab G*		
					V_d=4.4 (corrected)	*V_d=3.5 (error)*	
TAU	40	38.48	38.47	38.70	38.70	37.47	1.70
ALA		45.65	45.62	46.23	46.17	44.50	2.04
TAU	50	20.69	20.74	20.76	20.76	21.86	0.87
ALA		24.71	24.81	24.90	24.87	25.89	1.06
TYR		25.98	25.97	26.90	26.27	27.41	1.11
TAU	60	12.38	12.34	12.32	12.32	13.72	0.47
ALA		14.64	14.60	14.56	14.54	16.01	0.58
TYR		13.41	13.49	13.43	13.42	15.06	0.53
TRP		37.97	38.00	38.83	38.78	40.22	1.68
MET		38.41	38.45	38.81	38.78	40.27	1.69
TAU	70	8.48	8.36	8.35	8.35	9.47	0.29
ALA		9.74	9.59	9.34	9.54	10.76	0.34
TYR		8.48	8.35	8.28	8.27	9.46	0.29
TRP		17.33	17.20	17.01	17.00	19.10	0.71
MET		19.20	19.02	18.69	18.68	20.72	0.79
TAU	80	6.31	6.47	6.49	6.48	7.25	0.20
ALA		7.01	7.13	7.12	7.11	7.98	0.23
TYR		6.19	6.24	6.21	6.21	6.93	0.19
TRP		9.25	9.42	9.20	9.19	10.58	0.33
MET		10.58	10.80	10.49	10.48	11.95	0.38
Errors in t_R: C.V. (%)			1.2	1.9	1.5	10.2	
Errors in separation, as % of W			12	19	19	22	

[a] From best fit to isocratic data.
[b] Using DryLab G values of S and k_w.
[c] From direct calculations based on plate count from isocratic data.

The synthetic functions in Table I were used to carry out additional simulations by both programs: gradients A, B and C. Using gradient input calculated by LCSIM, all gradient simulations by DryLab G were in excellent agreement with LCSIM predictions, and there were no systematic errors for isocratic separations.

Features and applications of LCSIM

Solvent mixing and switching. Two similar mobile phase systems (*e.g.*, A, B and A′, B) may be "mixed" (A″, B) for simulations. Linear interpolation between the two sets of parameters (eqn. 1) for A, B and A′, B, in the proportions f and $1-f$, respectively, give a new set, which may be evaluated for isocratic resolution using the RTGRAPH plotting program[1], or for gradient methods using LCSIM. If A and A′ have the same organic content but different amounts of a solvent modifier (*e.g.*, THF), the overall retention will not be greatly different for A and A′, and the linear approximation will be valid for the mixture. The best of several trial f values will indicate the best THF concentration for the A″ solvent. Mid-run solvent switching to

TABLE V

RETENTION FUNCTIONS FROM EXPERIMENTAL AUTOTAG DATA

Solute	*Bevington fit*		*DryLab G (V_d=4.4)*[a]		*DryLab G (V_d=3.5)*[a]	
	A1	*A2*	*A1*	*A2*	*A1*	*A2*
TAU	676.259	−7.5016	692.819	− 7.5456	437.139	−6.4933
ALA	713.174	−7.1525	749.238	− 7.2393	481.526	−6.2676
TYR	1856.766	−8.9508	2077.876	− 9.1459	1085.475	−7.7712
TRP	12377.78	−9.8702	16055.34	−10.2626	6754.911	−8.7821
MET	5890.015	−8.6351	7372.582	− 8.9663	3703.564	−7.7620

[a] DryLab G internal parameters were converted as follows: $A1 = t_0 \exp(\ln 10 \cdot \log k_w)$; $A2 = -S(\ln 10)$. In all instances $A3 = t_0 + 2.25$ min, where $t_0 = 2.58$ min. Retention functions obey eqn. 2.

a different parameter set, which is required in many gradient separations, may be menu-selected at any timed event in the gradient scheme. Switching parameters may be useful also with retention data which are difficult to fit with a simple equation.

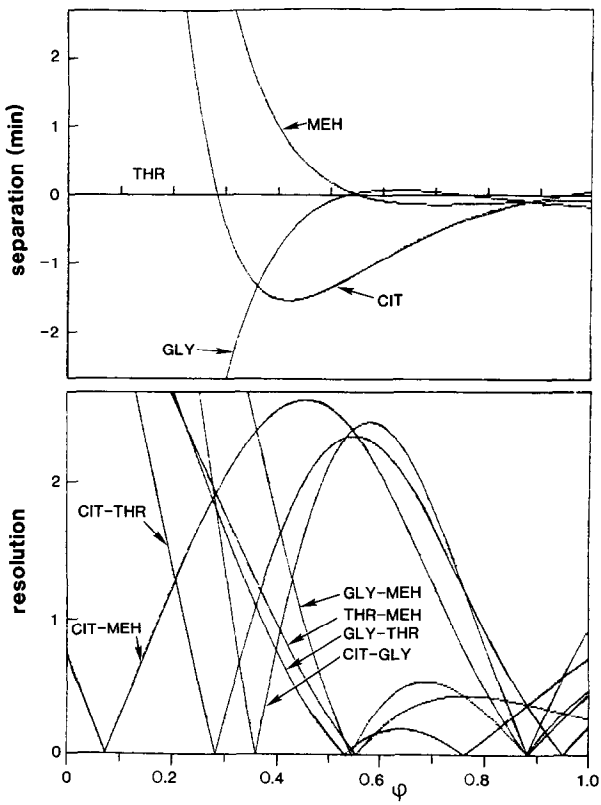


Fig. 1. Isocratic separation (top) and resolution (bottom) plots from RTGRAPH program for data acquired using Waters C_{18} Resolve column, as described in text and ref. 1, for CIT, GLY, THR and MEH.

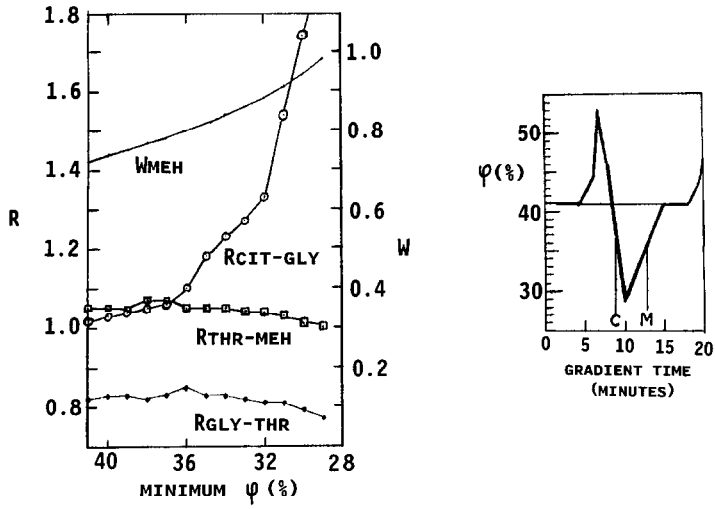


Fig. 2. Descending gradient scheme and results. Right, the most extreme perturbation in the multi-methods sequence. Markers show point of elution of CIT and MEH. Left, plots of resolution for all three pairs, and width for MEH, shown as a function of φ value at bottom of the valley decreasing from starting value 0.41.

Iterative fitting. With successive iterative simulations, the $A1$ parameters (eqn. 1) for each eluent can be updated to match the exact retention times for a new (or aging) column. For methods with solvent switching, $A1$ for each mobile phase mixture is multiplied by the same correction factor. The $A2$ and $A3$ parameters from previously acquired data are retained. The new set of parameters is labeled and stored as a file and may now be used by LCSIM as usual. $A1$ is adjusted because it is an extrinsic parameter; differences or loss of C_{18} loading and, therefore retentiveness, rather than changes in solvent dependence, would be expected with column replacement or aging. If this hypothesis were to be found invalid, the procedure described here would be accurate only for minor adjustments in gradients schemes.

Multi-methods programming example: planning a descending gradient. Rapid elution of CIT, GLY, THR and MEH in this order on Resolve C_{18} columns requires a descending gradient[1]. Separation and resolution plots from RTGRAPH for these eluents are shown in Fig. 1. As φ increases from 0.35 to 0.45, the resolution for CIT–GLY increases, whereas for the later eluting GLY–THR and THR–MEH pairs, the resolution worsens. Therefore it is an advantage to elute CIT–GLY at a higher average φ than that of the other two pairs. This can be done only with a descending gradient.

The multi-methods feature of LCSIM allows systematic mapping of gradient conditions for gradient separations. In this example, over the course of thirteen simulated runs the gradient events progressed from optimum isocratic conditions (φ = 0.41, Fig. 1) to the most extreme scheme illustrated in Fig. 2, an elevation to φ_e = 0.53, followed by a descent to φ_d = 0.29. Timing of the events is facilitated by the graphic output of LCSIM, which shows the point of elution along the gradient scheme (see ref. 1 for details and chromatograms); the elution points of CIT and MEH are indicated by markers in Fig. 2.

Plots of width for MEH and resolution of all three pairs are shown *vs.* the φ_d

TABLE VI

DESCENDING GRADIENT RESULTS

Parameter[a]	*CIT*	*GLY*	*THR*	*MEH*	
R	1.16	0.93	1.18		New (descending)
W	0.64	0.85	1.13	1.04	
R	1.02	0.82	1.05		Isocratic (φ = 0.41)
W	0.64	0.67	0.71	0.72	

[a] R = resolution; W = band width (min).

value. As the elevation of φ at 6 min and depression at 10 min become more pronounced, CIT–GLY resolution is enhanced, but the resolution of GLY–THR or THR–MEH is not affected, as long as $\varphi_d \geqslant 0.33$. Therefore, the scheme having this minimum value was chosen for the starting point for the next set of multi-method simulations.

This next set, in which φ at all gradient points was now incrementally lowered to improve the resolution of GLY–THR and THR–MEH, showed that starting the separation at $\varphi = 0.40$ was a good compromise for the resolution of all eluents in the group (Table VI, New). A substantial improvement in resolution over the original isocratic method has been achieved, although the accuracy of integration has been sacrificed, as the peaks are wider.

Late eluents. In this example resolution within the group TAU, ALA and TYR ($t_R \approx 27$ min) is to be enhanced without affecting the resolution of TRP–MET ($t_R \approx 32$ min). Resolution within the first group is enhanced by lower φ values (Fig. 3), but this worsens the resolution of TRP–MET. Bevington fits for TRP and MET (parameters in Table V) show greater solvent dependence for TRP, which will move more slowly than MET at low φ values; separation plots show that for $\varphi < 0.6$ the elution order is MET, TRP. However, as elution must take place in a reasonable time frame, the TRP, MET

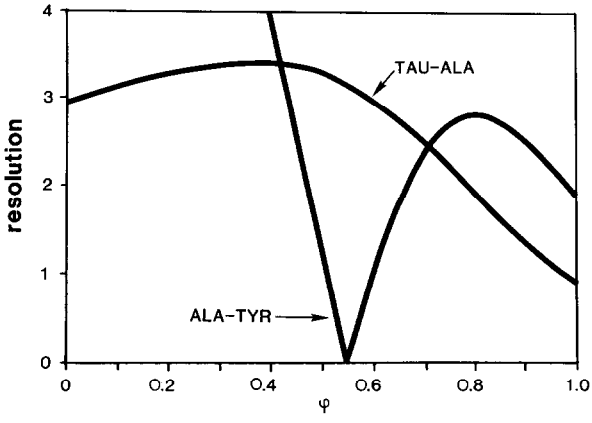
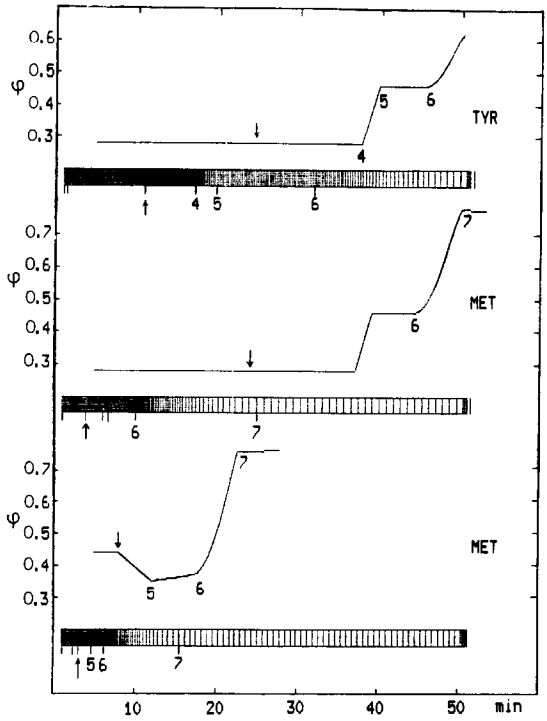


Fig. 3. Resolution plots from RTGRAPH for TAU–ALA and ALA–TYR obtained from converted DryLab G parameters (similar in general appearance to those obtained using parameters fitted to isocratic data). Column, Waters C_{18} Resolve Radial-PAK; mobile phase, A = 0.05 M phosphate–0.075 M acetate (pH 6.95) with 4% methanol; B = methanol–water (65:35, v/v).



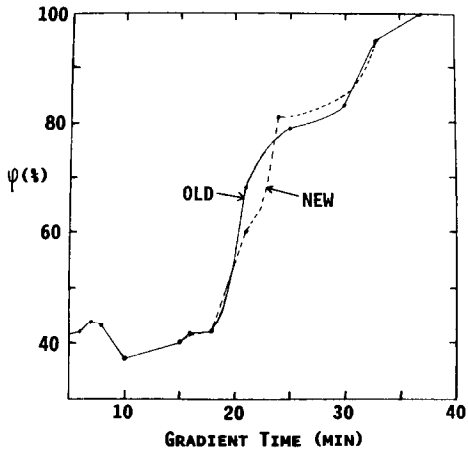


Fig. 4. Three graphic LCSIM outputs for TYR and MET derivatives to show effects of gradient scheme on availability of column plates for separation of closely eluting solutes at specific φ values. Upper two outputs long method; lower output, rapid method.

Fig. 5. Gradient schemes for resolution of plasma amino acids as OPA–mercaptoethanol derivatives on Waters Resolve Radial-PAK columns, as described in text.

order is chosen by having $\varphi > 0.7$. The TRP, MET elution order is the reason that rapid elution of CIT, GLY, THR and MEH must be carried out (as opposed to a longer method[1,9] using a lower φ value and a different resolution "window" for CIT···MEH in Fig. 1); in the graphic outputs (Fig. 4), note that in the long gradient scheme MET is half way through the column by the time φ has reached 0.75, whereas in the rapid scheme, MET has moved only about one quarter the length of the column.

In the improved gradient scheme, φ had to be decreased to elute TAU, ALA, and TYR, then more steeply increased higher than previously for the 25–30-min gradient time segment (Fig. 5). For accurate timing of the events the iterative fit feature was used to obtain valid parameters for the current column. On subsequent simulations and trial-and-error alterations of the gradient scheme, the new scheme was chosen (Fig. 5); real chromatograms show that the modification was successful in improving the resolution of TAU, ALA and TYR (Fig. 6). Table VII gives numerical data; note that while the separation of TRP–MET is less, the widths are smaller, so that the resolution is essentially unchanged.

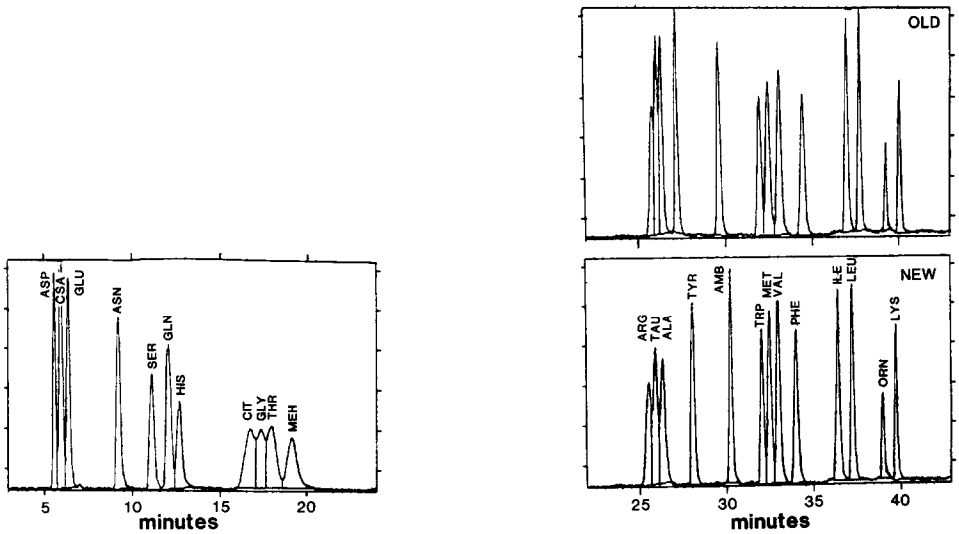


Fig. 6. Chromatograms of OPA–mercaptoethanol derivatives of plasma amino acid standard solutions (17 pmol each injected); left, early eluents; right, late eluents (old and new gradient schemes shown in Fig. 5 for resolution of TAU, ALA and TYR, as described in text).

CONCLUSIONS

Simulations with synthetic data and real separations presented in this paper show that both DryLab G and LCSIM are fairly accurate in predicting isocratic or

TABLE VII

SIMULATIONS WITH LCSIM AND EXPERIMENTAL DATA FOR OLD AND NEW GRADIENT SCHEMES

Gradient schemes are illustrated in Fig. 5.

Solute	*Old*					*New*				
	t_R *(min)*	*Sep (min)*	*W (min)*	*R*	φ_{el}	t_R *(min)*	*Sep (min)*	*W (min)*	*R*	φ_{el}
Simulations:										
TAU	25.98	–	0.44	–	0.60	25.92	–	0.55	–	0.57
ALA	26.30	0.32	0.41	0.74	0.65	26.36	0.44	0.55	0.80	0.59
TYR	27.13	0.83	0.25	2.55	0.72	28.08	1.72	0.34	3.86	0.66
TRP	31.94	–	0.35	–	0.79	32.04	–	0.30	–	0.82
MET	32.41	0.47	0.40	1.24	0.80	32.49	0.45	0.35	1.38	0.82
Experimental data:										
TAU	26.08	–	0.36	–		25.92		0.50	–	
ALA	26.34	0.26	0.37	0.72		26.36	0.44	0.52	0.86	
TYR	27.18	0.84	0.29	2.54		28.07	1.71	0.37	3.81	
TRP	31.94	–	0.42	–		32.04	–	0.37		
MET	32.45	0.51	0.47	1.14		32.48	0.44	0.41	1.14	

gradient retention times. Errors in the measurement of the system dwell volume will result in error of similar magnitude in predicting isocratic retention times with DryLab G, or gradient retention times with LCSIM. However, prediction of separation is usually not as susceptible to errors in dwell volume. Small errors in retention times of experimental gradient runs for input to DryLab G likewise can cause noticeable errors in the predicted retention times of isocratic runs. Excellent predictions based on Autotag data are possible if the dwell volume is corrected properly.

Application of some useful features of LCSIM have been described with OPA–mercaptoethanol-derivatized amino acids, derivatized with the precolumn Autotag method. It has been shown that DryLab G and LCSIM can be used together to access unique features of each program. Aquisition of gradient data is relatively rapid; DryLab G can use these data to supply internal parameters which can be used by both programs. LCSIM is useful for multi-solvent programming, has a useful graphics routine which lends insight into the course of the separation and allows updating of retention characteristics to a new or aged column.

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