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Computer simulation as an aid in method development for gas chromatography

I. The accurate prediction of separation as a function of experimental conditions

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ABSTRACT

Computer-simulation with commercially available software (DryLab GC) allows the prediction of isothermal or temperature-programmed gas chromatographic (GC) separation as a function of experimental conditions. In either case, two experimental runs are carried out initially, using a linear temperature program (heating rate different, all other conditions the same). Data from these two runs are entered into the computer, and separation can then be predicted for other conditions: different temperatures in the case of isothermal runs, or any kind of temperature program for programmed runs.

The reliability of resulting predictions was evaluated in the present study for several samples and a wide range in separation conditions. Retention time predictions were usually accurate within a few percent, and sample resolution was predicted within about ± 10%. The use of computer simulation should be a considerable help for the rapid development of superior GC methods.

INTRODUCTION

Computer simulation using DryLab software is proving to be a useful tool for method development in high-performance liquid chromatography (HPLC) [1–7]. Two experimental runs under standardized conditions are carried out initially, following which a personal computer can be used to optimize either mobile phase composition (%B) in isocratic separation or gradient steepness (b) in gradient elution. This approach can also be used to design complex, multisegment gradients, which are often of considerable value for improving resolution and/or shortening run time [1–10]. Much of the value of computer simulation in HPLC arises as a result of frequent changes in band spacing (values of α) when values of %B or b are changed [11].

In view of the value of computer simulation for HPLC method development, we have explored the similar application of this technique to gas chromatography (GC). GC separations in an isothermal or programmed-temperature mode are conceptually similar to corresponding separations by HPLC under isocratic or gradient conditions

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(*cf.* refs. 12 and 13). There are also several reports which show that band spacing[a] in GC can be varied by changes in either the temperature or programming rate [14–20] (although many chromatographers seem to be unaware of this possibility). These observations suggest that computer simulation similar to that now used for HPLC method development should also prove to be of value for GC.

Previously we have described software (DryLab GC) for the computer simulation of GC separations [21]. The present paper reports the application of this software to the separation of a number of different samples, in turn allowing an assessment of its accuracy for a wide range of conditions. A following paper [22] examines the general utility of controlling GC band spacing via selection of the best isothermal temperature or an optimized temperature program.

THEORY

Predictions of GC retention

Isothermal retention (values of the capacity factor k) in a defined GC system is related to temperature as

$$\log k = A + B/T \tag{1}$$

where A and B are constants for a given solute, and T is the column temperature (K); A and B depend on the entropy and enthalpy of vaporization, respectively [12,17]. Eqn. 1 assumes that A and B are independent of temperature, which is usually a reasonable approximation.

For the case of a linear temperature program (most often used in GC), the column temperature T is related to separation time t as

$$\begin{aligned} T &= T_0 + (T_f - T_0)(t/t_P) \\ &= T_0 + \Delta T(t/t_P) \end{aligned} \tag{2}$$

where T_0 and T_f refer to the initial and final temperatures, and t_P is the program time. Given values of A and B for the various solutes in a sample (for a defined GC system), it is possible to predict retention time t_R in separations based on linear (single-segment) temperature programs by means of the relationship [12,17]

$$1 = \int_0^{t_R} dt/[t_0(k + 1)] \tag{3}$$

Here t is the time after sample injection and the beginning of temperature programming, and t_0 is the column dead-time. Eqn. 3 assumes that band migration in temperature-programmed GC can be approximated as the sum of a series of (small) isothermal steps, each successive step being carried out at a slightly higher temperature.

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[a] By a "change in band spacing" we mean changes in relative values of the separation factor α for different band pairs, and possibly (but not necessarily) changes in band retention order.

An explicit solution for eqn. 3 has not yet been derived [17]. However, use of the so-called linear-elution-strength (LES) approximation [21],

$$\log k \approx (\text{constant}) - ST \tag{4}$$

where S is a constant for a given solute and GC system, allows an approximate solution which is suitable for rapid computer simulation using a personal computer (PC). Bandwidths W can also be predicted by means of

$$W = 4t_0(1 + k_e)/N^{1/2} \tag{5}$$

Here k_e is the value of k for the solute at the time of elution, and N is the column plate number. A value of k_e can be obtained from eqn. 1, since t_R defines the column temperature at the time the band elutes from the column (eqn. 2 with $t = t_R$). A value of N can then be obtained from eqn. 6 by using experimental values of t_R and W from one of the two starting experimental runs.

Eqn. 5 assumes that N and t_0 do not vary with temperature (which is of course an approximation). In that case, the width of a band *on a given column* just prior to elution will be constant for every solute and every temperature. The derivation of eqn. 5 follows from the definition of N and the relation of t_R to k at the time of elution (k_e);

$$N = 16(t_R/W)^2 \tag{6}$$

$$t_R = t_0(1 + k_e) \tag{7}$$

See also the discussion of ref. 23. With the addition of a correction for the extra-column volume of the GC system [21], eqn. 5 has been shown to give reliable predictions of bandwidth as a function of isothermal temperature or programmed-temperature conditions.

We have used the foregoing approach to construct a program (DryLab GC) for the computer-simulation of GC runs [21]. DryLab GC uses two experimental programmed-temperature runs as input for computer simulation —in the same way that computer simulation has been carried out for method development in gradient elution [8–10]. Predictions of separation can then be made for (i) isothermal runs at any temperature, (ii) temperature-programming for any starting temperature and heating rate, and (iii) multi-ramp temperature programs (where the heating rate is varied stepwise during the separation). A more detailed description of the theoretical basis of DryLab GC is given in ref. 21.

EXPERIMENTAL

Equipment

The gas chromatograph was an HP5890A (Hewlett-Packard; Avondale, PA, U.S.A.) equipped with split/splitless injection port and flame ionization detector. The system makes use of Hewlett-Packard's INET system network for control of the HP3396A integrator and HP7672A autoinjector. ChromPerfect (Justice Innovations, Palo Alto, CA, U.S.A.) was used for data analysis. Most injections were performed manually.

Software

The computer program DryLab GC is available from LC Resources (Lafayette, CA, U.S.A.). It is designed to run on any IBM-compatible personal computer; the addition of a math coprocessor is recommended.

Columns

Three fused-silica capillary columns were used in the present study: a non-polar column (SPB-1, Supelco, Supelco Park, PA, U.S.A.), a slightly polar column (DB-5, J & W Scientific, Folsom, CA, U.S.A.) and a polar column (Nukol, Supelco). Each column had the same dimensions (30 m × 0.025 cm I.D.) and film thickness (0.25 μm). The column dead-time (air-peak measurement) varies with temperature [21]; an average value of t_0 = 1.8 min was assumed in the present study.

Samples

Several different test mixtures were used to evaluate the present DryLab GC software. A number of samples were purchased from Supelco: (a) "non-polar test mixture": 2-octanone, *n*-decane, 1-octanol, *n*-undecane, 2,6-dimethylphenol, 2,6-dimethylaniline, *n*-dodecane and *n*-tridecane; (b) "phenol test mixture": 2,4,6-trichlorophenol, 4-chloro-3-methylphenol, 2-chlorophenol, 2,4-dichlorophenol, 2,4-dimethylphenol, 2-nitrophenol, 4-nitrophenol, 2,4-dinitrophenol, 2-methyl-4,6-dinitrophenol, pentachlorophenol and phenol; (c) "herbicide test mixture": Eptam, Sutan, Tillam, Ordram, Ro-neet, Trifuluralin, Atrazine, Terbacil, Sencor, Bromacil, Paarlan, Goal and Hexazinone; (d) "rapeseed oil mixture": methyl myristate, palmitate, stearate, oleate, linoleate, linolenate, arachidate, eicosenoate, behenate, erucate and legnocerate; (e) "barbiturate test mixture": barbital, amobarbital, aprobarbital, pentobarbital, secobarbital, hexobarbital, mephobarbital, phenobarbital, cyclobarbital, butabarbital and butalbital; (f) "pesticide test mixture": 16 chlorinated hydrocarbons, mainly derivatives of BHC, aldrin, endrin and DDT.

Various oil samples (spearmint, peppermint, lime) were obtained from Lorann Oils, Lansing, MI, U.S.A.; lemon oil was purchased locally. Two narrow-boiling gasoline samples (A, 200–300°F; B, 300–350°F) were a gift from the Unocal Research Center. Several random mixtures were assembled from our chemical stockroom: samples (A) cyclohexane, ethyl acetate, methylene chloride, isopropyl alcohol, 3-pentanone and *tert.*-amyl alcohol; (B) chlorobenzene, cyclohexanone, *n*-hexanol, cyclohexanol and *p*-dichlorobenzene; (C) 1,2-propanediol, acetophenone, *p*-dibromobenzene, nitrobenzene, *p*-nitrotoluene and benzyl alcohol; (D) cyclohexane, ethyl acetate, methylene chloride, *tert.*-amyl alcohol, *n*-hexanol, cyclohexanol, acetophenone and benzyl alcohol.

RESULTS AND DISCUSSION

Simulation of linear temperature-programmed runs

The potential accuracy of computer simulation for either HPLC or GC predictions is limited by similar factors; these have been discussed for HPLC in refs. 24 and 25 and for GC in ref. 21. In the case of DryLab GC, the experimental input data for computer simulation are from two linear, temperature-programmed runs having different heating rates: *e.g.*, 4 and 8°C/min. We can expect [21] that predictions for runs

with intermediate heating rates (*e.g.*, 4–8°C/min) will be more accurate than for extrapolated conditions (*e.g.*, <4 or >8°C/min). Similarly, the accuracy of extrapolated simulations will decrease whan the initial (experimental) runs have heating rates that are similar (*e.g.*, 4 and 5°C/min). Heating-rate ratios >3 are usually recommended, but ratios <3 were explored in the present study in order to magnify possible errors for extrapolated conditions. A further discussion of possible errors in GC computer simulation is provided in this paper.

Retention time predictions. A number of experimental runs were carried out for different samples with variation of the heating rate. These data allow comparisons between predicted and experimental results for a wide range of conditions (only heating rate varying). Some typical results are summarized in Table I and Fig. 1.

Results for the 13-component herbicide sample summarized in Table I show excellent agreement between experimental and predicted retention times; average errors in computer-simulated values of t_R are only 0.3–1.2%. As expected, the average error in t_R is less for the interpolated run (0.3%, inputs of 4 and 8°C/min) *vs.* the extrapolated runs; *i.e.*, 0.8–1.2% errors for the last two runs of Table I. Fig. 1A and B compares experimental *vs.* predicted chromatograms for the 6°C/min herbicide run, and Fig. 1C and D shows a similar comparison (6°C/min) for the barbiturate sample (data of Table II).

TABLE I

COMPARISON OF EXPERIMENTAL *VS.* SIMULATED RETENTION TIMES FOR HERBICIDE MIXTURE

Conditions: DB-5 column, linear 100–300°C temperature program, 1.0 ml/min flow-rate; DryLab GC used for simulations. Avg. error refers to the average of absolute errors for individual solutes.

Heating rate r (°C/min)		Retention times t_R (min)			
Inputs	Simulation	Expt.	Calc.	Error	
				t_R	Δt_R
4/8	6	11.40	11.43	0.03	0.01
		12.01	12.05	0.04	0.02
		13.61	13.67	0.06	0.00
		15.58	15.64	0.06	−0.01
		16.37	16.42	0.05	0.01
		17.73	17.79	0.06	0.00
		18.95	19.01	0.06	0.00
		20.16	20.22	0.06	0.01
		21.42	21.49	0.07	0.00
		22.98	23.05	0.07	−0.01
		23.79	23.85	0.06	0.01
		25.59	25.66	0.07	0.00
		28.27	28.34	0.07	
		Avg. error		±0.06 (0.3%)	±0.007 (0.5%)

(Continued on p. 6)

TABLE I *(continued)*

Heating rate r (°C/min)		Retention times t_R (min)			
		Expt.	Calc.	Error	
Inputs	Simulation			t_R	Δt_R
4/6	8	9.84	9.77	−0.07	−0.01
		10.32	10.24	−0.08	−0.01
		11.61	11.52	−0.09	−0.01
		13.10	13.00	−0.10	0.01
		13.62	13.53	−0.09	−0.02
		14.71	14.60	−0.11	0.00
		15.65	15.54	−0.11	−0.01
		16.59	16.47	−0.12	0.00
		17.54	17.42	−0.12	−0.01
		18.71	18.58	−0.13	0.01
		19.29	19.17	−0.12	0.00
		20.68	20.54	−0.12	−0.03
		22.79	22.64	−0.15	
		Avg. error		±0.11 (0.8%)	±0.01 (0.9%)
6/8	4	13.95	13.79	−0.16	−0.02
		14.83	14.65	−0.18	−0.05
		17.03	16.81	−0.23	−0.03
		19.92	19.66	−0.26	0.02
		21.26	21.02	−0.24	−0.03
		23.13	22.86	−0.27	−0.02
		24.89	24.60	−0.29	−0.02
		26.62	26.31	−0.31	−0.01
		28.51	28.19	−0.32	−0.02
		30.86	30.52	−0.34	−0.01
		32.13	31.80	−0.33	−0.05
		34.74	34.36	−0.38	−0.01
		38.24	38.12	−0.39	
		Avg. error		±0.29 (1.2%)	±0.02 (1.2%)

Differences in predicted *vs.* experimental values of t_R should be small, although errors of 3–5% are generally acceptable. For the purposes of method development and optimizing the separation, however, errors in *retention time differences* are more important. Resolution R_s is proportional to the difference (Δt_R) in t_R values for an adjacent pair of bands: $\Delta t_R = t_2 - t_1$, where t_1 and t_2 refer to values of t_R for the first and second band in a given band-pair. It can be seen in Table I that errors in Δt_R (0.007–0.02 min) are very much smaller than are errors in t_R (0.06–0.29 min), and this was observed in every case. That is, errors in GC computer simulation are highly correlated with retention time t_R (see discussion of ref. 21). This is fortunate, because it

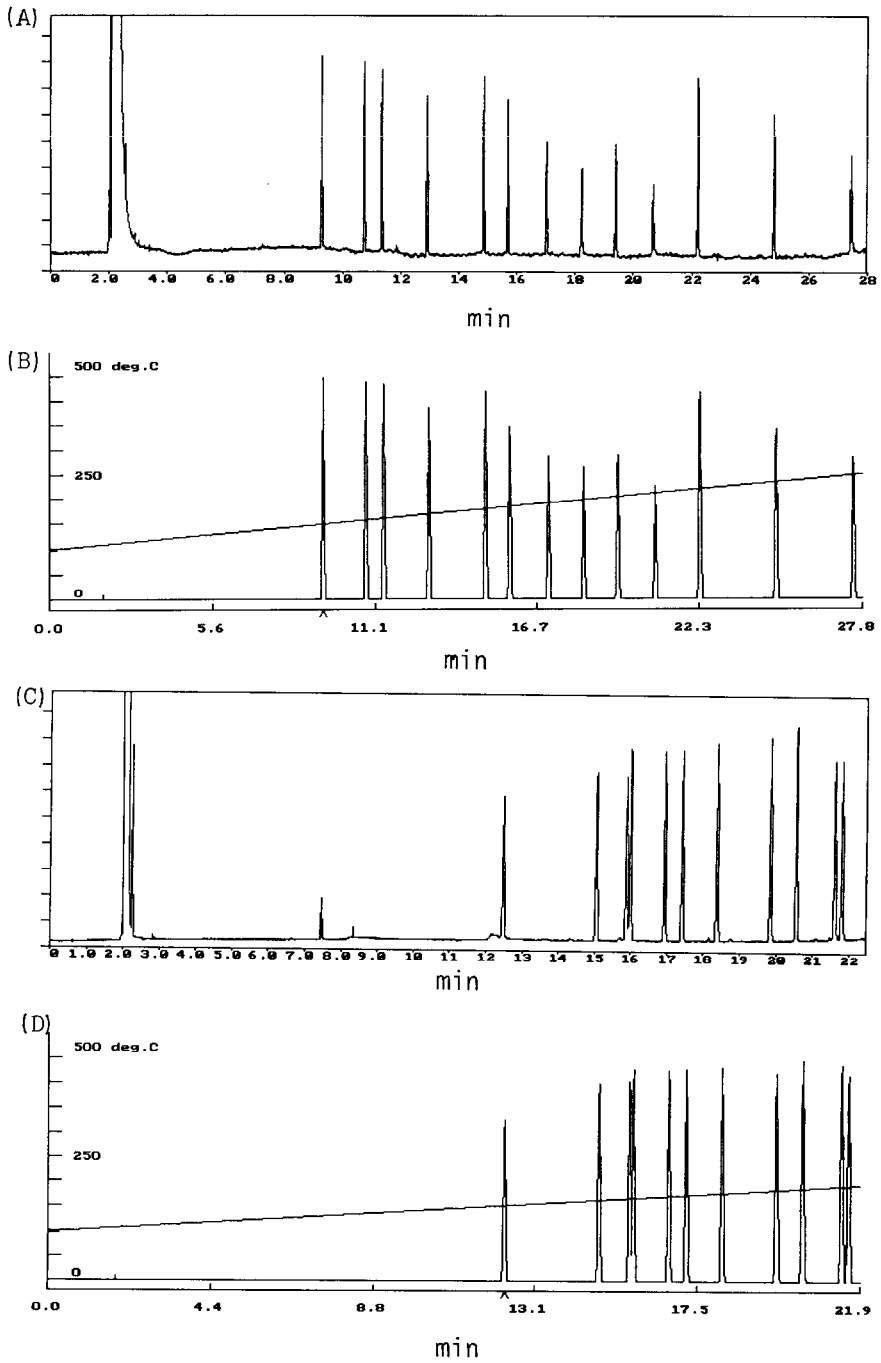

Fig. 1. Comparisons of experimental *vs.* computer-simulated (predicted) chromatograms for linear-program separations on DB-5 column. (A) Experimental chromatogram for herbicide sample of Table I (100–300°C, 6°C/min); (B) same, DryLab GC chromatogram (input data: 4 and 8°C/min); (C) experimental chromatogram for barbiturate sample of Table II (100–300°C, 6°C/min); (D) same, DryLab GC chromatogram (input data: 4 and 8°C/min).

TABLE II

SUMMARY OF COMPARISONS OF EXPERIMENTAL *VS.* PREDICTED RETENTION FOR LINEAR TEMPERATURE PROGRAMS AND DIFFERENT SAMPLES AND COLUMNS (AS IN TABLE I)

Sample (column/range)[a]	Heating rate r (°C/min)		Errors	
	Inputs	Simulation	t_R	Δt_R
Herbicides[b]	4/8	6	0.3%	0.5%
(DB-5/100–300°C)	4/6	8	0.8	0.9
	6/8	4	1.2	1.2
Non-polar[b]	4/8	6	0.2	0.9
(DB-5/100–300°C)	4/6	8	0.5	1.3
	6/8	4	0.6	1.6
Phenols[b]	4/8	6	0.4	0.8
(DB-5/100–300°C)	4/6	8	0.8	1.8
	6/8	4	1.0	2.1
Non-polar[b]	4/8	6	0.2	0.5
(Nukol/70–200°C)	4/6	8	0.4	1.2
	6/8	4	0.6	1.8
Phenols[b]	1/3	2	0.8	1.1
(Nukol/120–200°C)	1/2	3	1.6	1.7
	2/3	1	2.1	3.2
Rapeseed oil[b]	2/4	3	0.2	0.4
(DB-5/100–300°C)	2/3	4	0.4	0.9
	3/4	2	0.5	1.2
Barbiturates[b]	4/8	6	0.6	0.8
(DB-5/100–300°C)	4/6	8	1.5	0.6
	6/8	4	2.0	1.3
Sample A[c]	1/4	2	0.6	1.3
(Nukol/50–100°C)	1/2	4	1.7	0.4
	2/4	1	0.9	1.5
Sample B[c]	4/8	6	0.4	0.2
(Nukol/50–200°C)	4/6	8	1.3	0.7
	6/8	4	1.7	1.5
Sample C[c]	2/6	4	1.3	1.3
(Nukol/100–200°C)	2/4	6	3.0	2.0
	4/6	2	4.5	3.8
Sample D[c]	2/8	4	0.5	1.3
(Nukol/100–200°C)	2/4	8	1.2	2.4
	4/8	2	1.2	3.3
	Avg. errors[d]		±1.1	±1.4[a]

[a] Range refers to the change in temperature during the run.
[b] Supelco sample.
[c] Sample formulated by us.
[d] Average absolute errors.

means that predictions of resolution by computer simulation are more likely to be reliable —and therefore more useful for GC method development.

Similar comparisons as in Table I were carried out for several samples described in the Experimental section —using both the DB-5 and Nukol columns. These results are summarized in Table II. It is seen that predicted values of t_R and Δt_R are in every case in acceptable agreement with experimental values. Thus for interpolated heating rates, the average (absolute) errors in t_R and Δt_R were $\pm 0.4\%$ and 0.7%, respectively. Similarly, for extrapolated heating rates, the corresponding average errors were $\pm 1.2\%$ in t_R, and $\pm 1.4\%$ in Δt_R. These data suggest that computer simulation for GC may prove to be even more reliable than for HPLC (see HPLC comparisons of refs. 1–10, 24–26).

Bandwidth predictions. The prediction of bandwidth requires a value of the

TABLE III

SUMMARY OF COMPARISONS OF EXPERIMENTAL *VS.* PREDICTED BANDWIDTHS FOR VARIOUS SAMPLES AND COLUMNS (RUNS OF TABLE II); LINEAR TEMPERATURE PROGRAMS

See text for details.

Sample (column/range)[a]	Error (%)[b]
Herbicides (DB-5/100–300°C)	+10 ± 7
Non-polar (DB-5/100–300°C)	+17 ± 5
Phenols (DB-5/100–300°C)	+6 ± 4
Rapeseed oil (DB-5/100–300°C)	−25 ± 12
Barbiturates (DB-5/100–300°C)	−3 ± 3
Non-polar (Nukol/70–200°C)	−8 ± 4
Phenols (Nukol/120–200°C)	−6 ± 4
Sample B (Nukol/50–200°C)	−6 ± 4
Sample C (Nukol/100–200°C)	−9 ± 3
Sample D (Nukol/50–200°C)	−9 ± 5
Sample A (Nukol/50–100°C)	−8 ± 10
Overall average	−4 ± 5

[a] Range refers to the change in temperature during the run.

[b] Values are average percentage error and range in error values (1 standard deviation).

column plate number N (eqn. 5). Average values of N were measured for each column from isothermal runs, using well retained bands to avoid extra-column errors[a]. For the DB-5 column at 160°C, the column plate number was $N = 100\,000$. For the Nukol column at 100°C, $N = 70\,000$.

Table III summarizes our comparison of experimental *vs.* predicted bandwidths (W) for the various runs of Table II. Errors were calculated as $100[(W_{calc}/W_{expt}) - 1]\%$; *i.e.*, positive errors indicate that experimental bandwidths are narrower than predicted. For a given separation (*e.g.*, a specified sample, column and experimental conditions) the errors in predicted bandwidths were first averaged and then the standard deviation of the errors was determined. The overall (average) error in these predicted bandwidths is -4%, with an average deviation in each separation of $\pm 5\%$ from the average for that run.

Simulation of isothermal runs

Some samples are better separated isothermally, rather than via temperature programming. The DryLab GC software alerts the user to this possibility, based on the arbitrary requirement of $0.5 < k < 50$ for all bands in an isothermal separation. We have carried out several isothermal separations for the samples of Table I, in order to determine the accuracy of isothermal predictions based on temperature-programmed input data.

Retention time predictions. Table IV compares experimental *vs.* predicted values of retention for four isothermal runs which involve two different samples and two different columns. Two temperature-programmed runs were used as input for computer simulation (as previously). The average (absolute) error in predicted retention times t_R ranges from 1.3–4.4%, with an average value of $\pm 1.8\%$. Similarly, retention time differences (and resolution) show an average (absolute) error of 2–11%, with an average value of $\pm 8\%$. While these predicted retention times are not as accurate as those of Table II for temperature-programmed runs, they are adequate for the purposes of GC method development. A similar situation has been observed in computer simulation for HPLC, where the use of gradient runs as input data yields more accurate predictions of gradient runs than for isocratic runs.

Fig. 2 compares experimental and simulated chromatograms for the separation of the barbiturate sample at 170°C. Reasonable agreement between the two chromatograms is observed.

Bandwidth predictions. The four isothermal separations of Table IV exhibited errors in predicted bandwidths of: $+7 \pm 4\%$, $+4 \pm 2$, $+5 \pm 1\%$ and $-4 \pm 2\%$, respectively, for an overall average of $+3\%$ and an average deviation in each run of $\pm 2\%$. This excellent agreement reflects the use of isothermal values of the plate number N; see the above discussion of Table III.

Simulation of temperature-programmed runs with multiple temperature ramps

A particularly useful application of computer simulation in HPLC is for the design of complex, multi-segment gradients [1–10]. Such gradients are advantageous

[a] $N = 5.54(t_R/W_{1/2})^2$, where t_R is the retention time and $W_{1/2}$ is the bandwidth at halfheight. Extra-column band broadening effects and the dependence of N on k were corrected for by assuming an extra-column bandwidth $W_{ec} = 0.025$ min; see the discussion of ref. 21.

TABLE IV

COMPARISON OF EXPERIMENTAL *VS.* SIMULATED RETENTION TIMES FOR ISOTHERMAL SEPARATION OF DIFFERENT SAMPLES

Conditions: 1.0 ml/min flow-rate. DryLab GC used for simulations with 4 and 8°C/min runs as input.

Sample (column/range)[a]	Temperature[b] (°C)	Retention times t_R (min)			
		Expt.	Calc.	Error	
				t_R	Δt_R
Non-polar (DB-5/100–300°C)	110	3.99	4.06	0.07	−0.01
		4.11	4.17	0.06	−0.02
		5.13	5.17	0.04	0.02
		5.76	5.82	0.06	−0.06
		6.05	6.05	0.00	−0.09
		7.89	7.80	−0.09	−0.07
		8.92	8.76	−0.16	0.63
		13.50	13.97	0.47	
		Avg. error[c]		±0.12 (1.3%)	±0.13 (9.5%)
Phenols (DB-5/100–300°C)	160	2.73	2.67	−0.06	0.05
		2.86	2.85	−0.01	0.04
		3.43	3.46	0.03	−0.02
		3.36	3.37	0.01	0.05
		3.61	3.67	0.06	0.07
		4.34	4.47	0.13	0.14
		5.34	5.61	0.27	0.36
		8.12	8.75	0.63	0.12
		8.41	9.16	0.75	0.29
		11.68	12.72	1.04	0.53
		19.57	21.14	1.57	
		Avg. error		±0.41 (2.5%)	0.16 (9.5%)
Non-polar (Nukol/70–200°C)		3.50	3.56	0.06	0.06
		4.45	4.57	0.12	0.03
		6.12	6.27	0.15	−0.01
		6.37	6.51	0.14	0.18
		20.44	20.12	−0.32	
		Avg. error		0.16 (0.9%)	0.07 (1.7%)
Phenols (Nukol/120–200°C)	180	6.52	6.67	0.15	−0.11
		6.34	6.38	0.04	0.26
		9.10	9.40	0.30	0.15
		10.89	11.34	0.45	0.36
		14.57	15.38	0.81	
		Avg. error		0.35 (4.4%)	0.22 (10.9%)

[a] Supelco samples; range refers to temperature program for two experimental input runs.
[b] Isothermal run.
[c] Average absolute error.

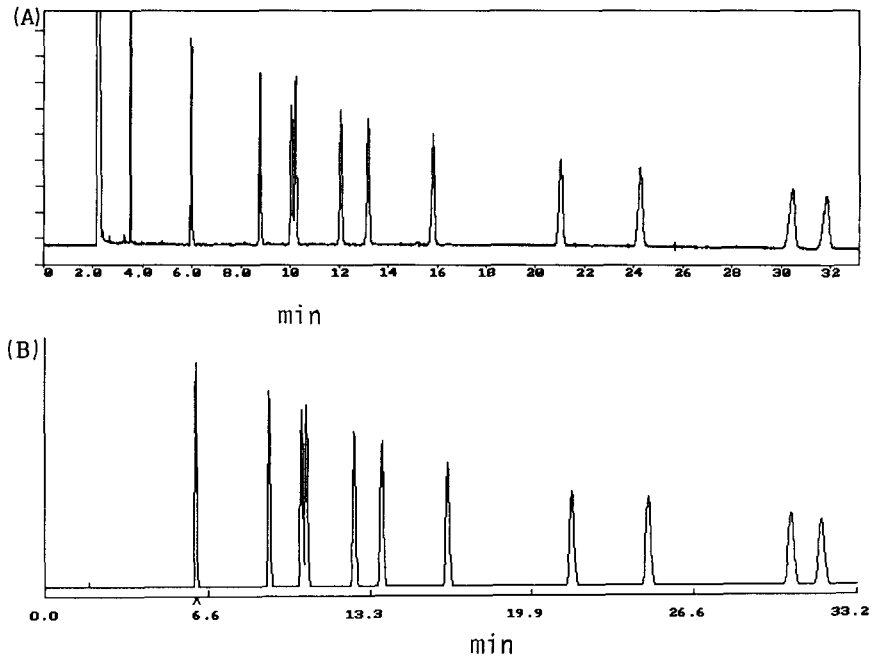

Fig. 2. Comparisons of experimental *vs.* computer-simulated (predicted) chromatograms for an isothermal separation. (A) Barbiturate sample at 170°C, DB-5 column, other conditions as in Table II; (B) same, DryLab GC chromatogram (input data: 4 and 8°C/min).

for a number of reasons. For example, steep gradients can be used to advantage for those parts of the chromatogram which have few, widely-separated peaks, in order to save run time. Likewise, flat gradients are able to increase the overall resolution of those parts of the chromatogram where the bands are more numerous and generally less well resolved. Perhaps the most rewarding application of segmented gradients, however, is the use of variations in gradient steepness at different parts of the chromatogram in order to optimize band spacing.

The similar application of segmented (multi-ramp) temperature programs in GC should prove equally useful. For this reason, several runs of this type were carried out for the samples of Table II, and the resulting chromatograms were compared with those obtained from computer simulation (starting with the usual two experimental runs as inputs to DryLab GC). In this way we were able to establish the relative accuracy of computer simulation for multi-ramp temperature programs.

Retention time predictions. Table V summarizes experimental *vs.* predicted retention times for a typical multi-ramp run: the barbiturate sample with a temperature program of 160/160/250/250°C in 0/14/18/22 min; *i.e.*, an initial isothermal hold followed by a 22.5°C/min temperature ramp followed by an isothermal hold. The retention times of the sample in this separation are distributed across the three segments of the temperature program, so that a good evaluation of the accuracy of multi-ramp predictions can be inferred from these data.

The data of Table V for this multi-ramp program exhibit larger errors in

TABLE V

COMPARISON OF EXPERIMENTAL *VS.* SIMULATED RETENTION TIMES FOR THE BARBITURATE SAMPLE AND A MULTI-RAMP TEMPERATURE PROGRAM

Conditions: DB-5 column, 1.0 ml/min flow-rate; 160/160/250/250°C in 0/14/18/22 min. DryLab GC used for simulations, with data for 100–300°C program in 25 and 50 min as input.

Retention times t_R (min)			
Expt.	Calc.	Error	
		t_R	Δt_R
7.68	8.33	0.65	0.51
11.93	13.09	1.16	−0.27
13.94	14.89	0.95	−0.11
14.21	15.05	0.84	−0.45
15.77	16.16	0.39	−0.12
16.30	16.57	0.27	−0.20
17.21	17.28	0.07	−0.23
18.32	18.16	−0.16	−0.12
18.84	18.56	−0.28	−0.12
19.60	19.20	−0.40	−0.01
19.75	19.34	−0.41	
Avg. error[a]		0.54 (4.5%)	0.22 (18%)

[a] Average absolute error.

predicted retention times (average of ±4.5%) than in the previous cases which involve either linear programs or isothermal separation (±0.2–3.5%). However, the corresponding errors in Δt_R are smaller (±0.22 min *vs.* ±0.54 min in Table V); the predicted error in resolution ±18% is marginally acceptable for method development (but note the following discussion in Table VI of very steep heating rates).

Table VI summarizes similar comparisons of experimental *vs.* predicted retention times for several multi-ramp separations. These data are arranged in order of increasing programming rate (°C/min, in parentheses, second column) for the steepest segment of the temperature program. It is seen that errors in Δt_R (and resolution) tend to increase for runs with steeper segments, as predicted for extrapolated heating rates. Thus, for segments with heating rates <20°C/min (first group of data in Table VI), the average error in Δt_R is ±5%. For segments with heating rates of 23–25°C/min (second group) or >33°C/min (third group), the average errors in Δt_R are ±9% and ±16%, respectively. These errors are still acceptable for method development purposes, but these examples do illustrate that larger errors are possible in the computer simulation of multi-ramp runs. It should also be noted that many workers avoid heating rates >20°C/min, because some GC systems are unreliable for very steep heating rates.

Fig. 3 compares experimental *vs.* predicted chromatograms for a multi-ramp separation of the herbicide sample (where the maximum programming rate is 33°C/min); reasonable agreement is observed for the two chromatograms.

Bandwidth predictions. For the runs of Table VI which do not involve

TABLE VI

SUMMARY OF COMPARISONS OF EXPERIMENTAL *VS.* PREDICTED RETENTION FOR MULTI-RAMP TEMPERATURE PROGRAMS AND DIFFERENT SAMPLES AND COLUMNS

Conditions: 1.0 ml/min; DryLab GC input runs as in Table II (4 and 8°C/min in most cases).

Sample (column)	Temperature program[a]	Errors	
		t_R	Δt_R
Non-polar (DB-5)	100/100/150/150°C (8) 0/5/11.25/16.25 min	±0.17 min (2.4%)	±0.02 min (1.9%)
Phenols (DB-5)	100/100/250/250°C (15) 0/8/18/23 min	±0.30 (2.2%)	±0.06 (4.2%)
Non-polar (Nukol)	70/110/110/200/200°C (20) 0/2/7/11.5/21.5 min	±0.11 (1.1%)	±0.09 (5.2%)
Phenols (Nukol)	120/160/160/200/200°C (20) 0/4/10/12/22 min	±0.29 (4.5%)	±0.09 (5.8%)
Sample C (Nukol)	100/180/180/200/200°C (20) 0/4/8/10/12 min	±0.34 (9.0%)	±0.07 (6.0%)
Sample D (Nukol)	50/60/60/200/200°C (23) 0/2.5/4.5/10.5/15 min	±0.29 (2.5%)	±0.14 (8.5%)
Barbiturates (DB-5)	160/160/250/250°C (23) 0/14/18/22 min	±0.54 (4.5%)	±0.22 (18%)
Rapeseed oil (DB-5)	200/200/300/300°C (25) 0/14/18/22 min	±0.42 (2.6%)	±0.11 (5.9%)
Herbicides (DB-5)	200/200/300/300°C (33) 0/5/8/12 min	±0.26 (3.6%)	±0.11 (18%)
Sample A (Nukol)	60/60/180/180°C (40) 0/2/4/5 min	±0.08 (6.0%)	±0.05 (18%)
Sample B (Nukol)	110/110/200/200°C (45) 0/4/6/8 min	±0.08 (3.8%)	±0.07 (13%)

[a] Numbers in parentheses are maximum heating rates (°C/min) for each separation.

programming rates >30°C/min, the overall (average) error in predicted bandwidths was 0 ± 15%. For temperature programs that involved steeper temperature ramps, the average error was −12 ± 21%. It appears that errors in bandwidth for multi-segment temperature programming are also somewhat greater (but acceptable) than for separations that involve linear programs.

Errors due to extrapolation and their empirical correction

The LES approximation used in DryLab GC does not seriously detract from the accuracy of predicted separations, as seen from the above discussion. However, the potential errors in computer simulation become larger, when the predicted separation is based on conditions that are far removed from those used for the initial two experimental runs used as input to DryLab GC. This is illustrated in Fig. 4 for the prediction of an isothermal GC separation. Here we assume isothermal input data, and

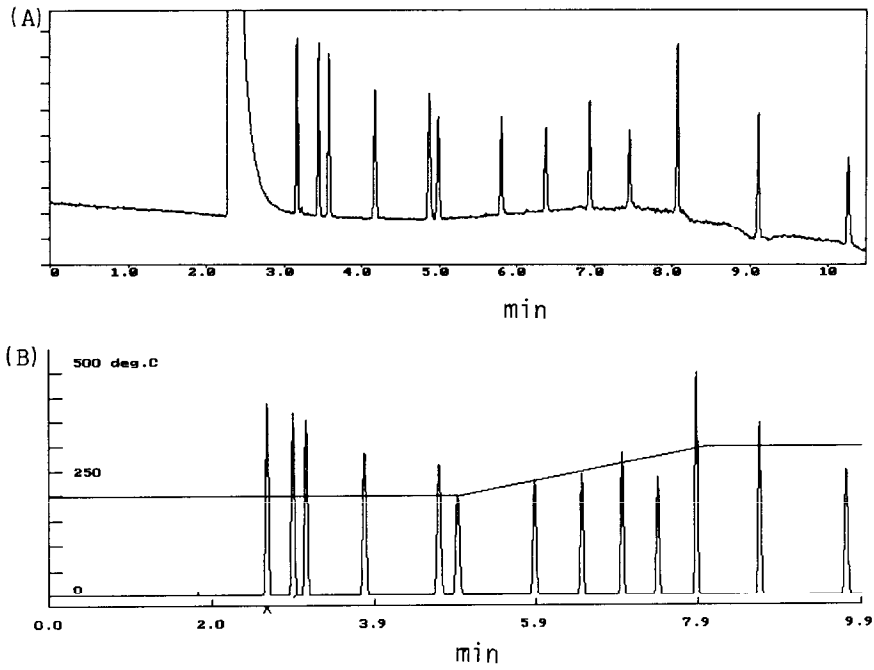

Fig. 3. Comparisons of experimental *vs.* computer-simulated (predicted) chromatograms for multi-segment temperature programming. (A) Herbicide sample, DB-5 column, 200/200/300/300°C in 0/5/8/12 min; other conditions as in Table VI; (B) same, DryLab GC chromatogram (input data: 4 and 8°C/min).

the curvature of the plots in Fig. 4 is exaggerated to better illustrate our point. A similar argument can be made for temperature-programmed runs (see discussion of Fig. 2 of following paper [23]).

Solute retention (log k) is plotted in Fig. 4A *vs.* temperature (solid curves), for two solutes that are to be separated. The input runs for computer simulation are carried out at 180 and 200°C (solid circles). The LES approximation is shown in Fig. 4A as dashed straight lines. It is assumed next that separation at 163°C is predicted by computer simulation (open circles), and the resulting chromatogram is shown in Fig. 4A. The experimental plots of log k *vs.* temperature indicate larger values of k at 163°C (solid squares) *vs.* those predicted by computer simulation (open circles). The experimental chromatogram therefore shows later elution of these two bands *vs.* the predicted (LES) separation, but little difference in resolution. This mirrors our previous comparisons of experimental *vs.* predicted separations (Tables I–VI).

Now consider a more complex case (Fig. 4B), where the two solutes to be separated show a change in band spacing as the temperature is varied. At 165°C the two bands have the same value of k, and the elution order of the two bands at lower temperatures (< 165°C) is reversed when the temperature is raised above 165°C. Again we assume that the initial experimental runs for input to computer simulation are 180 and 200°C (solid circles). Next assume that we predict the separation that will occur at 165°C (open circles of Fig. 4B); the predicted chromatogram shows baseline resolution of our two solutes. However, the experimental chromatogram exhibits complete

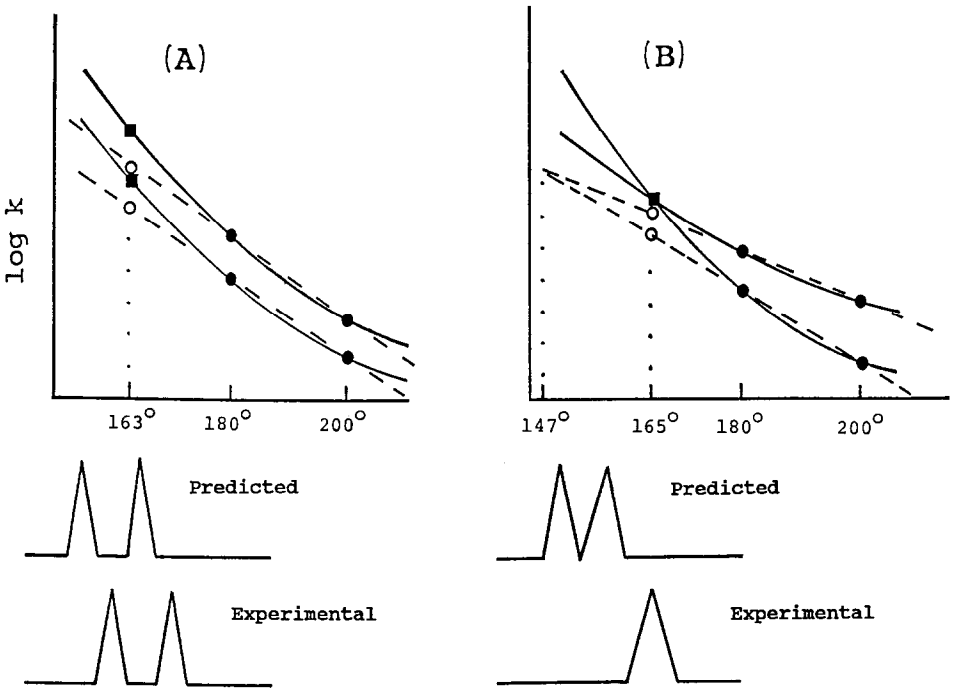

Fig. 4. Hypothetical (exaggerated) examples of errors in isothermal computer simulation due to use of the LES approximation. (A) Two solutes whose band spacing does not change with temperature; (B) two solutes whose band spacing changes with temperature. ● = Experimental input data for computer simulation; ○ = predictions of separation at a third temperature; ■ = experimental separation at a third temperature. See text for details.

overlap of the two bands, because of the error introduced by extrapolation beyond our starting data (closed circles).

The resulting error in computer simulation (Fig. 4B) is seen to be more serious than in the preceding example (Fig. 4A). To generalize, we can say that (i) extrapolation of experimental data as in Fig. 4 leads to greater possible errors, (ii) these errors can be magnified for the case where two bands exhibit large changes in band spacing as temperature is varied, and (iii) the practical effect of these errors is more serious for bands that are less well resolved. That is, if the average error in resolution is 10%, and if the average resolution (entire chromatogram) is $R_s = 5$, then the average error in R_s is ± 0.5 units, which does not appear very serious. If the predicted resolution for a critical (least resolved) band-pair is only 1.5, however, we might actually find R_s equal to 1.0 (or less) for the corresponding experimental run.

The errors illustrated in Fig. 4B can be corrected for (to a considerable extent) as follows. Note that the complete overlap of the two bands in Fig. 4B occurs at 165°C, whereas computer simulation (based on the LES approximation) predicts band overlap at 147°C. That is, the correct separation is predicted, but for the wrong temperature. The form of the LES approximation (see discussion of refs. 24, 25) is such as to make this generally true. That is, if an optimized separation is predicted for some

temperature T, and the experimental separation deviates significantly from that predicted, then a (usually) modest adjustment in the temperature should yield an experimental chromatogram that agrees with the separation predicted for temperature T. These small adjustments (in the right direction) are easily made, because of the predictable change in retention with temperature.

A similar situation exists for temperature-programmed runs. There small adjustments in heating rate may be required to obtain a good match between experimental and predicted chromatograms. With a little experience, most chromatographers should be able to carry out these "fine-tuning" adjustments with only one or two extra runs —in the occasional case where significant errors as in Fig. 4B are encountered.

CONCLUSIONS

A personal computer program (DryLab GC) is described for carrying out computer simulation as a means of facilitating method development for either isothermal or temperature-programmed GC. Based on two initial experimental runs as input to the computer, it is possible to predict separation as a function of experimental conditions: initial and final temperatures, temperature programming rate, multi-segment temperature programs, etc. Retention times, bandwidths and resolution can be displayed as tables, graphs or simulated chromatograms.

The accuracy of this program for predicting retention time and bandwidth (or resolution) was tested for several samples and two different columns, using variously (i) linear temperature programming, (ii) isothermal separation and (iii) multi-ramp (non-linear) temperature programming. On the basis of these comparisons of experiment and theory, it is concluded that the DryLab GC software is adequately reliable for method development. Retention times were predicted with an average accuracy of 1–2%, bandwidths are predicted with an average accuracy of about $\pm 5\%$, and resolution is predicted with an accuracy of about $\pm 10\%$.

SYMBOLS

All symbols for the present and following two papers [22,27].

a, b	constants in eqn. 2 of Part II
A, B	constants in eqn. 1 of Part I
b	temperature-program steepness parameter (eqn. 4, Part II); $b = t_0 r S$
GC	gas chromatography
i, j	solutes of Fig. 1, Part II
k	solute capacity factor (GC)
k'	solute capacity factor (HPLC)
$\underline{k}$	effective value of k during programmed-temperature separation; equal to value of k for band when it reaches the midpoint of the column (eqn. 4, Part II)
k_a, k_z	values of k for first and last bands in the chromatogram (eqn. 4, Part II)
k_e	value of k at elution
k_0	value of k for a solute at the beginning of a temperature programmed separation

LES	linear elution strength
LSS	linear solvent strength
N	column plate number
r	heating rate (°C/min)
RRM	relative resolution map
R_s	resolution of two adjacent bands
S	solute parameter of eqn. 5 (Part I)
S_i, S_j	values of S for solutes i and j (Fig. 1 of Part II)
t	time after sample injection and the beginning of the temperature program
t_0	column dead-time (min)
t_P	time of (linear) temperature program
t_R	retention time for temperature-programmed GC run
T	column temperature; usually in °C, except °K in eqn. 1
T_f	final temperature in temperature-programmed GC
T_0	initial temperature in temperature-programmed GC
W	baseline bandwidth (min)
W_{ec}	contribution to bandwidth from extra-column effect (min); see eqn. 14 of ref. 21
W_{expt}, W_{calc}	experimental and predicted values of W
α	separation factor
β	column phase ratio
$\Delta H_{v,i}$	enthalpy of retention (eqns. 1, 2 of Part II)
ΔR_s	a necessary change in R_s for a given band pair, in order to result in their adequate separation
ΔS	difference in S values for two adjacent bands (eqn. 4, Part II)
$\Delta S_{v,i}$	entropy of retention (eqns. 1, 2 of Part II)
Δt_R	difference in t_R values for two adjacent bands
φ	volume fraction of strong solvent B in binary mobile phase A/B (HPLC)
%B	% (v/v) of strong solvent B in binary mobile phase A/B (HPLC)

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