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Computer spreadsheet calculation of the optimum temperature and column lengths for serially coupled capillary gas chromatographic columns

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ABSTRACT

The application of computer spreadsheet calculations to the optimization of complex chromatographic separations on serially coupled capillary columns has been demonstrated. The equations used require a minimum of measured data for the prediction of retention times in the coupled systems. The calculations make no assumptions about equivalence of column diameters, and they can be easily extended to more than two columns, if desired. Temperature effects on flow-rate and on analyte capacity factors (k') are also taken into account. The spreadsheet allows convenient calculation of the minimum resolution at different combinations of column lengths and temperature. The method is shown to give reasonable agreement with measured retention times and excellent agreement with measured k' values.

INTRODUCTION

Much work has been published recently on the subject of optimizing capillary gas chromatographic (GC) separations through the use of serially coupled columns. A number of different variables have been manipulated to change the selectivity of the coupled system. Mathematical methods have been used to predict the temperature and pressure for an optimal separation for two columns connected directly [1–3] or with independent flow control [4–7]. Another potential mode of selectivity optimization is varying the lengths of the coupled columns. This method is practical only if calculations can give accurate predictions of retention times in the coupled system. Among the most active workers in this area are Buys and Smuts [8,9] and Purnell and co-workers [10–12], who emphasized the necessity of accounting for carrier-gas compressibility. These methods have been quite successful in predicting the retention times on coupled columns from the data on separate columns. Purnell determines the resistance to gas flow in a column by plotting the dead time at several different inlet pressures against a function of the pressure. This resistance factor is then used in calculation of the column dead-volume time at any specified inlet pressure. In the case of serially coupled columns, the resistance factors are used in calculation of the junction pressure for the two columns, from which further calculations give the ratio of dead-volume times on the front and back columns. The latter ratio is used in predicting

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the capacity factors (k') on the coupled system. The method may be applied to either packed or capillary columns, and it does not require that the columns to be connected have the same inside diameter.

A similar calculation has been employed successfully by Villalobos [13,14]. One difference in this method is that the retention time and resolution are calculated for the junction of the column as well as at the outlet. The method assumes that both columns involved have the same internal diameter, but this has been avoided by modifying the column lengths used in the calculation by a correction factor involving the fourth power of the ratio of column diameters.

We have been working independently on the problem of calculating the optimum lengths of dissimilar capillary columns which can be coupled to effect a desired separation. In the interest of simplicity of instrumentation, we have assumed a common mass flow-rate and a common temperature for the columns. While the calculations used are very similar to those of Purnell, they are limited to open-tubular columns, dealing primarily with the dimensions of the columns rather than with measured flow resistance. The algorithm begins with the specification of a desired volume flow-rate at the column outlet and predicts the head pressure and dead-volume time for each of the coupled columns. While in principle no chromatographic measurements are necessary to make these calculations, a single determination of flow-rate and dead-volume time for the separate columns at a known inlet pressure serves to determine the column I.D. more precisely and to increase the accuracy of the predictions. Once the dead-volume times are calculated, the retention times are calculated from known k' values on the individual columns as usual, except that the variation of k' with temperature is also taken into account. This paper shows how these equations can be used with a standard computer spreadsheet program to optimize separations in a coupled capillary system, with respect to both column length and temperature.

THEORY

The laminar flow occurring in a capillary GC column under typical operating conditions is described by Poiseuille's equation (see ref. 15)

$$-\frac{\mathrm{d}P}{\mathrm{d}x} = \frac{32\mu U}{D^2} \tag{1}$$

where P represents the pressure due to flow resistance, μ is the gas viscosity, U is the average velocity across the column diameter, x is the distance measured from the column head and D is the column inner diameter. Since the mass flow is constant and since for an ideal gas the volume is inversely proportional to the pressure, it follows that

$$U = \frac{P_o U_o}{P} \tag{2}$$

where P_o and U_o are the pressure and the flow velocity at the column outlet. Since it is the volume flow-rate (f) which is usually measured at the column outlet, U_o is conveniently replaced by

$$U_o = \frac{4f}{\pi D^2} \tag{3}$$

Combining eqns. 2 and 3 with eqn. 1 gives

$$-\frac{\mathrm{d}P}{\mathrm{d}x} = \frac{128\mu f P_o}{\pi D^4 P} \tag{4}$$

Since the viscosity of an ideal gas is invariant with pressure, μ can be treated as a constant in eqn. 4 for any given temperature. Integration of eqn. 4 for a column of length L operating at inlet pressure P_i then gives Poiseuille's formula (see ref. 16) for the relationship between pressure and flow-rate

$$f = \frac{(P_i^2 - P_o^2)\pi D^4}{256\mu P_o L} \tag{5}$$

Since all of the parameters on the right-hand side of eqn. 5 can be measured, the flow-rate for a capillary column under any combination of temperature, inlet pressure and outlet pressure can in principle be predicted. In practice, the fourth-power dependence on column diameter means that the uncertainty in the diameter usually leads to significant errors in the predicted flow-rate. However, a single measurement of the flow-rate under known conditions of temperature and pressure gives in effect a calibration of the column diameter, so that the flow-rate may be accurately predicted for other conditions. Alternatively, solution of eqn. 5 for P_i allows prediction of the inlet pressure required for a desired flow-rate.

Integration of eqn. 4 for an arbitrary fraction of the column length leads to an expression for the pressure as a function of the distance from the column head

$$P = \sqrt{P_i^2 - \frac{x}{L}(P_i^2 - P_o^2)} \tag{6}$$

The dead-volume time for a column operating under steady-state conditions can be calculated from a consideration of the volume V which passes any point in the interior of the column in a unit of time. Keeping in mind the inverse relationship between volume and pressure, the following expression relates the volume flow at any point within the column to the flow-rate measured at the outlet and to the linear velocity of the gas

$$\frac{\mathrm{d}V}{\mathrm{d}t} = \frac{\pi D^2}{4}\frac{\mathrm{d}x}{\mathrm{d}t} = f\frac{P_o}{P} \tag{7}$$

where t is the time. Combining eqns. 6 and 7 then gives

$$\frac{dx}{dt} = \frac{4f}{\pi D^2} \frac{P_o}{\sqrt{P_i^2 - \frac{x}{L}(P_i^2 - P_o^2)}} \tag{8}$$

Solution of eqn. 8 for t gives the time required for the carrier gas to advance a distance x down the column

$$t = \frac{\pi D^2 L}{6fP_o(P_i^2 - P_o^2)} \left\{ P_i^3 - \left[P_i^2 - \frac{x}{L}(P_i^2 - P_o^2) \right]^{3/2} \right\} \tag{9}$$

When the variable x in eqn. 9 equals L, t equals the dead time t_o, and the expression reduces to the equivalent of Purnell's equation (see eqn. 5 in ref. 11) for column dead time

$$t_o = \frac{\pi D^2 L}{6f} \left[\frac{P_i^3 - P_o^3}{P_o(P_i^2 - P_o^2)} \right] \tag{10}$$

Eqn. 10 can be used to make a second determination of the column diameter if the outlet flow-rate and dead-volume time are measured at known inlet and outlet pressures. It is equivalent to the diameter determination given by eqn. 5, except that the viscocity of the carrier gas does not have to be known. We have found that the most consistent measurements of D are obtained from eqn. 10 after determination of the dead time according to the method of Ambrus [17]. The value of D thus obtained can be used to calculate t_o for any desired length of the column at any desired flow-rate.

The equations described provide the means to calculate retention times on any combination of serially coupled column portions. The details of the method are as follows:

(1) Measure the length of each column to be used.

(2) *With each column individually*, connect the head to an injector operating at a known pressure, and after equilibration measure the flow-rate at the outlet, the column temperature, the laboratory temperature, and the barometric pressure.

(3) Connect the column to the detector and inject a test mixture of hydrocarbons for determination of dead time by the method of Ambrus.

(4) After correcting the measured flow-rate to the column temperature, substitute the measured parameters into eqn. 10 to determine the diameter of each column.

(5) Choose a flow-rate for the coupled system and specify the length of the tail section. Use eqn. 5 to calculate P_i for that section.

(6) Using the pressure calculated in step 5 as P_o, use eqn. 5 to calculate P_i for the next column in the series. For this calculation, the volume flow-rate must be corrected to the new value of P_o, and the values of D and L which apply to this column segment must be used.

(7) Repeat step 6 for any more column segments to be connected to the head end of the series.

(8) Use eqn. 10 to calculate t_o for each column in the series.

(9) Calculate the retention time for each analyte on each segment from the corresponding k' value for that segment by the usual equation

$$t_n = t_o(k'_n + 1) \tag{11}$$

The total retention time for component n is then given by the sum of the retention times on the connected columns. It should be noted that the use of eqn. 11 assumes that each original column is uniform, so that the k' values for any segment will be the same as those measured on the entire column.

The calculation of retention times allows a simple form of chromatographic optimization by maximization of the spacing between adjacent peaks. A more systematic approach is the use of window diagrams [18] applied to the α values of adjacent peaks ($\alpha = k'_2/k'_1$). The window-diagram approach may be very conveniently applied with a spreadsheet. However, since it is really resolution which is being optimized, we chose to derive diagrams for the resolution of neighboring peak pairs. This requires a knowledge of the theoretical plate number for each component to be analyzed. While prediction of theoretical plate numbers has also been accomplished [19], it would make the spreadsheet much more complex. We chose the simplifying assumption that a given column would give the same inherent plate number for all analytes. The lower apparent plate number for peaks of lower retention time was assumed to be due to extra-column band broadening. It has been shown that the extra-column variance may be determined by a plot of the observed peak variance σ^2 *versus* t^2, where the slope of the plot is the inverse of the inherent column theoretical plate number N and the intercept is the extra-column variance σ_e^2 [20]. The apparent plate number N_a for a given peak is then given by

$$N_a = \frac{N}{N\sigma_e^2/t^2 + 1} \tag{12}$$

In our initial work, the assumption was also made that if a column of length L had N theoretical plates, a fragment of length l taken from this column would have N_l plates, according to the following formula:

$$N_l = \frac{l}{L} N \tag{13}$$

The inherent plate number for the combined column was then given by the sum of N_l for the individual columns. It has since been shown by Guiochon and Gutierrez [19] that the correct form is given by

$$H_c L_c = \sum_{i=1}^{n} H_i L_i \tag{14}$$

where H represents the height equivalent of a theoretical plate, L the column length, subscript c the total coupled system, and subscript i the individual column segments which are connected. When, as is approximately true in the present work, the height equivalent of a theoretical plate for the column segments is the same, eqns. 13 and 14 lead to the same results. In the general case, the parameters H_c and L_c from eqn. 14 may be used to calculate the inherent plate number N_c for the coupled system, and the latter may be used in eqn. 12 to predict the apparent plate number as a function of retention time. Resolution is conveniently calculated by the usual expression

$$R_s = \frac{t_2 - t_1}{2(\sigma_2 + \sigma_1)} \tag{15}$$

While this treatment ignores the relationship between plate height and carrier velocity, this factor can be minimized by adjusting the inlet pressure to keep the calculated average linear velocity near the optimum value.

The other variable which may be conveniently controlled to optimize the coupled-column separation is temperature. It has already been shown (eqn. 5) that the only effect of temperature on the flow-rate for a capillary column lies in the temperature dependence of the carrier-gas viscosity. This is expressed in ref. 3 by the relationship

$$\mu = aT^{\alpha} \tag{16}$$

where a is a constant and T is the temperature. While the tabulated data for helium viscosity may be fitted to eqn. 16, a simple second-order regression gives a closer fit in the temperature range above 0°C. Expressing the viscosity as a function of temperature allows the relationship between pressure, flow-rate, and dead time to be calculated for any temperature. What remains for calculation of the selectivity is an expression for the relationship between k' and temperature. For the latter, we used the approach of measuring k' at three different temperatures and regressing log k' against the reciprocal of the absolute temperature. The regression parameters were used in the spreadsheet to express all of the k' values as a function of temperature. The chromatogram could thus be simulated for any combination of columns at any desired flow-rate and temperature.

EXPERIMENTAL

Chromatography was carried out on a Hewlett-Packard 5880 gas chromatograph with a split injector containing a packed insert. The insert contained about 1/4 in. of 2% SE-30 on 100–120-mesh Gas Chrom Q. The split flow was 50 ml/min, with column flow controlled by the constant head pressure. Helium was used as the carrier gas, and a flame-ionization detector was used.

Two fused-silica columns were used in this work, nominally 30 m × 0.32 mm I.D. The first contained a bonded methyl silicone phase (DB-1, J&W Scientific) of 1 μm thickness, while the second contained a bonded Carbowax phase (DBWax, J&W) of 0.5 μm thickness. The column length was determined by careful measurement of two unwrapped coils and by counting of the total number of coils.

The GC oven temperature was calibrated against a laboratory thermometer. The column flow-rate was determined by attachment of the injector end as usual, while the detector end was extended through the detector jet to the top of the chromatograph. It was connected through a septum to a soap-film flow meter, which was previously filled with helium to prevent errors due to differential diffusion of helium across the soap film. After thermal equilibration of the column at a known temperature, the flow-rate was measured carefully, and the column head pressure, barometric pressure and laboratory temperature were recorded. The measured flow-rate was corrected for the vapor pressure of water and was then corrected to the column temperature.

After connection of the tail of the column to the detector, the dead time was determined by injection of a mixture of *n*-alkanes ranging from C_5 to C_{12}. A regression of the retention times according to the method of Ambrus was used for determination of t_0. The measured pressures, flow-rate, column length and dead time were used in eqn. 10 to determine the actual column diameter.

The retention times of the solvents to be separated were determined by injection of a few microliters into a septum vial. After the liquid had evaporated, 5 μl of vapor was injected onto the chromatograph. Vapor injection was used to avoid retention time shifts that might result from overloading of the column.

The resolution calculations were carried out with Lotus 1-2-3, version 2.01, running on an AT&T PC 6300. One spreadsheet was used to calculate the k' values and the resolution of neighboring peaks for varying lengths of connected columns. Another was used to generate a simulated chromatogram, assuming Gaussian peaks and using peak variances calculated as described in the Theory section.

RESULTS

The measurements used for initial characterization of the two columns are summarized in Table I. It may be seen that the column diameters calculated according to eqn. 10 are in good agreement with the nominal values. The measured k' values on both columns at three temperatures for the set of 34 solvents studied are given in Table II. With these constants, retention times were calculated for serially connected combinations at 50°C, starting with the full DBWax column at the head and increasing the DB-1 column length at the tail from zero to the full 30 meters. The DBWax column length was then reduced to zero. The results are shown in Fig. 1, with the same calculation assuming the DB-1 column at the head shown in Fig. 2. While dramatic differences in relative retention times are predicted, it is clear that there is no combination which gives good spacing for all 34 component peaks. Window diagrams

TABLE I

MEASUREMENTS USED TO CHARACTERIZE THE INDIVIDUAL COLUMNS

Column 1 = 30 m of bonded methylsilicone; column 2 = 30 m of bonded Carbowax.

Column	P_i (p.s.i.g.)[a]	P_o (p.s.i.g.)[a]	L (m)	T_{col} (°C)	T_{room} (°C)	F (ml/min)[b]	t_o (min)	D_{calc} (mm)
1	26.4	14.4	31.43	50	23	2.326	1.48	0.323
2	26.2	14.2	31.94	50	23	2.237	1.59	0.325

[a] Absolute pressures.

[b] Measured at P_o and room temperature.

TABLE II

EXPERIMENTAL k' VALUES FOR TEST SOLVENTS ON THE INDIVIDUAL COLUMNS

Measurements were made at P_i = 12 p.s.i.g.

Peak No.[a]	Name	T = 40°C		T = 50°C		T = 60°C	
		$k'_{\text{col 1}}$	$k'_{\text{col 2}}$	$k'_{\text{col 1}}$	$k'_{\text{col 2}}$	$k'_{\text{col 1}}$	$k'_{\text{col 2}}$
1	Methanol	0.364	1.718	0.274	1.156	0.196	0.811
2	Ethanol	0.460	2.372	0.362	1.560	0.275	1.066
3	Acetonitrile	0.508	3.835	0.423	2.565	0.301	1.754
4	Acetone	0.542	0.864	0.450	0.609	0.333	0.464
5	Isopropanol	0.623	2.262	0.483	1.473	0.373	1.010
	n-Pentane	0.726	0.068	0.571	0.054	0.438	0.048
6	*tert.*-Butanol	0.787	1.803	0.612	1.183	0.464	0.811
	Methyl acetate	0.869	0.968	0.652	0.672	0.497	0.501
7	Methylene chloride	0.842	2.197	0.659	1.461	0.503	1.016
8	*n*-Propanol	1.135	5.447	0.827	3.426	0.614	2.244
9	Methyl ethyl ketone	1.483	1.712	1.090	1.177	0.810	0.849
10	Ethyl acetate	1.831	1.550	1.320	1.050	0.954	0.756
11	*n*-Hexane	1.824	0.146	1.347	0.117	0.987	0.092
12	Chloroform	1.831	4.534	1.360	2.931	1.000	1.965
13	Tetrahydrofuran	2.076	1.265	1.535	0.899	1.124	0.675
14	Ethylene dichloride	2.316	6.443	1.697	4.085	1.235	2.710
	n-Butyl chloride	2.574	1.052	1.866	0.741	1.353	0.551
15	*n*-Butanol	2.888	12.735	2.021	7.675	1.425	4.819
	Benzene	2.888	2.359	2.095	1.632	1.516	1.159
16	Cyclohexane	3.141	0.417	2.277	0.306	1.647	0.253
	Dioxane	4.027	6.061	2.837	3.959	2.000	2.710
17	*n*-Heptane	4.593	0.320	3.194	0.243	2.222	0.191
18	Methyl isobutyl ketone	5.528	3.971	3.788	2.621	2.614	1.804
	Pyridine	5.528	15.414	3.788	9.680	2.614	6.239
19	Toluene	7.404	5.065	5.076	3.353	3.497	2.313
20	*n*-Butyl acetate	11.374	6.851	7.375	4.338	4.853	2.865
	n-Octane	11.558	0.722	7.577	0.514	5.020	0.253
21	Ethyl benzene	17.117	9.861	11.178	6.319	7.379	4.205
22	*m*-Xylene	18.502	10.922	12.021	6.968	7.895	4.627
23	*p*-Xylene	18.645	10.411	12.082	6.659	7.941	4.422
24	*o*-Xylene	22.397	15.039	14.408	9.473	9.405	6.196
	n-Nonane	29.082	1.615	17.955	1.107	11.320	0.787
	n-Decane	73.181	3.615	42.554	2.356	25.536	1.605
	n-Undecane	184.150	8.074	100.854	5.025	57.601	3.268

[a] Peak numbering for subset used in separation optimization.

confirmed this observation, with the minimum α value never exceeding 1.01. Variations in the temperature changed the selectivity somewhat, but no conditions could be found for simultaneous separation of all components with these columns.

Because of the complexity of the separation, we decided to limit the optimization to a subset of the solvents which were of most interest in our laboratoy. The components selected are those which are numbered in Table II. The plot of σ^2 *vs.* t^2 for these components on the DB-1 column gave a straight line, with the intercept corresponding to σ_{external} = 0.018 min and the slope to a column plate number of

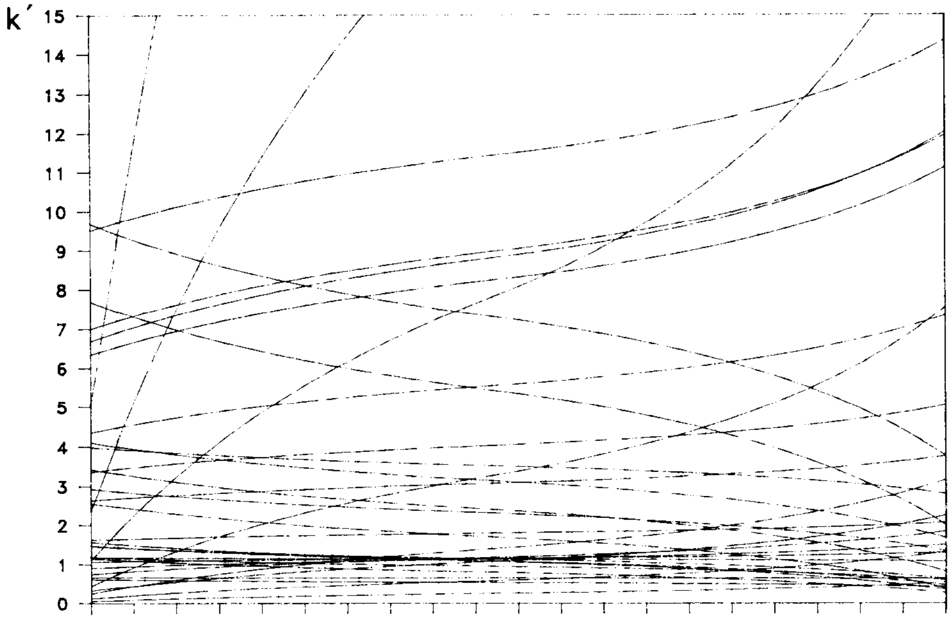


Fig. 1. Calculated k' values for 34 solvents on column 2 coupled ahead of column 1. The left side of the graph represents column 2 alone, with the length of column 1 increasing in the x-direction to its full length at the midpoint of the x-axis. Column 2 then decreases until the right side represents column 1 alone.

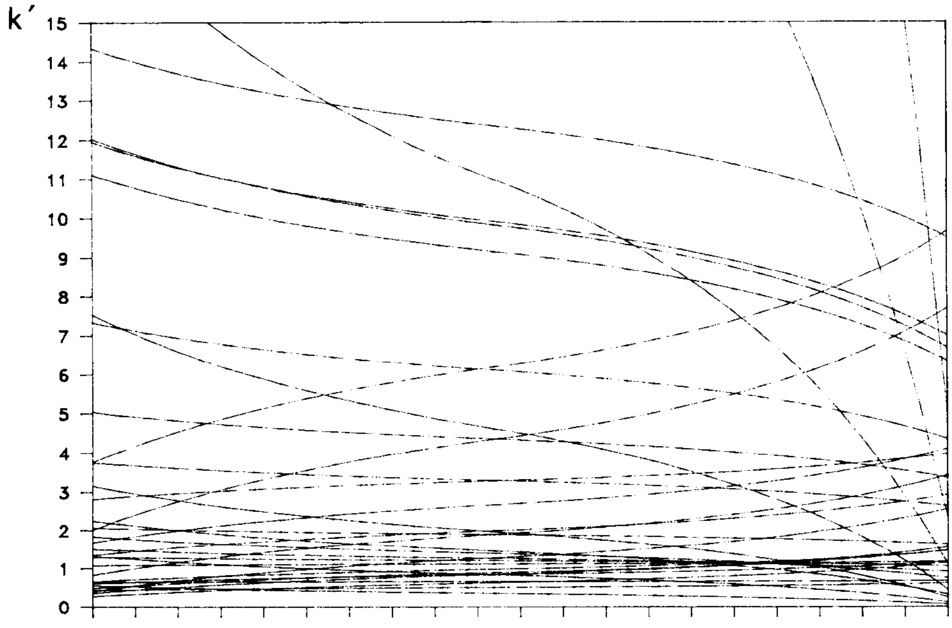


Fig. 2. Calculated k' values for 34 solvents on column 1 coupled ahead of column 2. The plot is the same as Fig. 1 except for the reversal of column order.

70 000. The corresponding plot for the DBWax column gave a poorer fit, primarily due to significant broadening of the peaks for some of the more-polar components. The broadening was accompanied by increased tailing, indicative of residual unblocked hydrogen-bonding sites on the DBWax column. The assumption of a common plate number for all components is thus not really true for this column, and the fit to a straight line was poorer. However, the linear regression gave the same σ_{external} of 0.018 min, and the column plate number was 55 000. With these constants, the spreadsheet was programmed to calculate the retention times for each set of conditions, to sort the peaks in order of increasing retention time and to calculate the resolution of all adjacent peaks. The minimum resolution was tabulated for each case. A plot of this value *versus* column length gave a window diagram which suggested areas where calculations should be made at shorter intervals.

In order to test the validity of the theory, we first calculated the retention times expected for connection of the complete DBWax column ahead of the DB-1 column. For a temperature of 50°C and an outlet flow-rate of 3 ml/min, the method predicted a column head pressure of 24.2 p.s.i.g. The comparison of the retention times found under these conditions with those predicted is shown in Table III. While the peak order

TABLE III

COMPARISON OF CALCULATED AND EXPERIMENTAL RETENTION TIMES FOR SOLVENT SUBSET ON THE SERIALLY CONNECTED FULL COLUMNS

Values were measured at 50°C; P_i = 24.2 p.s.i.g.; retention times in min; number code for solvents as in Table II.

Solvent	t_{calc}	t_{exp}	k'_{calc}	k'_{exp}
1	5.70	5.22	0.81	0.79
2	6.58	6.09	1.09	1.08
3	8.54	7.96	1.71	1.72
4	4.87	4.53	0.55	0.55
5	6.58	6.09	1.09	1.08
6	6.17	5.74	0.96	0.96
7	6.77	6.26	1.15	1.14
8	10.75	9.94	2.41	2.40
9	6.76	6.26	1.15	1.14
10	6.81	6.26	1.16	1.14
11	5.02	4.66	0.59	0.59
12	10.44	9.67	2.31	2.31
13	6.77	6.26	1.15	1.14
14	13.09	12.13	3.16	3.15
15	20.36	18.89	5.46	5.46
16	6.56	6.09	1.08	1.08
17	7.55	7.01	1.40	1.40
18	12.85	11.94	3.08	3.08
19	15.84	14.72	4.03	4.03
20	20.57	19.14	5.53	5.54
21	29.05	27.05	8.22	8.25
22	31.34	29.18	8.95	8.98
23	30.83	28.75	8.79	8.83
24	39.09	36.37	11.41	11.43

agrees very well with that predicted, the observed times are all slightly shorter than expected. A possible explanation for the discrepancy is uncertainty of the column-head pressure, since the gauge was not calibrated against any outside standard. In addition, variations in the outlet (atmospheric) pressure on different days were not included in the calculations. Nevertheless, the last two columns of Table III show that the agreement between predicted and experimental k' values is very good, with an average deviation (root mean square, r.m.s.) of 0.8%.

With the success of the prediction for connection of the whole columns, we returned to the question of optimizing the separation. Calculations were made with variations in the column order, the lengths and the temperature. In the length calculations, the full length of one column was assumed while the other was varied. The temperature was varied over the range which gave a reasonable predicted total run time. Even with the analysis limited to the subset of solvents, complete resolution could not be obtained for all components simultaneously. The best conditions which could be found (17.8 m of DBWax column attached at the head of the 31.4-m DB-1 column, temperature 48°C) gave a predicted minimum resolution of 0.40. This occurred between the peaks for *tert.*-butyl alcohol and ethanol, with nearly the same resolution between ethanol and isopropyl alcohol.

To test the prediction, we cut a 17.8-m length from the DBWax column and attached it to the front of the DB-1 column. With a pressure of 17 p.s.i.g. and a column temperature of 48°C, the retention times found are given in Table IV, along with the predicted values. While again the observed times are systematically shorter than those predicted, the general agreement is good except in three areas. The ethanol–*tert.*-butanol (peaks 2 and 6), *m*-xylene–*p*-xylene (peaks 22 and 23) and acetonitrile–cyclohexane (peaks 3 and 16) pairs were found to be coincident. Since especially the latter peak pair seemed outside the expected range of variation, we looked for a cause. The compounds were chromatographed on the 17.8-m section of DBWax column alone, and the k' values were found to be larger than those measured on the original column by an average factor of 1.04. This is evidence that the initial assumption of invariant k' as a function of column length is not completely valid. Either a variation in stationary phase thickness along the length of the column or a variation in column diameter could give rise to this result. We have no evidence as to which factor is most significant, but if the variation is in column diameter, the derivation of eqns. 5 and 10 is not valid, and additional deviation between predicted and experimental chromatograms would be expected.

The revised k' values measured on the 17.8-m section of DBWax column were used in a repeat of the calculations. Essentially the same optimum separation was predicted, but with a DBWax section of 17.2 m. The DBWax section was therefore shortened to 17.2 m and re-attached to the DB-1 column. The retention times and k' values for a run with this combination at 48°C and 17.1 p.s.i.g. are given in Table V, compared with the predicted values. Once again, the measured retention times are systematically shorter than those predicted, but the k' values are in generally good agreement. The acetonitrile–cyclohexane pair now separate as predicted. The ethanol–*tert.*-butanol pair still elute as a single peak, although the k' values measured separately are slightly different. The lack of resolution may be partly due to the lower plate number mentioned earlier for these polar compounds on the DBWax column.

The spreadsheet was also used to generate a simulated chromatogram for the

TABLE IV

COMPARISON OF CALCULATED AND EXPERIMENTAL RETENTION TIMES FOR SOLVENT SUBSET AT THE PREDICTED OPTIMUM SERIAL COMBINATION

17.8 m of column 2 at head, 31.4 m of column 1 at tail; values were measured at 48°C; P_i = 17.2 p.s.i.g.; retention times in min; number code for solvents as in Table II.

Solvent	t_{calc}	t_{exp}	k'_{calc}	k'_{exp}
1	4.48	4.23	0.71	0.70
2	5.12	4.83	0.96	0.94
3	6.43	6.27	1.46	1.52
4	4.04	3.89	0.54	0.56
5	5.21	4.97	0.99	1.00
6	5.03	4.83	0.92	0.94
7	5.45	5.20	1.08	1.09
8	8.24	7.86	2.15	2.16
9	5.77	5.51	1.21	1.21
10	5.98	5.73	1.29	1.30
11	4.83	4.62	0.85	0.86
12	8.39	8.02	2.21	2.22
13	6.09	5.86	1.33	1.36
14	10.40	9.95	2.98	3.00
15	15.56	14.90	4.95	4.99
16	6.52	6.27	1.50	1.52
17	7.90	7.58	2.02	2.05
18	11.84	11.35	3.53	3.56
19	14.77	14.16	4.65	4.69
20	19.79	18.99	6.57	6.63
21	28.27	27.13	9.81	9.90
22	30.45	29.21	10.65	10.74
23	30.17	29.21	10.54	10.74
24	37.47	35.93	13.33	13.44

predicted separation, assuming Gaussian peaks and the plate numbers calculated above. The simulated and actual chromatograms are compared in Fig. 3. Except for the peak tailing for some components in the actual chromatogram, the two compare very well. The plot of σ^2 *vs.* t^2 for the coupled columns gave a scatter comparable to that for the DBWax column alone, with an intercept corresponding to $\sigma_{external}$ of 0.025 min and a slope corresponding to 102 000 theoretical plates. The slight increase of $\sigma_{external}$ (0.4 s) may not be significant, but it could be evidence for extra variance caused by coupling of the columns. The plate number agrees well with the 99 600 predicted by the calculation, with the major outliers being acetonitrile and the alcohols as for the DBWax column alone.

CONCLUSIONS

Standard spreadsheet programs running on a personal computer can be used to make very successful predictions of the chromatograms which will result from the serial coupling of capillary columns. A simple measurement of column dead time and of k' values for the analytes on each column is sufficient for the prediction of the separation for any desired combination of lengths. Determination of k' values at three

TABLE V

COMPARISON OF CALCULATED AND EXPERIMENTAL RETENTION TIMES FOR SOLVENT SUBSET AT THE REVISED PREDICTED OPTIMUM COMBINATION

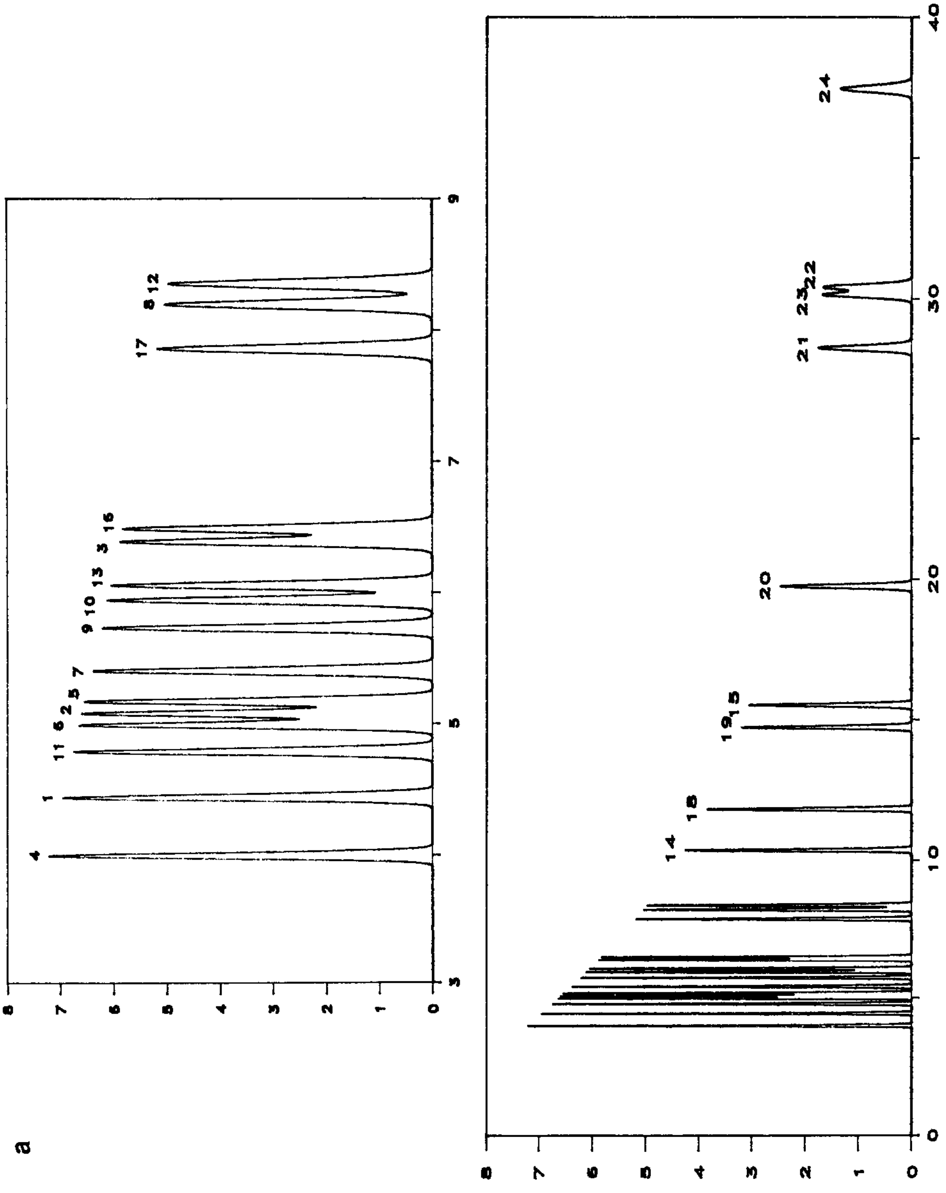
17.2 m of column 2 at head, 31.4 m of column 1 at tail; values were measured at 48°C; P_i = 17.2 p.s.i.g.; retention times in min; number code for solvents as in Table II.

Solvent	t_{calc}	t_{exp}	k'_{calc}	k'_{exp}
1	4.44	4.11	0.72	0.68
2	5.08	4.76	0.97	0.95
3	6.39	6.03	1.48	1.47
4	4.00	3.82	0.55	0.56
5	5.17	4.86	1.01	0.99
6	4.99	4.73	0.94	0.93
7	5.41	5.10	1.10	1.09
8	8.20	7.64	2.19	2.12
9	5.73	5.41	1.23	1.21
10	5.94	5.63	1.31	1.30
11	4.79	4.57	0.86	0.87
12	8.36	7.84	2.25	2.21
13	6.06	5.77	1.35	1.36
14	10.37	9.70	3.03	2.97
15	15.53	14.47	5.04	4.92
16	6.48	6.20	1.52	1.53
17	7.86	7.51	2.06	2.07
18	11.81	11.16	3.59	3.56
19	14.74	13.94	4.73	4.70
20	19.76	18.70	6.68	6.65
21	28.25	26.73	9.98	9.93
22	30.43	28.77	10.83	10.76
23	30.15	28.42[a]	10.72	10.62[a]
24	37.46	35.35	13.57	13.45

[a] Retention time estimated from unresolved shoulder.

different temperatures further allows calculation of the chromatogram for any combination at any desired temperature. The columns need not be of the same diameter, and the method could easily be extended to the coupling of three or more columns, if desired. The primary limitation is the assumption of constant diameter and stationary phase thickness along each column, which has not proved quite true in practice.

The equations utilized in this study are also useful for work with a single capillary column. After measurement of the dead time at one column-head pressure and temperature, it is simple to calculate the head pressure required for a given dead time at any desired temperature. With gas chromatographs using pressure-controlled flows, this allows prediction of the pressure required to keep the average linear velocity within the optimum range for maximum plate number. It also allows a check on whether the column I.D. matches the nominal value. With the wide availability of personal computers, such methods can be a routine tool of the gas chromatographer.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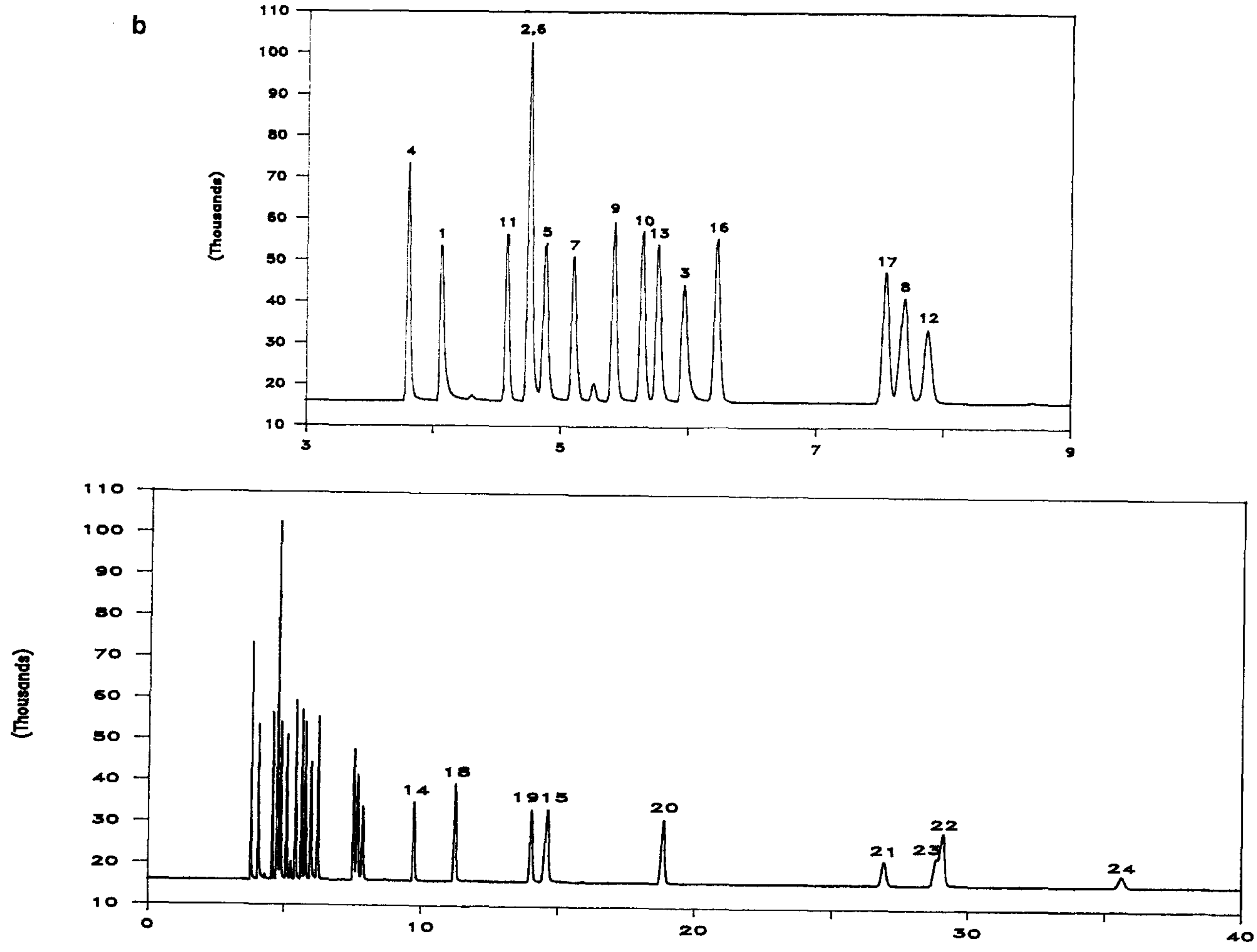

Fig. 3. Comparison of predicted and actual chromatograms for the subset of 24 solvents on the optimized system (time in min). (a) Predicted chromatogram (17.2 m of column 2 coupled ahead of 31.4 m of column 1, 17.1 p.s.i.g., 48°C; peak labels as in Table II); (b) actual chromatogram.

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