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Theoretical and experimental comparison of serially linked and mixed-packing gas–liquid chromatography columns

J. H. PURNELL* and M. H. WATTAN
Department of Chemistry, University College of Swansea, Singleton Park, Swansea SA2 8PP (UK)
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ABSTRACT

Theory developed in this laboratory allows accurate prediction of solute capacity factors for serial operation of columns A and B from basic data for A and B alone. Window analysis then identifies the relative and total column lengths required for baseline resolution of all mixture components. The relative lengths required depend on column sequence, A (front)/B (back) or B (front)/A (back), and normally differ significantly although the resulting chromatograms are identical. Provided the theoretical plate height (H) carrier velocity ($\bar{u}$) relationships for A and B are not too dissimilar, the total column length required is the same in either mode. It is shown theoretically that an optimised mixed packing (A + B) provides exactly the same chromatogram, though the necessary packing ratio does not correspond to either optimised serial column length ratio. Thus, depending on the packing (length) ratio used, an experimental comparison may indicate that the mixed packing approach is superior, or *vice versa*.

Where the $H/\bar{u}$ relationships of A and B differ greatly, total column lengths may vary from one mode to the other to produce the same optimised separation. Generally, the effect is more marked for the serial systems. However, even in this situation, total analysis time will, in certain circumstances, be less for the serial system.

The experimental results presented confirm the theoretical arguments.

INTRODUCTION

In seeking controlled improvement in selectivity of separation in gas–liquid chromatography (GLC) by the use of two (or more) liquid substrates (solvents), three practical routes are open to us:

(a) the use of mixed liquids (A + B) on a single solid support (S); or,

(b) of intimately, mixed or highly striated, packings (A + S) and (B + S) in a single column; or,

(c) serial operation of two columns, (A + S) and (B + S).

We have shown [1–4] that for a number of commonly used GLC solvents, the results obtained with mixed solvents and mixed packings are essentially identical, which implies independent action by A and B when mixed. However, we would generally expect interaction of A with B, hence, some change in solvent properties on mixing. Mode a is, therefore, generally less reliable than mode b and since the one approach is as technically easy as the other, the latter is generally to be preferred. Since

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the solvents A and B must act independently in a mixed packing the net retention volume (V'_R) of any analyte (solute) must be the sum of the independent contributions (V'_{RA} and V'_{RB}),

$$V'_R = V'_{RA} + V'_{RB} = w_A V^0_{gA} + w_B V^0_{gB} \tag{1}$$

where w represents a solvent weight and V^0_g is the specific retention volume of the solute with the relevant solvent. The overall specific retention volume $V^0_g = [V'_R/(w_A + w_B)]$ is thus [5,6],

$$V^0_g = W_A V^0_{gA} + W_B V^0_{gB} \tag{2}$$

where W represents a solvent weight fraction ($w/\Sigma w$). It is a simple matter to show that this can alternatively be written,

$$K^0_R = \varphi_A K^0_{RA} + \varphi_B K^0_{RB} \tag{3}$$

where K^0_R represents the stoichiometric partition coefficient and φ is the volume fraction of one or other solvent ($V_l/\Sigma V_l$).

Thus, measurements of either V^0_g or K^0_R for any solute with test columns containing either solvent A or B allows immediate calculation of the corresponding quantity for any known mixture of A and B packings. The retention of each solute is clearly simply linearly related to the quantity of each solvent or packing present.

In decided contrast, the retention parameters of any solute eluted from the same pair of columns linked in series are not simply related to substrate composition. This arises because the two columns do not operate over the same pressure (flow velocity) regime and so, compressibility of the carrier gas contributes to the overall retention. Further, the overall retention of any given solute depends upon the mode of operation *i.e.* whether A precedes B or *vice versa*. The general equation for the serial operation of two columns, now designated for convenience, front (F) and back (B), where front defines the inlet end of the system, is [7–14]

$$k' = \left[\frac{Pk'_F + k'_B}{P + 1}\right] \tag{4}$$

where k' represents the capacity factor for a solute with either column and $P = t_{dF}/t_{dB}$, where t_d is the dead time (elution of unsorbed component). Using columns of A and B, either of course, may be F or B. Only if $P = 1$ is k' the arithmetic average of k'_F and k'_B and the same in alternative modes, [A(F)/B(B)] and [B(F)/A(B)].

Values of k'_A and k'_B for solutes are readily determined with test columns exactly as in mixed packing applications. P, however, cannot be calculated without further experimental information. First, we need to know the value of the junction pressure p arising when we run the system with column A at the inlet ($p_i \rightarrow p$) and column B at the outlet ($p \rightarrow p_o$), or *vice versa*. Secondly, because there may be differences in packing density or column dimensions, we need to know the relevant column dead volumes (V_M) and resistance to flow factors, R_F, the latter being defined by [15]

$$R_F = \frac{[p_i^2 - p_o^2]}{p_o u_o} = \frac{[p_i^2 - p_o^2]}{\bar{p}\bar{u}} \tag{5}$$

where $\bar{p}$ and $\bar{u}$ are the compressibility averaged pressure and gas velocity, respectively. While we can use eqn. 5 to evaluate R_F it is much more convenient to use the equation

$$t_d = \frac{2LR_F}{3}\left[\frac{(p_i^3 - p_o^3)}{(p_i^2 - p_o^2)^2}\right] \tag{6}$$

A plot of t_d against the bracketted function gives a straight line of slope $(2LR_F/3)$ and if flow-rates, corrected to column temperature (F_c) are measured simultaneously with t_d, V_M can be also be calculated.

For any column pair, the junction pressure is then given by [9,14]

$$p^2 = \left[\frac{p_i^2 - l_F[p_i^2 - (V_{MB}R_{FF}/V_{MF}R_{FB})p_o^2]}{1 - l_F[1 - (V_{MB}R_{FF}/V_{MF}R_{FB})]}\right] \tag{7}$$

where l_F is the length fraction of the front column $[L_F/(L_F + L_B)]$. We note that this equation is, as also are subsequent equations presented, independent of any assumption of, or need to measure, other column parameters such as column diameters or phase ratios, a considerable advantage.

Finally, we can calculate P for any solute for any column lengths and inlet pressure *via.* [9,14]

$$P = \left[\frac{L_B R_{FB}}{L_F R_{FF}}\right]\left[\frac{V_{MF}}{V_{MB}}\right]^2\left[\frac{p_i^3 - p^3}{p^3 - p_o^3}\right] \tag{8}$$

and, so, can calculate k' for all solutes in either mode A(F)/B(B) or B(F)/A(B).

We turn now to the matter of analytical interest, the choice of mixed packing ratio, or of relative column lengths in serial operation, that will provide the desired separation of the mixture to hand. The method of choice is the window analysis method developed by Laub and Purnell [16]. For mixed packings this is a straightforward procedure. From eqns. 2 and 3 we see that V_g^0 and K_R^0 are both linear functions of controllable column variables, so, we need know only the values of either for the solutes measured with the columns of the pure solvents, A and B, and can then use the relevant equation to calculate k' for all mixture components at any value of W_A or φ_A. Alternatively, we may construct a plot by simply connecting the relevant values for a solute with solvents A and B with a straight line on a scale of W_A or $\varphi_A = 0$ to 1. It is clearly a simple matter to evaluate the relevant retention parameter for each solute at a number of intermediate values of W_A or φ_A by interpolation from the plot or by use of the appropriate equation and so, for all pairs of solutes, the ratios $V_{g2}^0/V_{g1}^0 = K_{R2}^0/K_{R1}^0 = \alpha$, the relative retention. The plot has the merit of indicating immediately which pairs offer no difficulty of separation and so can be left out of the calculations. The values of α are then plotted against W_A (or φ_A) and, since we are uninterested at this point in the order of elution we choose to keep $\alpha \geqslant 1$. Typically then, the lower envelope of the plot takes the form of a number of approximate triangles (windows), the highest of which defines the largest value of the lowest value of α among all solute pairs, and the corresponding value of W_A or φ_A. Knowing α at the optimum window

peak, we then calculate the number of theoretical plates required (N_{req}) to provide base-line separation of all components *via* [17]

$$N_{req} = 36\left[\frac{\alpha}{\alpha - 1}\right]^2\left[\frac{1 + k'}{k'}\right]^2 \tag{9}$$

where k' is now the capacity factor of the second to elute of the most difficult pair to separate. Clearly, if this pair is separated, so too are all others.

During the test experiments that yielded the basic data, we can determine the attainable theoretical plate height (H) and, hence, from N_{req}, the total column length needed for base-line separation. We thus know all that is necessary to achieve the optimisation of the desired separation with the chosen packings at the test temperature. Given further information the optimisation can be extended [18–20] to solvent choice, temperature, analysis time, and so forth. Window analysis is now widely employed to optimise various aspects of chromatographic, or other, analysis although it is frequently designated by some other name.

As eqn. 4 shows, in serial column operation, k' is not a simple linear function of the controllable variable, l_F. Thus, to apply the technique of window analysis as described above would now appear to require measurements of k' for all mixture components at a number of values of l_F. This would be tedious. Fortunately, as Purnell and Williams [9] have shown, we can get around this problem as follows.

Let us identify a quantity $f(f_F + f_B = 1)$ which linearises k', thus,

$$k' = f_F k'_F + f_B k'_B \tag{10}$$

Equating the right hand side with that of eqn. 4 shows that

$$P = f_F/(1 - f_F) \tag{11}$$

Substituting in eqn. 8 and rearranging gives

$$p^3 = \left[\frac{p_i^3 - f_F(p_i^3 - \gamma p_o^3)}{1 - f_F(1 - \gamma)}\right] \tag{12}$$

where

$$\gamma = \left[\frac{L_F R_{FF}}{L_B R_{FB}}\right]\left[\frac{V_{MB}}{V_{MF}}\right]^2 = \left[\frac{\bar{R}_{FF}}{\bar{R}_{FB}}\right]\left[\frac{\bar{V}_{MB}}{\bar{V}_{MF}}\right]^2 \tag{13}$$

the superior bars defining a quantity per unit column length.

This then leads, finally, to an expression for the value of l_F that corresponds to any given value of f_F, and P at specified p_i and p_o (eqn. 12) *viz*

$$l_F^{-1} = \left[\frac{\bar{R}_{FF}\bar{V}_{MB}}{\bar{R}_{FB}\bar{V}_{MF}}\left[\frac{(p^2 - p_o^2)}{(p_i^2 - p^2)}\right] + 1\right] \tag{14}$$

We can now proceed with the window analysis as before by plotting k' as a linear function of f_F and so identify the highest window and the corresponding value of f_F. Fixing on values of p_i and p_o we then calculate the corresponding value of p (eqn. 12) and, thence, evaluate l_F *via* eqn. 14.

The foregoing allows us to draw an important conclusion regarding the relative merits of mixed packings and the analogous serial columns. For the same packings the two methods share the same window peak, *i.e.*, α at the optimum is the same in either approach and so provided we work at the same H, the total column length needed for base-line resolution of the mixture is exactly the same although the relative amounts of packing needed may be very different. But, further, since the value of α at the optimum occurs at the same values of k' for the most difficult pair to separate, all other k' must also be the same in either mode. That is, the optimised chromatograms will be identical in every respect. This is an important observation in the context of choice or comparison of method. It also explains why the few attemps to compare the methods experimentally [21–25] have produced conflicting conclusions. They produce identical chromatograms only when operated so as to optimise separation. In other experimental conditions, *e.g.* if a pair of serial test columns were run, then unpacked and repacked as a mixed packing column, the experiments would yield different chromatograms and, depending on circumstances, column sequence in the serial mode for instance, would indicate that one or other method was superior in terms of resolution.

In what follows we provide experimental proof of the foregoing.

EXPERIMENTAL

The experiments were all conducted with a Perkin-Elmer F.33 chromatograph equipped with flame ionization detection. The carrier gas was nitrogen and mixture components (0.1–1.0 μl) were eluted separately to establish the relevant capacity factors and methane was used to measure dead times. The column packings used were 10% (w/w) of squalane (SQ) and of diisobutylphthalate (DBP) supported on 100–120 mesh Chromosorb G AW DCMS and were packed in stainless steel tube of 0.32 cm outside diameter. Carrier flow-rates were measured with a soap-bubble meter and corrected for water vapour pressure and to column temperature (90°C) to yield fully corrected values (F_c). All values of t_d, k' and V_M reported are averages of a number of measurements.

The solvents were obtained from Phase Separations (Queensferry, UK) and the mixture components from BDH (Poole, UK). All were used as delivered. Solvent/support loadings were doubly checked by thermogravimetric analysis (TGA) and Soxhlet extraction.

RESULTS

Table I lists values of k' for twelve hydrocarbons eluted by nitrogen at 90°C from both the SQ and DBP columns. With each solvent there is at least one solute pair that cannot be separated at any realistic column length.

The pressure, dead time and flow-rate studies yielded the values of R_F and V_M listed in Table II while concurrent measurements of H as a function of flow-rate

TABLE I

k' FOR NAMED SOLUTES ELUTED AT 90°C BY NITROGEN FROM 10% (w/w) COLUMNS OF SQUALANE (SQ) (180 cm) AND DIISOBUTYLPHTHALATE (DBP) (170 cm) ON 100–120 MESH CHROMOSORB G AW DCMS

Component	Solute	k'(SQ)	k'(DBP)
1	*n*-Hexane	4.92	1.24
2	Benzene	8.80	5.37
3	*n*-Heptane	11.6	3.48
4	Methylcyclohexane	15.5	6.32
5	Toluene	18.7	15.1
6	*n*-Octane	26.6	8.05
7	Ethyl benzene	39.3	30.6
8	Non-1-ene	52.3	20.4
9	*o*-Xylene	52.3	39.5
10	*n*-Propyl benzene	78.7	58.1
11	Dec-1-ene	113.8	43.3
12	*n*-Decane	130.4	39.5

provided good Van Deemter plots the minima for both columns occurring at $H_{min} = 0.065$ cm and $\bar{u} = 4.3$ cm s^{-1}.

Fig. 1 shows plots of k' against f_{SQ} constructed simply by connecting the values of k' at $f_{SQ} = 0$ and $f_{SQ} = 1$. We see a number of values of f_{SQ} at which lines cross ($\alpha = 1$) and so expect to find numerous windows on the α/f_F plot. Fig. 2 shows that there are seven with one outstandingly superior to all others ($f_{SQ} = 0.872$; $\alpha = 1.12$). This value of α with the relevant $k' \approx 12$ (see Fig. 1) gives, via eqn. 9, the value $N_{req} = 3260$. At $H = 0.065$ cm (the Van Deemter minimum for both columns) the total column length required is thus 212 cm to provide baseline separation of all components.

We calculate the corresponding relative column lengths, as described earlier, as follows:

Mode 1: SQ(F)/DBP(B); $f_F = 0.872$; $P = 6.812$; $\gamma = 1.288$ and, with $p_o = 14.6$ p.s.i. and $p_i = 34.6$ p.s.i., $p = 19.14$ p.s.i. giving $l_F = 0.813$ ($L_{SQ} = 172$ cm; $L_{DBP} = 40$ cm).

Mode 2: DBP(F)/SQ(B); $f_F = 0.128$; $P = 0.146$; $\gamma = 0.776$ and, with $p_o = 14.6$ p.s.i. and $p_i = 34.6$ p.s.i., $p = 33.46$ p.s.i. giving $l_F = 0.0915$ ($L_{SQ} = 192.5$ cm; $L_{DBP} = 19.5$ cm).

TABLE II

R_F AND V_M VALUES FOR COLUMNS AND CONDITIONS OF TABLE I

Column	$10^{-6}R_F$ (Nsm^{-3})	V_M (ml)	$10^{-6}\bar{R}_F$ (Nsm^{-4})	$\bar{V}_M$ (ml m^{-1})
SQ	5.56	3.86	3.09	2.14
DBP	4.71	3.99	2.77	2.34

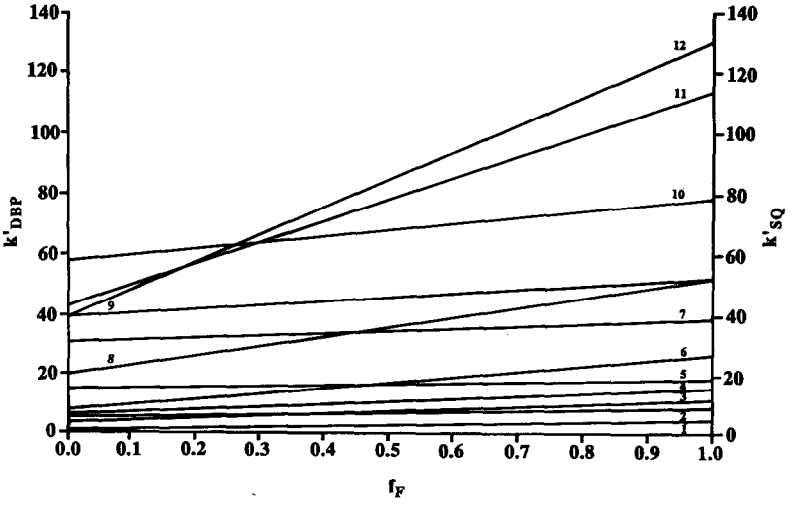

Fig. 1. Plots of k' against f_{SQ} constructed by plotting data of Table I at $f_{SQ} = 0$ and 1 and connecting with straight line. Note numerous cross-overs which indicate a value of $\alpha = 1$ so that separation of all components cannot be achieved.

Fig. 3 shows the chromatograms obtained in the alternative modes. They are clearly baseline resolved and identical, as theory demands.

Turning now to the mixed packing alternative. Provided that the free volumes per g of each packing are not too dissimilar then eqn. 2 can be represented as

$$k' = W_A k'_A + W_B k'_B \tag{15}$$

For the two packings used here the free volume discrepancy is not more than 5% so that eqn. 15 can be used with little approximation. Thus, had we represented Fig. 1 as

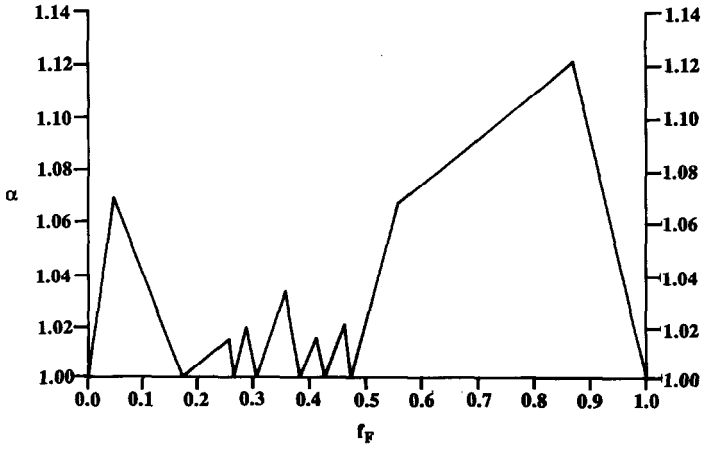

Fig. 2. Window diagram constructed from data of Table I (eqn. 10). Each window defines a region of f_F wherein total separation of all components is possible. Highest window defines most favourable α (shortest column) and corresponding f_F.

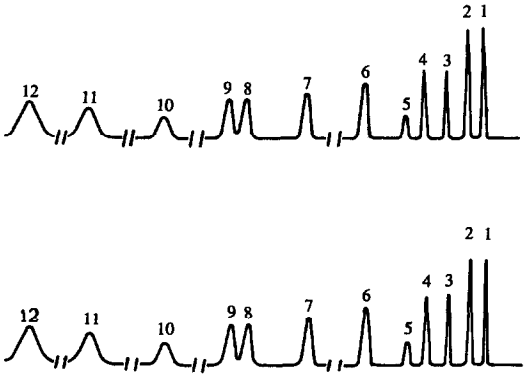

Fig. 3. Optimised serial separations at 90°C. Upper: SQ(F) 172 cm/DBP(B) 40 cm; lower: DBP(F) 19.5 cm/SQ(B) 192.5 cm. Time scale interrupted for convenience of presentation.

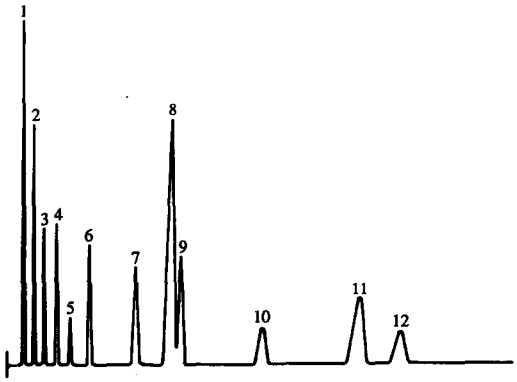

Fig. 4. Optimised mixed column separation at 90°C. Column, 212 cm; weight ratio of 10% (w/w) packings SQ/DBP = 6.81.

TABLE III

k' FOR ALL SOLUTE COMPONENTS WITH SERIAL COLUMNS, WITH MIXED PACKING AND AS THEORETICALLY CALCULATED FOR CONDITIONS OF OPTIMISED SEPARATION

Component	Serial columns average k'	Mixed packing k'	Any mode calculated k'
1	4.92	4.90	4.45
2	8.00	7.80	8.40
3	11.1	11.5	11.2
4	15.0	14.8	14.7
5	18.6	18.5	18.3
6	24.6	24.7	24.3
7	38.1	38.3	38.2
8	47.8	47.9	48.2
9	50.7	50.8	50.7
10	75.7	75.7	76.1
11	106	106	104
12	120	120	119

a plot of k' against W_{SQ}, the numerical value of the weight fraction at the best window peak would have equalled that of f_F cited earlier (0.872). Thus, to complete the comparison sought we now require a 212 cm mixed packing column with the 10% packings in the ratio SQ:DBP = 6.81. Fig. 4 shows the chromatogram obtained with this column at the same p_i and p_o as employed with the serial systems. To all intents and purposes, baseline resolution is again achieved and the three chromatograms are visually identical. The most telling information, however, is that detailed in Table III where we list the average values of k' for the two serial modes, those for the mixed packing and those calculated *via* the equations presented earlier. The agreement is seen to be virtually exact.

DISCUSSION

The theoretical arguments advanced earlier are fully confirmed. They allow us to calculate precisely the capacity factors arising in mixed packing or serial operation in either mode and, further, tell us that in the conditions of optimised separation the actual chromatograms obtained are identical. As indicated, provided the theoretical plate heights attainable are not too dissimilar in the two approaches, the overall column length needed for the optimised separation is also the same for the mixed packing and either serial mode. This situation will be encountered fairly frequently in practice. Thus, choice of method will commonly depend on other criteria. One obvious factor is that associated with solvent volatility. In the serial array, solvent from both columns will be transported but that from the front column will be deposited initially in the back column. Thus, the basic retention characteristics of both front and back columns will change with time. As this happens the system moves away from the optimum and progressive loss of resolution will be observed. There will also be transfer in the mixed packing bed but here, as indicated earlier, the deposition of *e.g.* A in B, will lead to little change in α in most cases. Thus, the mixed packing is likely to maintain resolution far longer. If, therefore, one or other of the chosen solvents is excessively volatile, the operating lifetime of the mixed packing column should be superior.

The most significant effect that influences choice of method, however, arises where $H/\bar{u}$ differs significantly from column to column. There are two cases to consider. First, if the van Deemter curves for the serial and mixed packings are very different, not only will different total column lengths be needed but different gas velocities may be necessary. Whilst this will not influence k' values in the optimised separation it may lead to significant difference in the total time of analysis. Where the $H/\bar{u}$ differences between the separate packings are significant the situation is more complex. This aspect has been treated theoretically by Purnell and Williams [5,6] but no experimental study has yet been reported. They showed that in certain circumstances the value of $H/\bar{u}$ for a serial column could be significantly greater than the corresponding values of $H/\bar{u}$ for either column alone. Broadly, this arises when the columns are of disparate length and the shorter column is both more retentive and substantially less efficient. In contrast, all published evidence is that $H/\bar{u}$ for a mixed packing lies between the values characterising the separate test columns. The mixed packing column would then be superior to the serial column in terms of the length required for optimised analysis. Extention of their analysis allowed comparison of the alternative modes when the optimised separation is conducted in conditions yielding

minimum analysis time (far above the Van Deemter minimum). In this situation, it emerges that, notwithstanding the efficiency issue illustrated above, conditions could exist in which either mode, serial or mixed, might provide faster analysis. Clearly, any future experimental comparison involving analysis time as one criterion of optimisation must take this observation into account.

Where the main aim is to achieve separation the foregoing clearly indicates that the mixed packing approach will normally be preferred in GLC–GLC since, if nothing else, it is more convenient. However, it cannot be used with confidence with GLC–gas–solid chromatography combinations due to potential deactivation of the gas–solid component following solvent transfer. Equally, with open-tube columns, mixed substrates present practical problems and serial operation will be preferred.

Finally, it is evident that serial systems provide opportunity to operate the columns at different temperatures. This developing technique, for which commercial equipment is already available, is subject to exactly the theory and procedures described here, as we have shown earlier [14]. Since, in this case, separate temperature regimes are superimposed on the compressibility effect, the empirical approach to optimisation is tedious, and possibly unrewarding and a theoretically based procedure becomes even more desirable.

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