Journal of Chromatography, 468 (1989) 99–114
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMSYMP. 1543

CONTEMPORARY LIQUID CHROMATOGRAPHY COLUMN DESIGN

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SUMMARY

The development of chromatographic column theory over the last thirty years is described and the impact of the plate theory and rate theory on column design discussed. The concept of the reduced chromatogram is employed to develop equations for optimum column length, optimum column diameter, optimum particle size and minimum analysis time. The equations are used to identify the practical limits of column design and column performance, and the design of a family of columns for general liquid chromatography analysis is recommended.

INTRODUCTION

Column technology today has reached a very advanced stage of development. It is now possible to define the optimum column (in terms of length, radius and particle diameter of the packing) that will separate a given mixture, employing a particular phase system, in the minimum time and with the minimum mobile phase consumption. Such optimum columns can be defined for separations carried out by both gas chromatography (GC) and liquid chromatography (LC) and for both packed and capillary columns[1,2]. A protocol for column design has been established[1] and the constraints imposed upon the separation by the instrument specification limits in terms of analysis time, sample size and solvent consumption are now recognized and understood. Furthermore, it is now possible to predict, with some accuracy, future improvements in high-speed separations and in the resolution of highly complex mixtures, that will be achievable with conventional chromatographic systems which employ pressure induced mobile phase flow-rates.

Modern column technology has evolved over a period of more than thirty years, but it is only over the last five years that the various aspects of column theory and practice have been brought together in a rational form to provide a sound basis for column design. In this paper, the development of column theory will be traced over the last thirty years, together with the progress in column technology that has resulted. In particular, its impact on column design in LC will be discussed at each stage. Finally, the future practical limits of performance that can be expected from packed columns employing pressure-induced flow-rates will be considered.

0021-9673/89/$03.50

THE DEVELOPMENT OF CHROMATOGRAPHIC THEORY

The first major contribution to the theory of chromatography was the plate theory which was developed by Martin and Synge[3] and extended by Keulemans[4]. In its original form, the plate theory explained retention in the terms of distribution coefficient and allowed the variance of a peak to the estimated as inversely proportional to the number of theoretical plates in the column. Consequently, the plate theory *per se* provided an equation that allowed the efficiency of a column to be calculated *after* it had been constructed, but this did not help much in column design. However, in 1959 Purnell[5] used the plate theory to develop an equation that allowed the number of plates required to effect any given separation to be calculated from the separation ratio of the closest eluted pair and the capacity factor of the first eluted peak of the pair. The equation of Purnell is given as follows

$$n = 16(1 + k')^2/k'^2(\alpha - 1)^2 \quad (1)$$

where n is the number of theoretical plates required, α is the separation ratio of the closest eluted pair and k' is the capacity factor of the first member of the closest eluted pair. (In this respect eqn. 1 differs slightly from that of Purnell, in that it employs the k' value of the first eluted peak as opposed to that of the second eluted peak.)

The relationship between the required number of theoretical plates to effect a separation as a function of the separation ratio and the k' value of the first eluted peak is shown in Fig. 1.

Purnell's equation demonstrated for the first time the need for extremely high efficiencies to separate solute pairs having *small* separation ratios eluted at low k' values. In Fig. 1, it is seen that to resolve a pair of solutes having a separation ratio of 1.01 and eluted at a k' of 1 requires more than half a million theoretical plates. The effect of k' is also a little surprising. The efficiencies required to separate a solute pair having a separation ratio of 1.06 and eluted at a k' of 5 is only one third of that required at a k' of unity. As a consequence, the need for high efficiencies to separate solute pairs eluted at low k' values encourages the choice of a solvent (or solvent mixture) that will provide greater retention.

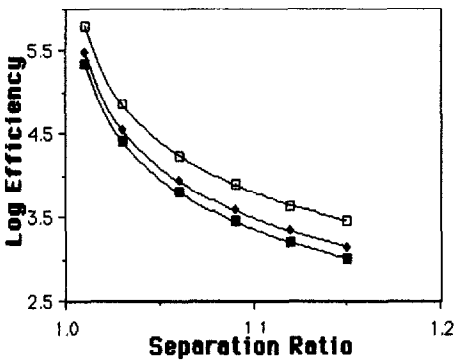


Fig. 1. Graphs of efficiency against separation ratio for different values of k': □, $k' = 1$; ◆, $k' = 2.5$; ⅜, $k' = 5$.

Eqn. 1 was of primary importance, as it allowed the efficiency needed to achieve any given separation to be calculated, but this was only a beginning. Before proceeding further, it is necessary to introduce the concept of the *reduced chromatogram*. Any chromatogram of a complex mixture of solutes can be reduced to a simple separation that will concisely describe the chromatographic problem. An example of a reduced chromatogram is shown in Fig. 2.

The reduced chromatogram consists of four peaks, first the dead volume peak, then a pair of peaks that are the two eluted closest together and thus the most difficult to separate. This pair of peaks is termed the *critical pair*. The column must be designed to separate the critical pair and, if this is satisfactorily achieved, then all the other peaks, which by definition are less difficult to separate, will also be resolved. The fourth peak in the reduced chromatogram is the last peak in the mixture which must be eluted to complete the analysis.

However, it shoukd be pointed out that a given column, operated at a given flow-rate, can exhibit a range of efficiencies depending on the nature of the solute that is chosen to measure it[6]. (This dependence of column efficiency on solute type will be discussed later.) Consequently, under exceptional circumstances, the predicted conditions for the separation of the critical pair may *not* be suitable for another pair and complete resolution of all solutes may not be achieved. This could occur if the separation ratio of another solute pair, although *larger*, is very *close* to that of the critical pair but contains solutes, for example, of very different molecular weight. However, the probability of this situation arising is extremely remote and will not be considered in this review. Thus, from the reduced chromatogram and eqn. 1 the number of theoretical plates required to separate any given mixture can be calculated.

The next development in the theory of chromatography that was essential for column design was the rate theory, first introduced by Van Deemter in 1956. Van Deemter *et al.*[7] put forward an equation relating the variance per unit length of a column (which can be shown to be numerically equivalent to the height of the theoretical plate) to the linear velocity of the mobile phase and physical properties of the solute, mobile phase and column.

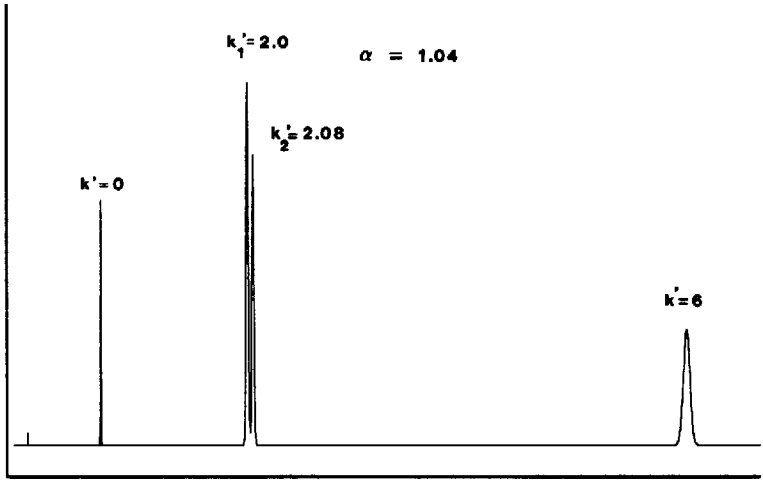


Fig. 2. The reduced chromatogram. Peaks: (1) $k'_0 = 0$; (2) $k'_1 = 2.0$; (3) $k'_2 = 2.08$; (4) $k'_3 = 6.0$. $\alpha = 1.04$.

Their equation took the following form.

$$H = A + B/u + Cu \tag{2}$$

where H is the variance per unit length of the column; A, B and C are constants for a particular chromatographic system, and u is the linear velocity of the mobile phase.

For a given column, a given mobile phase and a given solute, the expressions for the constants A, B and C are as follows:

$$A = 2\gamma d_p$$

where γ is a packing constant and d_p is the particle diameter of the packing.

$$B = 2\lambda D_M$$

where D_M is the diffusivity of the solute in the mobile phase and λ is a packing constant.

$$C = f_1(k')d_p^2/D_M + f_2(k')d_f^2/D_S$$

where d_f is the film thickness of the stationary phase and D_S is the diffusivity of the solute in the stationary phase.

Thus

$$H = 2\gamma d_p + 2\lambda D_M/u + f_1(k')d_p^2u/D_M + f_2(k')d_f^2u/D_S \tag{3}$$

Since the work of Van Deemter, a number of equations have been developed that purport to describe the variance per unit length of a column as a function of the linear mobile phase velocity[8–11]. However, each was carefully examined by Katz *et al.*[12], and it was concluded that the original equation of Van Deemter was as good as any, and better than most, for the precise prediction of the variance per unit length of a column particularly for linear velocities in the neighbourhood of the *optimum velocity*. Katz *et al.*[12] also showed that the resistance to mass transfer in the stationary phase contributed very little to the variance of the eluted peak, due to the very small value for d_f (the surface of the silica or bonded silica constituting the stationary phase). It was also established that $f_1(k')$ was very similar to the value derived for capillary columns, *viz.*

$$f_1(k') = (0.37 + 4.69k' + 4.04k'^2)/24(1 + k')^2 \tag{4}$$

Consequently eqn. 3 becomes

$$H = 2\gamma d_p + 2\lambda D_M/u + f_1(k')d_p^2u/D_M$$

or

$$H = 2\gamma d_p + 2\lambda D_M/u + (0.37 + 4.69k' + 4.04k'^2)d_p^2u/24(1 + k')^2D_M \tag{5}$$

An height equivalent to a theoretical plate (HETP) curve drawn from experimental data fitted to the Van Deemter equation, is shown in Fig. 3. It is seen that the fit is excellent. The Van Deemter equation can also provide a value for the optimum mobile phase velocity that must be employed with any given column. The optimum velocity will give the minimum variance per unit length and thus the maximum column efficiency. It was suggested by Knox and Saleen[13] and subsequently confirmed by Katz *et al.*[1] that the optimum velocity is that velocity which must be employed with the optimized column of minimum length to provide the minimum analysis time. To obtain the optimum velocity. Eqn. 4 is differentiated with respect to u and equated to zero. Thus, by solving for u it is seen that

$$u_{opt} = (B/C)^{0.5}$$

or

$$u_{opt} = \{2\lambda D_M/[f_1(k')d_p^2/D_M]\}^{0.5} \tag{6}$$

The minimum plate height, which is obtained at the optimum velocity, can be determined by substituting for u_{opt} in eqn. 2 from eqn. 6

$$H_{min} = A + 2(BC)^{0.5}$$

$$= 2\gamma d_p + 2[2\lambda D_M(0.37 + 4.69k' + 4.04k'^2)d_p^2/24(1 + k')^2 D_M]^{0.5} \tag{7}$$

Now, as the variance per unit length of the column is equal to the ratio of the column length (l), to the efficiency then, eqn. 7 can also provide an expression for the column length.

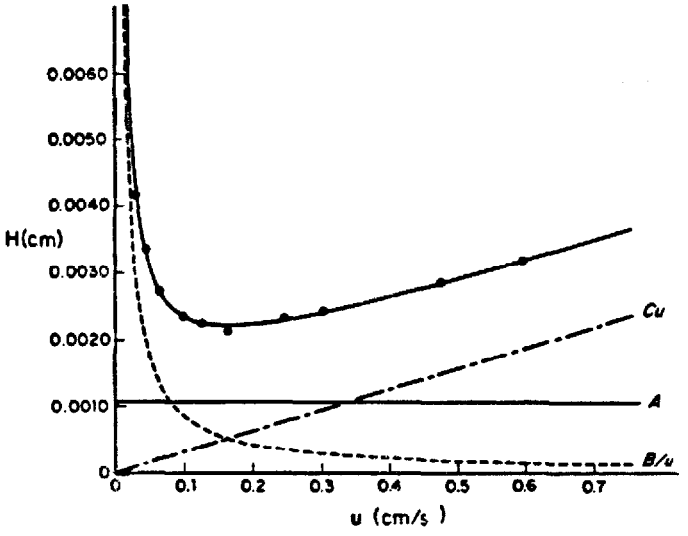


Fig. 3. Graph of HETP against linear mobile phase velocity (points curve fitted to the Van Deemter equation).

$$l = nH_{min} = n[A + 2(BC)^{0.5}]$$

$$= n\{2\gamma d_p + 2[2\lambda D_M(0.37 + 4.69k' + 4.04k'^2)d_p^2/24(1 + k')^2 D_M]\}^{0.5} \quad (8)$$

Eqn. 8 provides a value for the length of the column that is necessary to achieve the separation of the critical pair in the reduced chromatogram. However, this equation still contains one *undefined* variable, the particle diameter, d_p, the rest (*i.e.* γ, λ, D_M and k') being determined by the choice of the phase system and the quality of the packing. Consequently, it is now necessary to obtain an expression for the optimum particle diameter to be used in the packing.

Now, the particle size of the packing controls not only the value of H but also the permeability of the column. According to D'Arcy's law,

$$u = P\psi d_p^2/\eta l \quad (9)$$

where ψ is D'Arcy's constant for a packed bed, η is the viscosity of the mobile phase and P is the applied pressure, or, when the optimum mobile phase velocity is employed

$$u_{opt} = P\psi d_p^2/\eta l$$

or

$$l = P\psi d_p^2/\eta u_{opt} \quad (10)$$

However, from eqn. 8 it is also seen that:

$$l = nH_{min}$$

Equating expressions 8 and 10

$$nH_{min} = P\psi d_p^2/\eta u_{opt} \quad (11)$$

Substituting for H_{min} and u_{opt} from eqns. 6 and 7, respectively

$$n[A + 2(BC)^{0.5}] = P\psi d_p^2/\eta(B/C)^{0.5} \quad (12)$$

Solving for the particle diameter, d_p

$$d_p = (2\eta n D_M/\psi P\{\lambda[2\gamma/f(k')]^{0.5} + \gamma\})^{0.5} = d_{opt} \quad (13)$$

Thus, the particle diameter of the packing can be determined. It should be emphasized that this is the *optimum* particle diameter which will give the *minimum* analysis time.

It is now a simple substitution procedure to obtain an equation for the minimum analysis time (t), which is given by

$$t = (1 + k'_2)l/u_{opt} = (1 + k'_2)nH_{min}/u_{opt} \quad (14)$$

or

$$t = (1 + k'_2)n[A + 2(BC)^{0.5}]/(B/C)^{0.5} = (1 + k'_2)n[A(C/B)^{0.5} + 2C] \quad (15)$$

$$t = (1 + k'_2)n\{2\gamma d_{opt}[(0.37 + 4.69k' + 4.04k'^2)^2_{opt}/24(1 + k')^2 D_M^2 2\lambda]^{0.5} + \\ + 2[(0.37 + 4.69k' + 4.04k'_2)d^2_{opt}/24(1 + k')^2 D_M]\} \quad (16)$$

where r is the column radius and σ_A is the extra column dispersion in milliliters resulting from dispersive processes taking place *external to the column* in, *e.g.*, the sample valve, connecting tubes, detector cell etc. It is seen that the column radius depends only on the extra column dispersion of the chromatograph, the separation ratio of the critical pair, and the optimum particle diameter of the packing.

In summary the pertinent equations for column design are as follows:

Column efficiency $(n) = 16(1 + k')^2/k'^2(\alpha - 1)^2$

Particle diameter $(d_{opt}) = (2\eta n D_M/\psi P\{2\lambda[2\gamma/f(k')]^{0.5} + 2\gamma\})^{0.5}$

Optimum velocity $(u_{opt}) = \{2\lambda D_M/[f_1(k')d_p^2/D_M]\}^{0.5}$

Column length $(l) =$
$n\{2\gamma d_{opt} + 2[2\lambda D_M(0.37 + 4.69k' + 4.04k'^2)d^2_{opt}/24(1 + k')^2 D_M]\}^{0.5}$

Analytical time $(t) =$
$(1 + k'_2)n\{2\gamma d_{opt}[(0.37 + 4.69k' + 4.04k'^2)d^2_{opt}/24(1 + k')^2 D_M^2 2\lambda]^{0.5} +$
$+ 2[(0.37 + 4.69k' + 4.04k'^2)d^2_{opt}/24(1 + k')^2 D_M]\}$

Column diameter $(r) = [0.09\sigma_A(\alpha - 1)/d_{opt}]^{0.5}$

DISCUSSION

The equations given above demonstrate that there is one *unique* column that will resolve a given mixture in the minimum time and this column must be packed with particles of optimum diameter and operated at the optimum mobile phase velocity. As a result, there appears to be some conflict with traditional ideas on the subject which have usually assumed that for fast separations, velocities above the optimum should be employed and for high resolution and high efficiencies particle diameters should be made as small as possible. These misconceptions have arisen partly as a result of disregarding the fact that there is a limited inlet pressure available from the pump and partly from attempting to obtain fast separations from columns of fixed length. As a consequence of limited inlet pressure the particle diameter cannot be beyond that which will permit the optimum velocity to be realized. If higher efficiencies are required, the column must be made longer, and to achieve this, the column permeability must be increased by making the particle diameter larger.Velocities higher or lower than the optimum would increase the HETP and thus the required resolution would not be obtained.

However, if, for some reason, the length of the column cannot be changed then, for samples where the separation ratio of the critical pair is relatively high and the column has an efficiency in excess of that required, very fast separations can be achieved by operating at very high linear velocities. However, it must be emphasized that under these circumstances, although the separation will be fast, the analysis time will *not* be the minimum. The separation would be made even faster by reducing the particle size of the packing and employing a shorter column that could now operate at the optimum velocity. Unfortunately, as will be discussed later, the optimum particle size for a very simple separation may be smaller than the minimum available or below that which can be packed with known techniques. Under such circumstances, non-optimized columns with excess efficiency, operated at high velocities may, be the only way to reduce the analysis time to the required level.

The design equations will now be used to demonstrate the unique properties of optimized columns. For the most part, the main variable that will be employed will be the separation ratio of the critical pair, as this will demonstrate how the column properties vary with the difficulty of separation. In the examples given, the following values for the other pertinent variables will be assumed: packing constant (λ), 0.5; packing constant (γ), 0.6; diffusivity of the solute in the mobile phase (D_M), $3.5 \cdot 10^{-5}$ cm^2/s; mobile phase viscosity(η), 0.023 poises; D'Arcy constant (ψ), 35; capacity factor of the first of the critical pair (k'_1), 2.5; and capacity factor of the last eluted peak (k'_2), 5.0.

The values for the packing constants of 0.5 and 0.6 for λ and γ, respectively, are those predicted from theory by Giddings[15] and generally attainable by modern packing procedures. The value taken for the diffusivity of the solute in the mobile phase is for benzyl acetate in a mixture consisting of 5% (w/w) ethyl acetate in *n*-heptane, typical for many solute–solvent systems. The viscosity value taken is for the same solvent mixture. The D'Arcy constant was taken from measurements made on a number of columns packed with particles of different diameter by Katz *et al.*[1]. The k' values taken are also fairly typical for many routine chromatography analyses.

Employing eqn. 16 the analysis times were calculated for the resolution of three samples, the critical pairs having separation ratios of 1.02, 1.04 and 1.06 respectively. The results, plotted as curves relating analysis time to particle diameter are shown in Fig. 4. Included are curves relating the optimum particle diameter (calculated for three different inlet pressures) to the separation ratio of the critical pair. The other curves were calculated for an inlet pressure of 3000 p.s.i. Examination of Fig. 4 shows that there is, indeed, an optimum particle diameter that will provide the minimum analysis time and this optimum increases in magnitude with the difficulty of the separation. This again appears to be in conflict with accepted principles. However, it is clear that the more difficult separations must be accomplished with particles of larger diameter to provide adequate column permeability and, thus, permit the use of the necessary longer columns.

The more simple the separation, the more critical becomes the need to employ the optimum diameter if the minimum analysis time is to be achieved. Furthermore, it is seen that, if the particles are too small, the column will be operated below its optimum velocity, due to inadequate inlet pressure. Consequently, the variance per unit length will be increased as a result of the dominance of the longitudinal diffusion term in the Van Deemter equation, and a longer column will be necessary in order to

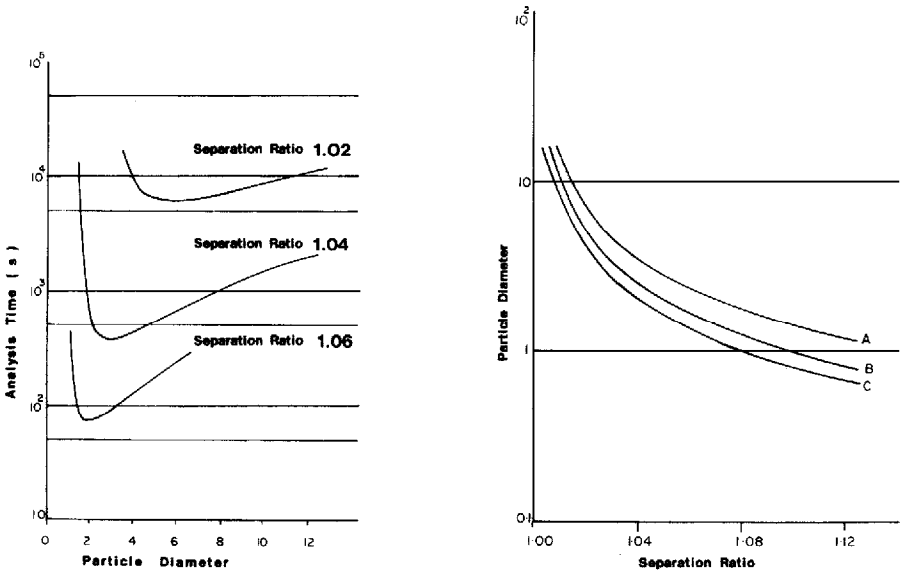


Fig. 4. (1) Graphs of analysis time against particle diameter (μm) for the separation of different solute pairs having different separation ratios. (2) Graphs of optimum particle diameter (μm) for minimum analysis time against separation ratio. Key: A = 2000 p.s.i.; B = 4000 p.s.i.; C = 6000 p.s.i.

attain the necessary efficiency. In a similar manner, if the particles are too large the dispersion will be greater at the optimum velocity due to the increased magnitude of the resistance to the mass transfer term in the Van Deemter equation. Consequently, the column must again be made longer to provide adequate efficiency and, as a result, the analysis time will also be extended.

It is seen from the second graph that, over the range of separation ratios chosen, the magnitude of the optimum particle diameter extends from *ca.* 0.8 μm to about 20 $\mu\mu$. It is also seen that, providing the inlet pressure available is above 2000 p.s.i., the effect of pressure on analysis time is not nearly as significant as might be expected. At present, particles of less than about 2 μm are not readily available and are fairly difficult to pack employing the usual slurry methods of packing.

It is also interesting to determine how the column length and analysis time for optimum columns change with the separation ratio of the critical pair. Employing 8 and 16, curves relating optimum column length and analysis time to the separation ratio of the critical pair were constructed and are shown in Fig. 5.

Fig. 5 shows that the analysis time can range from 2–3 s to *ca.* 2.8 h for separation ratios of 1.12 (a very simple separation) to 1.03 (a moderately difficult separation). An analysis time of 2.8 h appear long, but if the critical pair has a separation ratio of 1.03 and the maximum inlet pressure available is 6000 p.s.i. then this must be tolerated as no other column will provide a faster analysis.

Long analysis times appear as anathema to most chromatographers and many seem to think that by some clever design of column all mixtures, however complex, can be separated in a few minutes. Nothing could be farther from the truth. The cost of a chromatographic separation is paid for in two "currencies", time and pressure. This

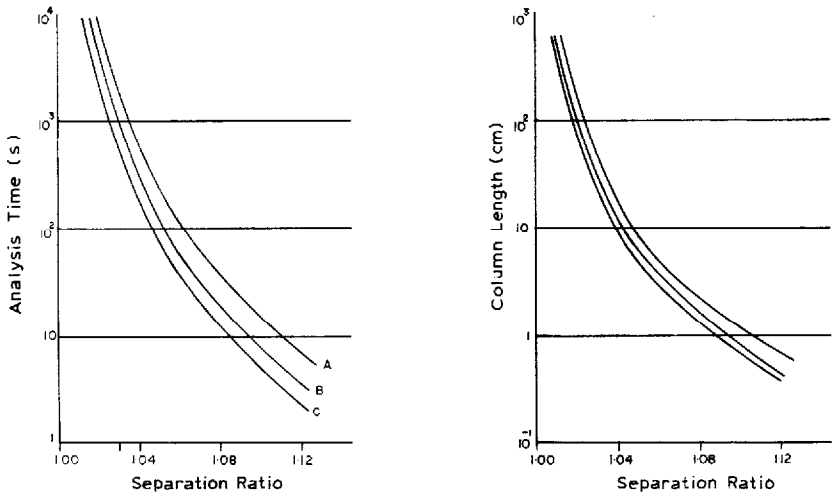


Fig. 5. (1) Graphs of analysis time obtained by the use of optimum diameter particles against separation ratio. (2) Graphs of column length against separation ratio for columns packed with particles of optimum diameter. Key: A = 2000 p.s.i.; B = 4000 p.s.i.; C = 6000 p.s.i.

was clearly stated by Golay[16] in 1960 when he introduced the Performance Index and, as the inlet pressure of any chromatographic system has a practical limit, time is the only variable left to expend to ensure resolution. *The impatient chromatographer must seek a simple sample.*

The relationship between the optimum column radius and the separation ratio of the critical pair is given by eqn. 16 and is graphically represented in Fig. 6. The standard deviation (S.D.) resulting solely from extra column dispersion was taken to be $2.5 \cdot 10^{-3}$ ml.

Fig. 6 shows that the optimum column radius increases linearly with the separation ratio of the critical pair. The optimum radius (which will depend on the extra column dispersion of the apparatus) ranges from about 5 mm for a separation ratio of 1.12–0.5 mm for a difficult separation of 1.02. Thus, complex mixtures that are *difficult* to separate would be carried out on long, thin columns and *simple* separations carried out on short, wide columns.

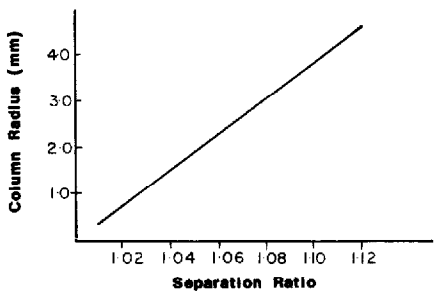


Fig. 6. Graph of optimum column radius against the separation ratio of the critical pair.

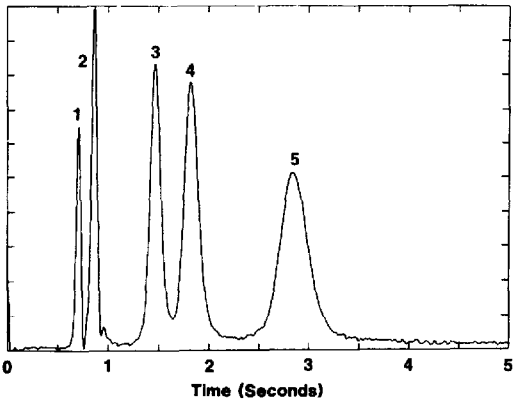
EXAMPLES OF EXCEPTIONAL COLUMN PERFORMANCE

Very high-speed separations and high-resolution columns can only be realized by designing the column to be optimum, or near optimum for the particular analysis to be carried out. An example of a high-speed separation taken from the work of Katz and Scott[17] and carried out on a near optimum column is shown in Fig. 7.

It is seen that the separation of five solutes is accomplished in less than 4 s. The column used was 2.5 cm long and this was the shortest that could be packed efficiently by the equipment available at that time. The particle diameter was 3 μm and this was also the smallest diameter packing available that had a sufficiently narrow particle size distribution for quality packing. The column diameter of 2.6 mm was appropriate for the extra column dispersion present in the instrument. The separation ratio of the critical pair (peaks 3 and 4) had a separation ratio of 1.5 and thus required particles of less than 1 μm in diameter for optimum performance. This would also require a column length of less than 1 cm. As a consequence the actual column used would have had an excess of efficiency for the separation required, if operated at the optimum velocity. Thus, to achieve a rapid separation, the linear velocity was increased to 3.3 cm/s (the rationale for this methods of operation has been previously discussed). This held the speed record in LC for a number of years.

Separations such as this, although demonstrating one aspect of the efficacy of the technique, are really part of chromatography 'show biz' as there are very few application where analyses of this speed are required. Perhaps such speeds might find use in work associated with fast reaction kinetics, or continuous toxicity monitoring, but in most analytical laboratories, results produced at this speed would provide an embarrassing problem of accurate interpretation and sensible subsequent action.

Very fast separations in LC can only be achieved for simple mixtures where there is little chromatographic challenge, that is to say, for mixtures were the critical pairs have large separation ratios. For example, the first pair of peaks in Fig. 7 has a separation ratio of *ca.* 5, and thus, even at the low k' value for the second peak of 0.2 very few theoretical plates are required to effect a separation. In Fig. 8, the first two



	k'	N	N/s
1 p-xylene	0	1100	1450
2 Anisole	0.2	1080	1200
3 Nitrobenzene	1.0	840	560
4 Acetophenone	1.5	800	430
5 Dipropyl Phthalate	2.9	450	160

Fig. 7. High-speed chromatography. Packing, Hypersil 3 μm; column, 2.5 cm × 0.26 cm I.D.; Linearly Velocity 3.3 cm/s. N = column efficiency.

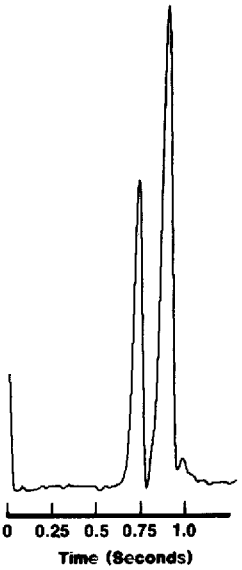


Fig. 8. Subsecond separation on a 2.5-cm column. Peaks: 1 = *p*-xylene, 2 = anisole.

peaks are shown as an isolated chromatogram and includes some analytical data taken from the same woerk of Katz and Scott[17] and are given in Table I.

It is seen that the separation is complete in about 900 m and the first peak is eluted in about 750 ms. Although the separation was very fast, demonstrating the limits of chromatographic speed at that time (1982), it would have very little practical use for the same reasons as those given for the parent chromatogram. It is interesting to note, however, that retention time and area precision of measurement would be quite satisfactory for many analyses, if such speeds were ever called for.

At the other extreme, very-high-efficiency columns, capable of resolving very difficult mixtures, will exhibit analysis times of many hours. In Fig. 9 a chromatogram from a column providing 160 000 theoretical plates shows the separation of a sample of cinnamon bark oil.

The column used was 10 m × 1 mm I.D. It was packed with particles of 20 μm in diameter, which is close to the optimum particle size for this length of column. The flow-rate employed was 38 μl/min, which was significantly above the optimum. At the optimum flow-rate the column gave 250 000 theoretical plates which is the efficiency to

TABLE I

CHROMATOGRAPHIC DETAILS FOR THE SEPARATION SHOWN IN FIG. 8.

	Normalized peak area			*Retention time (ms)*		
	Mean (6 runs)	*σ (S.D.)*	*%σ (R.S.D.)*	*Mean (6 runs)*	*σ (S.D.)*	*%σ (R.S.D.)*
p-Xylene	44.82	0.54	1.2	730	2.2	0.4
Anisole	55.2	0.55	1.0	891	5.9	0.7

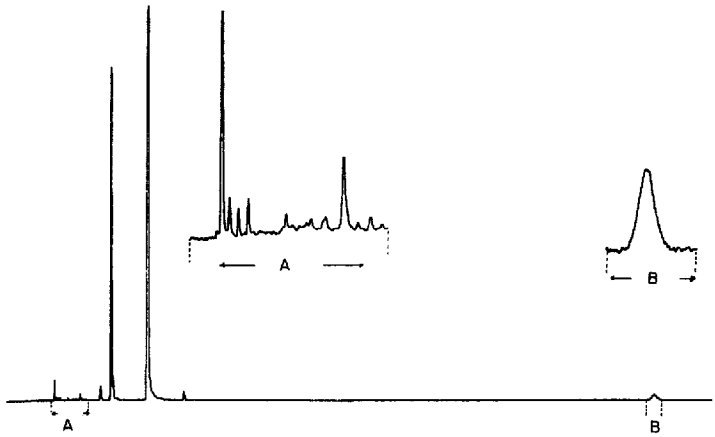


Fig. 9. Chromatogram of cinnamon bark oil. Column, 10 m × 1 mm I.D.; packing, Partisil 20; mobile phase, ethyl acetate–*n*-heptane (3:97, v/v); sample volume, 0.5 μl, flow-rate, 38 μl/min.

be expected from a 10-m column packed with 20-μm particles. The analysis time was over 52 h, but this was largely a result of the large k' value of the last eluted peak. It is interesting to note from the enlargement of the last peak, that even when eluted at a k' of about 50, the peak profile is still symmetrical. The enlargement of the early part of the chromatogram emphasizes the high resolution that can be obtained from the column. The last two, small peaks, shown in the enlargement are very well resolved and have a separation ratio of only about 1.07, the first solute being eluted at a k' value of less than unity.

Thus, modern chromatographic theory provides the equations necessary to predict the optimum column design and operating conditions necessary to resolve any given sample mixture. Unfortunately, due to the practical constraints of the apparatus and the limited availability of certain packing materials, it is often not possible to fabricate the optimum column. Furthermore, even if the column is fabricated, the separation may take an impossible length of time to complete. It follows that it is necessary to know the practical limits of column dimensions and operating conditions and, equally important, how these limits restrict the range of sample complexity that can be satisfactorily handled by LC analyses.

THE COLUMN OF THE FUTURE

Future LC columns of the conventional type, employing pressure-induced flow-rates will, perhaps a little disappointingly, not differ greatly from those used at present. Analysis times may be reduced to a few milliseconds for the separation of solutes having relatively large separation ratios, by employing small columns packed with the smallest available particles and operated at very high mobile phase velocities. However, the areas of application of such columns will, indeed, be very limited. At the other end of the scale, 1 or 2 million theoretical plates are obtainable from long, thin columns, packed with particles of 20 or 30 μm in diameter, but these will involve analysis times extending over several days if real samples are to be analyzed.

In Table II the properties of a number of optimized columns, suitable for the

TABLE II

PROPERTIES OF SOME OPTIMIZED LC COLUMNS

Separation ratio	*Column efficiency*	*Particle diameter (μm)*	*Column length (cm)*	*Column radius (cm)*	*Analysis time*
1.001	$3.1 \cdot 10^7$	110	615 016	$3.5 \cdot 10^{-3}$	84 years
1.005	$1.3 \cdot 10^6$	22	7667	0.02	7.2 days
1.010	313 000	11	613	0.035	10.4 h
1.050	12 500	2.2	4.9	0.175	1 min
1.100	3140	1.1	0.6	0.35	3.8 s
1.200	784	0.5	>0.1	0.73	270 ms

separation of samples covering a wide range of difficulty, are given. These results were obtained by the use of the equations previously presented. The basic data used in the calculations are the same as those previously defined, except for pressure, which was taken as 3000 p.s.i.

The results shown in Table II clearly indicate the practical range of separations that are amenable to LC analysis. It is obviously that a column 6 km long with an analysis time of 84 years would only be useful in some "time dimension" other than our own. In fact, the column 76 m long, less than 1 mm in diameter with an analysis time of over 7 days is only just feasible. Furthermore, the problem would, indeed, have to be very important, if its construction and use were to be justified. The real practical column limit for high resolution starts with the 6-m column, just under 1 mm in diameter, packed with 11-μm particles and requiring an analysis time of about 10 h. This column would separate samples where the separation ratio for the critical pair was as low as 1.01. At the other extreme, a column less than 1 mm long, packed with 0.5-μm particles and providing separations in less than 300 ms is also not practical tp construct or operate, and the availability of closely graded particles, less that 1 μm in diameter is, at best, a speciality at this time. In fact, particles less than 2 μm in diameter are still somewhat of a novelty. As a consequence, the fastest practical column for the separation of relatively simple mixtures, is probably the 5-cm column, *ca.* 4 mm in diameter, packed with 2.2-μm particles, which would complete separations in about one min. In fact, a family of three or four columns, spanning the range of separation ratios between 1.01 and 1.10 would be the most practical for analytical purposes. However, before this column family is considered, there should be some discussion on the maximum inlet pressures to the column. In the calculations necessary to provide the data given in Table II, an inlet pressure of 3000 p.s.i. was assumed. Pumps are readily available that will provide pressures of 6000 or even 10 000 p.s.i. but, unfortunately, it is not the pump that controls the operating pressure of the column system. The sample valve is the component most liable to leakage at high pressures, particularly after prolonged use. Valves are also manufactured for use at 6000 p.s.i. and even more, but unfortunately, in continuous use, the life of such valves tend to be limited. Nevertheless, most commercially available valves will work for long periods at 3000 p.s.i. without leaking, and for this reason, this pressure was chosen as appropriate for the calculation. Finally, it must be said that columns a little outside the range

TABLE III

PRACTICAL FAMILY OF COLUMNS FOR GENERAL LC APPLICATIONS

	Separation ratio	*Column efficiency*	*Particle diameter (μm)*	*Column length (cm)*	*Analysis time (min)*
1	1.02	78 400	5.5	77	40
2	1.03	34 840	3.7	23	8
3	1.05	12 500	2.2	4.9	1

suggested could well be practical, if higher pressures could be used, long analysis time were tolerated, or smaller particles became readily available, together with satisfactory packing procedures for them. Higher pressures would result in considerable heat generation, so the column would need to be well thermostated with fluids of high heat capacity.

The following set of three columns is recommended to cover a practical range of LC separations (Table III). They can be packed with either silica gel or a bonded phase of choice.

The columns are not available as standard items, and both columns 1 and 2 would probably need to be packed in separate lengths and then joined. Column 1 could be packed in three 25-cm lengths and column 2 in two 12-cm lengths. Particle sizes close to those required for columns 1 and 2 are readily available and the 2.2-μm particles for column 3 are becoming available through certain manufacturers. Optimum column diameters are not given, as they will depend on the type of chromatograph with which the columns are to be associated. It is likely that a compromise value for the column diameter that might be satisfactory for the majority of instruments would be 2 mm. It should be emphasized, however, that the extra-column dispersion of the instrument should be known and the optimum column diameter should be calculated and used. Finally, it must be said that for routine analyses, where the same sample is analyzed many times over long periods, then it is well worth identifying the optimum column for the particular analysis, employing the equations given above, and have it custom-made. The result would be significant economic savings in both time and solvent consumption.

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