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LIQUID CHROMATOGRAPHY COLUMN DESIGN

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SUMMARY

A liquid chromatography column design protocol is described utilizing three data bases which are defined as performance criteria, instrument constraints and elective variables. The optimum column length, column radius and particle size of the packing to provide minimum analyses time can be calculated from the information contained in these three data bases. An explicit equation is also derived to permit the optimum particle diameter to be calculated. It is shown that if the inlet pressure is limited, *small* particles are only suitable for use in short columns for simple separations. Conversely, difficult separations can only be achieved with larger particles packed in long columns. In liquid chromatographic analyses, operating at 6000 p.s.i. as opposed to 4000 p.s.i. results in a proportional reduction in analysis time (about 30%). It follows that a maximum inlet pressure of 4000 p.s.i. appears to be quite adequate and is to be recommended for general liquid chromatographic analysis. The optimum k' value of the first solute of the critical pair of a complex mixture can range between 2 and 6; furthermore the optimization procedure compensates for changes in diffusivity by corresponding changes in optimum particle diameter and optimum column length. Consequently the numerical value of solute diffusivity is not critical. The quality of the packing remains important even for fully optimized columns and consequently, the best packing procedures should always be employed.

INTRODUCTION

Liquid chromatography is now a mature analytical technique that is based on a well developed theoretical background and as a consequence is carried out with fairly efficient liquid chromatographic equipment. The high level of performance achievable by liquid chromatography today, however, is the direct result of over two decades of careful study into the basic theory of chromatographic separations. Explicit equations are now available to calculate the optimum physical properties of the column that are necessary to achieve a particular separation in a minimum time together with the best method of operation. Even now, however, the various aspects of chromatographic theory have not been brought together to provide a comprehensive protocol for column design. It is the purpose of this paper to draw together the various aspects of chromatographic theory and to manipulate the various explicit equations available to provide such a practical column design protocol.

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The design concept

A successful chromatographic analysis is the result of a separation process involving a large number of interacting variables, some of which are under the control of the chromatographer, some of which are not. The nature of the sample presented for analysis, the sample throughput of the analytical service and the cost effectiveness of the service laboratory, all make their individual demands on the chromatographic system, and in particular, the design of the chromatographic column. The needs of the analyst, therefore, have to be known before the column can be designed and these needs can be defined under the title of performance criteria. The performance criteria must state explicitly (where appropriate in numerical form) the requirements of the analyst. There are three data bases necessary for column design and the performance criteria demanded by the analyst is the first of these sources of design data.

The chromatograph employed for the analysis will have operating specifications that have been determined by the design and method of manufacture of the instrument and will probably differ significantly from one manufacturer to another. The specifications of the instrument set by the manufacturer control and limit the ultimate performance obtainable from the column with which it is used. It is likely that the specific instrument characteristics that control the overall system performance will be maintained sensibly constant by the manufacturer for the lifetime of the product. This would allow any column system designed for optimum use with the instrument also to have a reasonable life span. The instrument specifications provide the second data base necessary for optimum column design and is given the term instrument constraints. It is important to realize, and this will become increasingly apparent, that it is the instrument constraints that ultimately control and limit the optimum performance of the column.

Finally, the analyst is left with some choice in the strategy that is to be used in the analysis by way of the chromatographic media selected, and in the level of some operating variables that are considered appropriate and necessary. However, as most are defined under performance criteria and instrument constraints the analyst is not left with a very wide choice of variables to select from. This might be considered an advantage, however, as the fewer decisions that are left in the hands of the operator, the less skill is required and thus the cost of staff is reduced and the analytical service becomes more cost effective. Nevertheless, the range of variables left to the choice of the analyst constitutes the third data base for column design and will be termed elective variables. The column is thus designed and the operating conditions identified from the information provided by the three data bases. Furthermore, a given set of column specifications that has been derived on the basis of these three data sets will also provide a complementing set of analytical specifications. A diagram representing the overall column design protocol is shown in Fig. 1.

Performance criteria

Chromatography is a separation technique and a satisfactory chromatographic analysis demands, *a priori*, the appropriate separation of the sample into its constituents. In order to achieve this separation, the appropriate phase system must be chosen to move the individual components apart from one another. The column must then be designed with sufficient efficiency that the pair of solutes which elute closest together (the critical pair) are eluted discretely. The pair of solutes in the mixture that

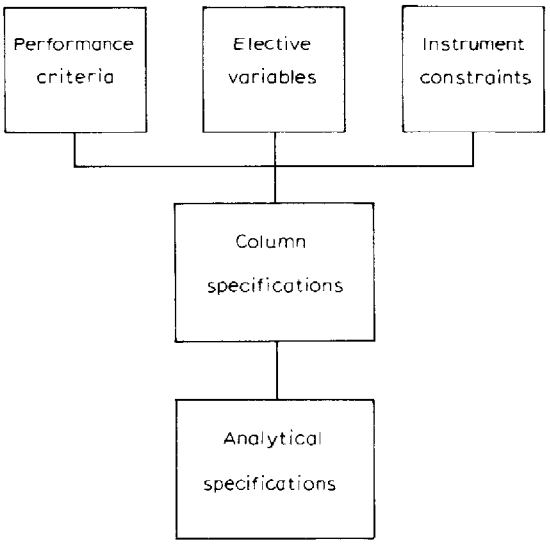

Fig. 1. Column design protocol.

is most difficult to separate determines, therefore, the resolution demanded of the column. It follows that the resolution, defined in an appropriate numerical form, constitutes the first performance criterion. The efficient analytical laboratory manager also requires the maximum possible throughput of samples and thus the second performance criterion is that the separation is accomplished in the minimum time. The laboratory should also operate as economically as possible and therefore the consumption of solvent should also be minimal. Finally to conserve sample and to be able to analyse trace materials the maximum mass sensitivity should be maintained. The performance criteria can therefore be summarized:

(1) A defined resolution must be obtained
(2) The analysis must be completed in the minimum time
(3) The analysis must be completed with the minimum solvent consumption
(4) The maximum mass sensitivity must be achieved.

Instrument constraints

Certain operating limits are inherent in any liquid chromatograph, these limits may vary with the purpose for which the instrument is intended. For example the preparative chromatograph will have very different operating characteristics from those of the analytical chromatograph. The first and obvious operating limit will be the maximum column inlet pressure that the pump will provide. It will be seen that the magnitude of the maximum inlet pressure available will determine the optimum column length, the optimum particle diameter of the material with which it can be packed and as a consequence the minimum analysis time. In a like manner, the maximum and minimum flow-rates that the pump can provide, will, under certain circumstances determine the column diameter that can be employed to maintain a given linear mobile phase velocity. In this dissertation, however, the limitations of maximum/minimum flow-rate will not be examined. Another extremely important

instrument specification is the total dispersion that takes place in the sample valve, connecting tubes and detector cell. It is clear that there would be no point in designing a column to elute peaks that were less dispersed than that which would result from instrument dispersion alone. Under such circumstances the resolution obtained would be determined by the instrument dispersion and not the column. Instrument dispersion is one of the most important instrument specifications to be taken into account in column design as it is the major factor that determines column radius and consequently solvent consumption. Finally, the speed of response of the overall detecting system needs to be specified and utilized in the overall instrument design. However, the influence of detector time constants on high speed separation has already been dealt with by Katz and Scott[1] and so will not be repeated here. The instrument constraints can therefore be tabulated as follows:

(1) The maximum inlet pressure
(2) The extra-column dispersion
(3) The minimum flow-rate
(4) The maximum flow-rate
(5) The response time of the detecting system

Elective variables

The choice of variables remaining with the operator, as stated before, is somewhat restricted and in fact is confined to the selection of the phase system. Preliminary experiments must be carried out to identify the best phase system that will separate the mixture under consideration. The best phase system will be that which provides the greatest separation ratio for the critical pair of solutes and at the same time ensure a minimum value for the capacity factor for the last eluted solute. Unfortunately, at this time theories that predict the optimum solvent system that should be used to separate a given solute mixture are sparse and can be highly inaccurate to say the least. *Ipso eo* the best phase system is usally obtained by an experimental procedure involving much trial and error. Nevertheless, on arriving at a satisfactory phase system there are still a few choices left to the user some of which can be very important. It will be seen later, for instance, that solvent viscosity plays an important part in controlling analysis time. Consequently, where possible a phase system should be chosen that not only provides the necessary selectivity but also provides a solvent mixture of low viscosity; a mixture of *n*-hexane and methyl acetate would be preferable to a mixture of *n*-heptane and ethyl acetate both being capable of providing a comparable selectivity. In a similar manner the phase system will also control the diffusivity of the solute in the molecular phase. The elective variables available to the operator can therefore be summarized as follows:

(1) The separation ratio of the critical pair
(2) The capacity factor of the first peak of the critical pair
(3) The separation ratio of the last peak to the first of the critical pair
(4) The solvent viscosity
(5) The solute diffusivity

Column specifications and operating conditions

Employing the conditions defined in the three data bases together with established chromatographic theory, the physical properties of the column and the con-

ditions under which it should be operated can be calculated to meet the defined performance criteria. Assuming therefore, that the separation ratio of the critical pair is to be defined in the elective variables, the minimum column length and particle diameter must be calculated to provide the required resolution and the minimum analysis time. In order to meet the performance criteria of maximum mass sensitivity and minimum solvent consumption, the column radius must also be calculated. The flow-rate and linear mobile phase velocity automatically follow from the previously determined optimum column conditions and the maximum inlet pressure available is defined under instrument constraints. However, the magnitude of both the column flow-rate and the linear mobile phase velocity would be conveniently included in column specifications and operating conditions. The column specifications and operating conditions can therefore be tabulated as follows:

(1) Column length
(2) Optimum particle diameter
(3) Column radius
(4) Column flow-rate
(5) Linear mobile phase velocity

Analytical specifications

The analytical specifications must prescribe the ultimate performance in appropriate numerical values to ensure that the performance criteria have been achieved. The separation of the critical pair would require a given column efficiency and therefore this should be given for the optimum column in theoretical plates. The second most important analytical requisite will be that the separation is achieved in the minimum analysis time and, therefore, the analysis time must also be reported under analytical specifications. The third most important criterion is that the solvent consumption should be minimal and therefore the solvent consumption per analysis should also be specified.

Finally, for convenience, the total peak capacity that is achieved by the optimized chromatographic system needs also to be quoted. It follows that the analytical specifications can be reported as follows:

(1) Column efficiency in theoretical plates
(2) Analysis time
(3) Solvent consumption for a single analysis
(4) Total peak capacity

The basic chromatographic theory pertinent to column design

The pertinent and valid equations that are used in column design have been developed sporadically over the last 20 years or so. The first equation of primary importance was reported by Purnell[2] for gas chromatography (GC) in 1959 and gave an expression for the number of theoretical plates, N, necessary to effect a given separation. The equation derived by Purnell for GC is equally pertinent for liquid chromatography, *viz.*

$$N = [4(1 + k')/k'(\alpha - 1)]^2 \quad (1)$$

where k' is the capacity ratio of the *first* of the initial pair of eluted solutes and α is

the separation ratio of the critical pair of solutes. The analysis time, t, required to effect the separation is given by

$$t = (1 + k'_1)\, L/u \tag{2}$$

where L is the length of the column, u is the linear mobile phase velocity and k'_1 is the capacity ratio of the last eluted peak.

Eqn. 2 has been put forward in different ways by a number of workers in the field in the early days of GC for example Desty and Goldup[3] and Scott and Hazeldean[4].

As a result of the definition of the height of the theoretical plate, H:

$$L = NH \tag{3}$$

Now there have been a number of explicit equations developed describing H in terms of the basic physical properties of the chromatographic system which in chronological order were those of Van Deemter *et al.*[5] (1956), Giddings[6] (1961), Huber and Hulsman[7] (1967), Kennedy and Knox[8] (1972) and Horváth and Lin[9] (1976). In 1975, Halasz *et al.*[10] published results suggesting that the Van Deemter equation was probably the most accurate form of the equation for H and this was confirmed more recently by an extensive number of measurements made by Katz *et al.*[11]. It follows that for column design the equation of Van Deemter will be used, *viz.*

$$H = A + B/u + Cu \tag{4}$$

where $A = 2\lambda d_p$, $B = 2\gamma D_m$, $C = f(k')\, d_p^2/D_m$ (λ and γ are constraints), d_p = particle diameter, D_m = diffusivity of the solute in the mobile phase and u = linear velocity of the mobile phase. It should be noted that the C term only includes a resistance to mass transfer factor in the mobile phase as Katz *et al.*[11] have shown that the resistance to mass transfer in the stationary phase has a minimal contribution to the plate height.

From eqns. 3 and 4:

$$L = N(A + B/u + Cu) \tag{5}$$

Another important equation used in column design is that of D'Arcy that describes the flow of fluid through a packed bed

$$L = \psi P d_p^2/\eta u \tag{6}$$

or

$$L = DP/u \tag{7}$$

where $D = \psi d_p^2/\eta$ (η = the viscosity of the solvent and ψ = constant) and P is the column inlet pressure. Consequently if the efficiency N is to be achieved with a column length of L, then by equating eqns. 5 and 7 and solving for u:

$$\bar{u} = A/2C \pm [A^2/4C^2 - 4(B/C - DP/N)]^{1/2}/2 \tag{8}$$

where $\bar{u}$ is the linear velocity required to achieve the separation in the minimum time with a limited inlet pressure of P. Eqns. 1, 2, 5 and 8 are fundamental in column design. In the subsequent calculations the following established practical values for the pertinent variables were assumed[11] unless otherwise stated: $\lambda = 0.5$, $\gamma = 0.6$, $D_m = 3.5 \times 10^{-5}$ cm^2/sec, $\eta = 0.025$ P, $\psi = 35$, $k' = 2.5$ and $f'(k') = (1 + 6k' + 11k'^2)/24(1 + k')^2$

CALCULATIONS, RESULTS AND DISCUSSION

The effect of particle size on analysis time

Employing eqn. 8 to calculate the mobile phase linear velocity, $\bar{u}$, by assuming a given column inlet pressure, the column length, L, and consequently the analysis time, t, was calculated using eqns. 5 and 6 respectively. Calculations were performed for particles of three different sizes over a range of separation ratios from 1.01 to 1.08. The results of these calculations are shown in Fig. 2 where the analysis time is plotted against separation ratio for particle sizes of 3, 5 and 10 μm. It is seen quite clearly that the analysis time increases very rapidly as the separation ratio of the critical pair becomes smaller, *i.e.*, as the separation becomes more difficult. The rapid increase in analysis time with reduction in the α value of the critical pair is no more than would be expected. The effect of particle diameter however is not so obvious. It is seen that particles 3 μm in diameter provide the shortest analysis time for separation ratios down to about 1.03. Solute pairs having separation ratios between

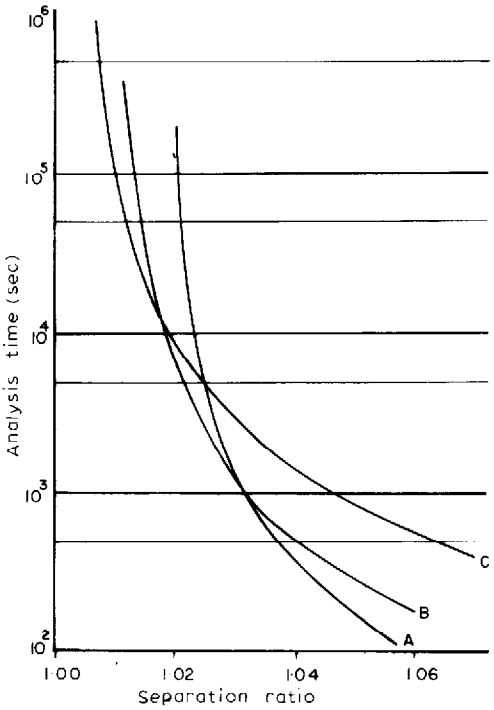

Fig. 2. Graphs of analysis time against separation ratio for columns packed with particles of different diameters: A, 3; B, 5; C, 10 μm. Inlet pressure: 3000 p.s.i.

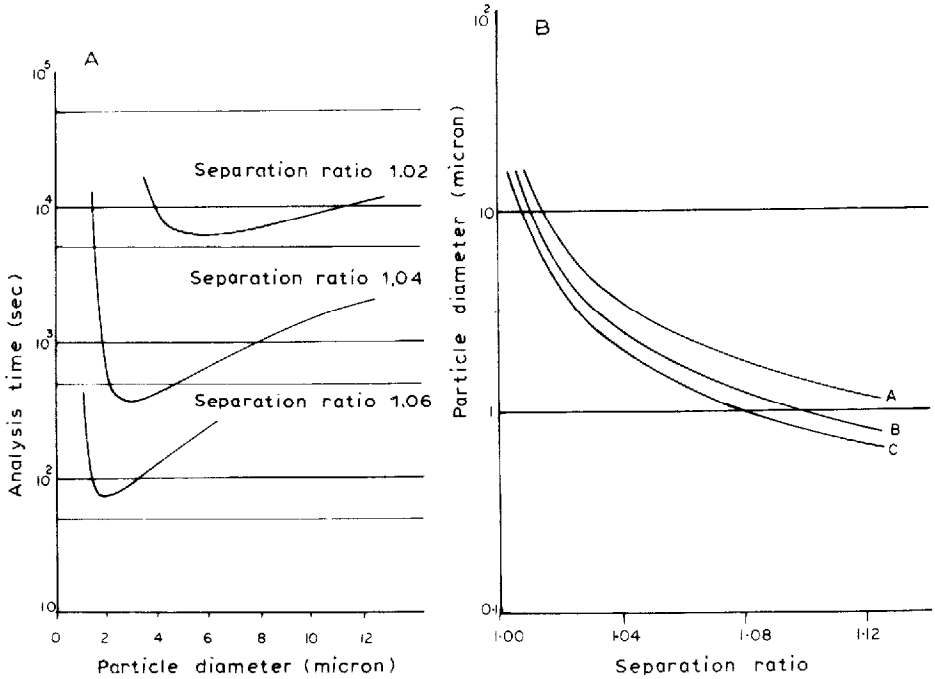

Fig. 3. A, Graphs of analysis time against particle diameter for the separation of different solute pairs having different separation ratios. B, Graphs of optimum particle diameter for minimum analysis time against separation ratio. Inlet pressure: 2000 (A), 4000 (B), 6000 (C) p.s.i.

about 1.02 and 1.03 will be separated in the shortest time by employing particles 5 μm in diameter whereas solute pairs having separation ratios 1.01 and 1.02 would require particles having diameters of 10 μm to achieve the separation in the minimum time. The more difficult the separation (the lower the separation ratio) the larger must be the particle diameter for minimum analysis time. This is a direct result of having a limited inlet pressure; as the separation becomes more difficult, the more theoretical plates are required to effect the separation and consequently the longer the column must be. *Ipso eo* if the pressure is limited then the particle size must be increased to permit the necessary solvent flow through the longer column. The natural corollary of this is that there will be an optimum particle diameter for any given separation that will permit the analysis to be completed in the minimum time.

Employing the same equation, curves relating analysis time to particle diameter were constructed for solute pairs having different separation ratios 1.02, 1.04, and 1.06 and the results are shown in Fig. 3A. It is seen that indeed there is an optimum diameter that provides the minimum analysis time for a given solute pair. It is also apparent that the minimum in the analysis time curve is much sharper for small particles separating simple mixtures than for larger particles separating more difficult mixtures (α = 1.02). Consequently for optimum performance in terms of analysis time the particle diameter is more critical for simple separations than for the more difficult separations. Employing an iterative technique and with the aid of the ubiquitous computer, graphs can be constructed relating optimum particle diameter for

minimum analysis time to the separation ratio of the critical solute pair. The results obtained from such calculations for three different inlet pressures are shown in Fig. 3B. It is seen that very small particles of 1 or 2 μm in diameter should only be used for very simple separations whereas the high efficiencies necessary for the separation of difficult mixtures require the use of particles having relatively large diameters. The majority of separations carried out today have separation ratios for the critical pair of 1.1 or even more. It is seen from Fig. 3B that for optimum performance the particle diameter should be $\leqslant$ 1 μm. Particles 1 μm in diameter are not available at present and neither are packing procedures developed for use with them. It follows that the smallest particles available, namely 3 μm, will have to be used which also means that optimum performance cannot be achieved at present for simple separations. It should also be noted that raising the inlet pressure from 4000 to 6000 p.s.i. has a relatively small effect on the magnitude of the optimum particle diameter.

Chromatographic performance when operating with optimum particle diameters

Curves relating analysis time and separation ratio together with those relating column length with separation ratio for columns packed with particles of optimum diameter are shown in Fig. 4A and 4B. The values were calculated using the same equations and the same iteration procedure as that described previously. Curves were constructed for three different inlet pressures 2000, 4000 and 6000 p.s.i. It is seen that minimum analysis time ranges from 2 or 3 sec when separating solute pairs having separation ratios of 1.12 to 3 h for the separation of a solute pair having a separation ratio of 1.01. It is also interesting to note that increasing the inlet pressure from 4000 to 6000 p.s.i. only reduces the analysis time by about 30%. Such an improvement

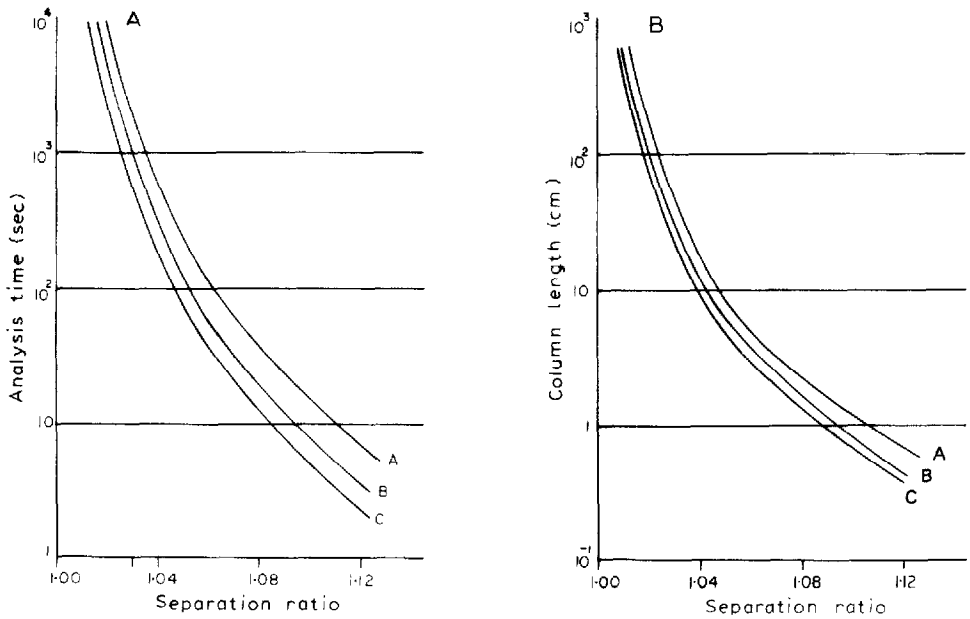

Fig. 4. A, Graphs of analysis time obtained by the use of optimum diameter particles against separation ratio. B,Graphs of column length against separation ratio for a column packed with particles of optimum diameter. Inlet pressure: 2000 (A), 4000 (B), 6000 (C) p.s.i.

may well not be worthwhile considering the price to be paid in terms of both instrument complexity and cost. At lower pressures fewer demands would be made on pump seals, non-return valves and sample valves making the equipment easier and less costly to make. If particles of optimum, or near optimum size are employed, 4000 or even 3000 p.s.i. inlet pressure may prove to be an excellent compromise between that which is theoretically desirable to that which is practically acceptable.

It is interesting to calculate the linear mobile phase velocity that is used with the particles of optimum diameter to achieve the minimum analysis. Knox and Saleen[12] suggested that the minimum analysis time could only be obtained using the optimum linear mobile phase velocity but as they employed an empirical equation for H it was not possible to confirm this.

Differentiating eqn. 4 with respect to u and equating to zero it can easily be shown that:

$$u_{opt} = [2\gamma D_m^2/f'(k')d_p^2]^{1/2} = (B/C)^{1/2} \quad (9)$$

During the iteration procedure by the computer the value of $\bar{u}$ can be calculated at the optimum particle diameter from eqn. 8 and the optimum mobile phase velocity calculated from eqn. 9. Values of the separation ratio, α, $\bar{u}$ and u_{opt} are given in Table I. It is seen that the contention by Knox and Saleem[12] that the optimum particle diameter must be employed with the optimum linear velocity to provide the minimum analysis time is indeed correct. However, by having an explicit equation for H an equation for the optimum particle diameter can now be obtained. At the optimum mobile phase velocity the value for H is at a minimum and by substituting for u from eqn. 9 in eqn. 4:

$$H_{min} = A + 2(BC)^{1/2} \quad (10)$$

TABLE I

MOBILE PHASE LINEAR VELOCITY BY COMPUTER ITERATION AND BY DIRECT CALCULATION

Separation ratio	*Linear velocity (cm/sec)*	
	From computer iteration	*By direct calculation*
1.01	0.0586	0.0586
1.02	0.1170	0.1173
1.03	0.1759	0.1756
1.04	0.2345	0.2341
1.05	0.2934	0.2924
1.06	0.3517	0.3512
1.07	0.4097	0.4104
1.08	0.4674	0.4699
1.09	0.5262	0.5282
1.10	0.5842	0.5874
1.11	0.6460	0.6428
1.12	0.7035	0.7024

TABLE II

OPTIMUM PARTICLE DIAMETERS BY COMPUTER ITERATION AND BY DIRECT CALCULATION

Separation ratio	*Particle diameter (μm)*	
	From computer iteration	*By direct calculation*
1.01	11.63	11.89
1.02	5.81	5.95
1.03	3.88	3.96
1.04	2.91	2.97
1.05	2.33	2.38
1.06	1.94	1.98
1.07	1.66	1.70
1.08	1.45	1.49
1.09	1.29	1.32
1.10	1.16	1.19
1.11	1.06	1.08
1.12	0.97	0.99

Substituting for H from eqn. 10 in eqn. 3 and equating to eqn. 6, simplifying and solving for d_p it can be seen that:

$$d_{p(opt)} = (2\eta N D_m/\psi P\{\lambda[2\gamma/f'(k')]^{1/2} + \gamma\})^{1/2} \quad (11)$$

Eqn. 11 provides a means of calculating the optimum particle diameter in absolute terms without employing an iterative procedure. In Table II values for the optimum particle diameter calculated by the computer using an iterative procedure are given for a series of values of the separation ratio α, together with the optimum values of d_p calculated by eqn. 11. It is seen that excellent agreement is obtained and that eqn. 11 can be employed with confidence in column design to calculate the optimum particle diameter.

Fig. 4B shows the relationship between column length and separation ratio for columns packed with particles of optimum diameter. It is seen that an optimized column for separating mixtures where the critical pair has a separation ratio of greater than 1.08 is less than 1 cm in length. Once more the practical use of particles 1 μm in diameter packed in columns less than 1 cm long comes into question. It is true that very short columns are fairly easy to pack with small particles but the practical value of reducing the particle size to 1 μm and packing them in a column less than 1 cm long remains to be established.

It is suggested that 2-μm particles packed into columns 2 cm long which would be about optimum for separating solute pairs having a separation ratio of about 1.08 might be the practical limit in both particle size and column length. It is now of interest to examine the effect of other chromatographic parameters on analytical performance when using particles of optimum size.

The effect of capacity factor on analysis time for optimized columns

In Fig. 5A the analysis time is shown plotted against capacity factor, k', for

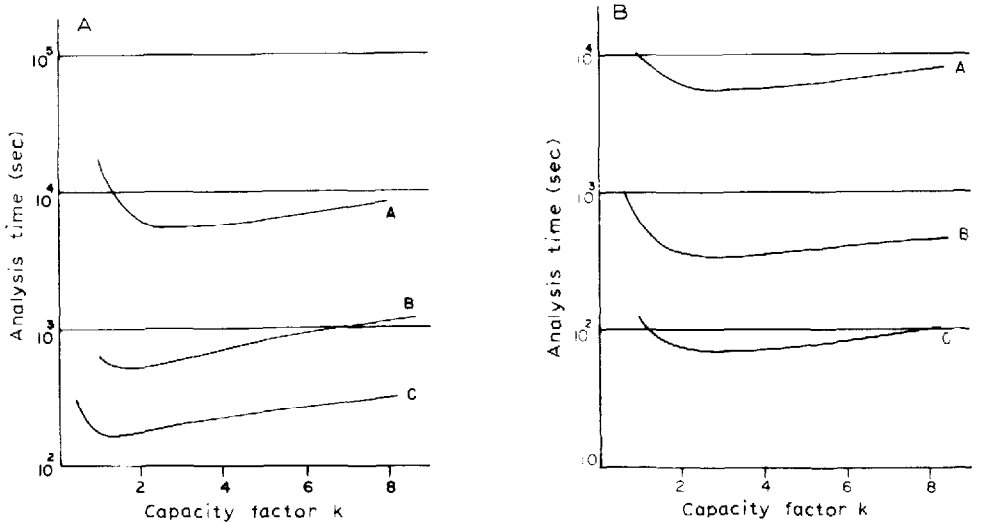

Fig. 5. Graphs of analysis time against capacity factor for solute pairs of different separation ratios (A, d_p = 5 μm) and for columns packed with particles of optimum diameter (B). Inlet pressure: 3000 p.s.i. α = 1.02 (A), 1.04 (B), 1.06 (C).

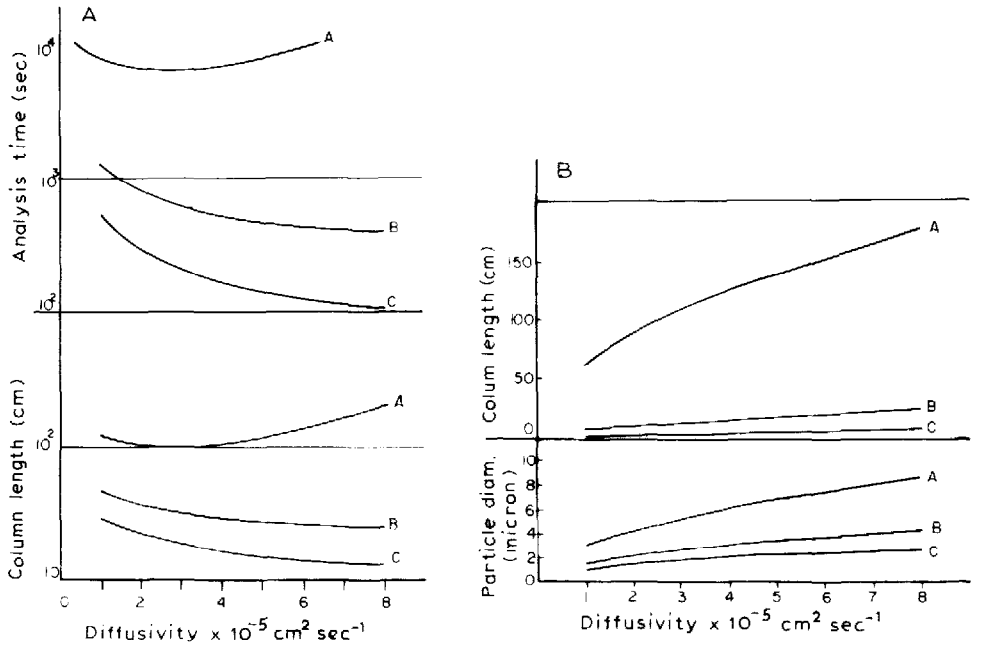

Fig. 6. A, Graphs of analysis time and column length against solute diffusivity for the separation of solute pairs of different separation ratios. Particle diameter: 5μm. Inlet pressure: 3000 p.s.i. Separation ratios: 1.02 (A), 1.04 (B), 1.06 (C). B, Graphs of column length and optimum particle diameter against solute diffusivity for solute pairs of different separation ratios. Inlet pressure: 3000 p.s.i. Curves: A, α = 1.02, analysis time 5970 sec; B, α = 1.04, analysis time 373 sec; C, α = 1.06, analysis time 74 sec.

a non-optimized column packed with 5-μm particles and operated at an inlet pressure of 3000 p.s.i. It is seen that there is a minimum for the analysis time at a particular value of k' and this value ranges from about 1.2 for $\alpha = 1.06$ to about 2.5 for $\alpha = 1.02$. However if the columns are packed with particles of optimum diameter (Fig. 5B) the optimum value of k' becomes sensibly constant at about 2.5–3. Values that compare well with those predicted by Grushka and Cooke[13] and Martin *et al.*[14]. It should also be noted that the curve relating analysis time with k' is very flat subsequent to a k' value of about 2 and so for optimized columns the value of k' is not critical and may be as great as 4 or 5 without significantly increasing the analysis time. This is the result of a lower efficiency being required for resolution at higher k' values and thus a shorter column can be used.

The effect of diffusivity on analysis time for optimized columns

In Fig. 6A the analysis time and column length are shown plotted against diffusivity for an non-optimized column packed with particles 5 μm in diameter and operated at an inlet pressure of 3000 p.s.i. The diffusivity ranges from $1 \cdot 10^{-5}$ to $8 \cdot 10^{-5}$ cm^2/sec which encompasses a molecular weight range of about 1000–60 in a hexane–5% (v/v) ethyl acetate solvent mixture as the mobile phase. It is seen that there are indeed optimum values of D_m for minimum analysis time and the optimum values increase as the separations become simpler (as α changes from 1.02 to 1.06). It is also seen the column length also has a minimum value for a given diffusivity. From Fig. 6B it is seen that for an optimized column the situation is quite different and for a given separation ratio the analysis time remains constant for each value of α and for all values of diffusivity as shown at the top of Fig. 6B. However, as is seen from the two curves relating particle diameter and column length with diffusivity this is due to the fact that with an optimized column both the particle diameter and the column length are increased to compensate for any change in diffusivity and consequently the same analysis time is maintained.

The effect of column packing factors on the performance of optimized columns

The quality of the packing, as opposed to its physical properties is reflected solely in the values of the constants λ and γ in the Van Deemter equation. Various theoretical estimates have been made as to the numerical values of the constants that represent a well packed column. Those of Giddings[15] are probably the best estimates for λ and γ which were 0.5 and 0.6 respectively. Nevertheless, to accommodate a range of values for λ that would encompass the extremes of packing quality a range of values from 0.2 to 1.3 were considered possible. Consequently, curves relating analysis time and multipath factor over the range of $\lambda = 0.2$ to $\lambda = 1.3$ for values of $\alpha = 1.02$, 1.04 and 1.06 and an inlet pressure of 2000 p.s.i. were constructed and shown in Fig. 7A. It is seen that as λ increases and the quality of the packing deteriorates the analysis time increases significantly. It follows that the best packing procedure must always be employed, *i.e.*, λ should be as small as possible. The need for high quality packing is even more important for optimized systems. In Fig. 7B the effect of λ on analysis time is shown for columns packed with particles of optimum diameter and it is seen that the increase in analysis time with λ is even greater and consequently the quality of the packing even more critical. This is particularly true for the simpler separations where $\alpha = 1.04$ or 1.06 and it is clearly seen that the slope of the curves relating analysis time with λ is greater in Fig. 7B than in 7A.

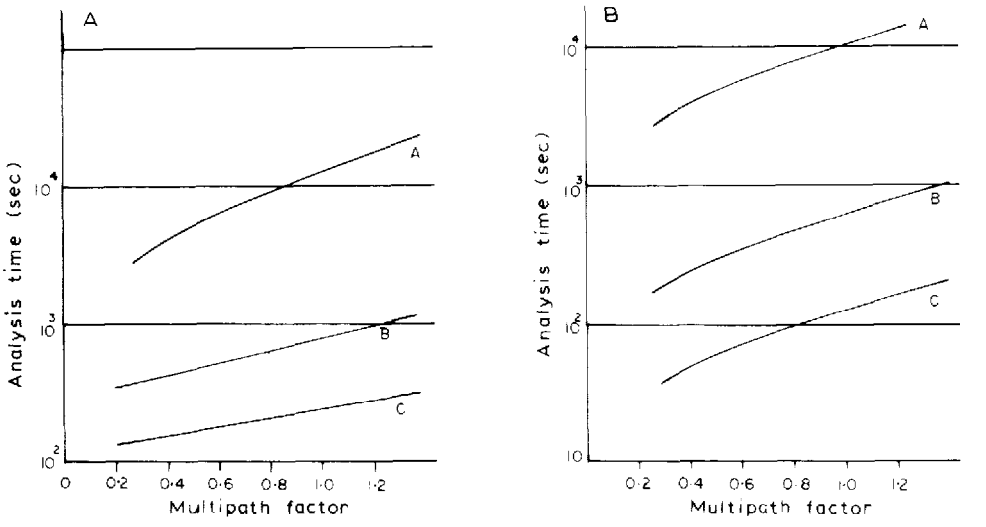

Fig. 7. Graphs of analysis time against multipath factor for solute pairs of different separation ratios (A, inlet pressure 2000 p.s.i., d_p = 5 μm) and for columns packed with particles of optimum diameter (B, inlet pressure 3000 p.s.i.). α = 1.02 (A), 1.04 (B), 1.06 (C).

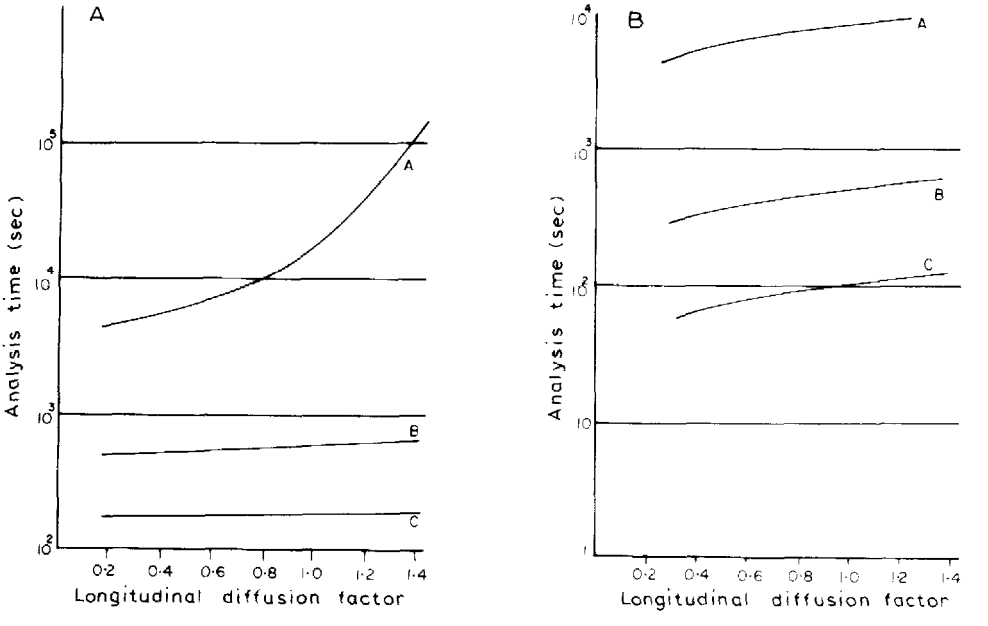

Fig. 8. Graphs of analysis time against longitudinal diffusion factor for solute pairs of different separation ratios (A, d_p = 5 μm) and for columns packed with particles of optimum diameter (B). Inlet pressure: 3000 p.s.i. α = 1.02 (A), 1.04 (B), 1.06 (C).

The effect of the γ factor involved in the longitudinal diffusivity term in the Van Deemter equations is different. In Fig. 8A analysis time is plotted against different values of γ for a non-optimized column packed with 5-μm particles operated at an inlet pressure of 3000 p.s.i. Three curves are shown for values of α of 1.02, 1.04 and 1.06. It is seen that for the simpler separations where $\alpha = 1.04$ and 1.06 the analysis time is virtually insensitive to changes in the value of γ. However the effect of α on analysis time for an α value of 1.02 is very significant indeed, but this curve is where the particle diameter of 5 μm is very far from the optimum value of about 10 μm. In Fig. 8B the effect of the value of γ on the analysis time is shown for a column that is packed with particles of optimum diameter. It is seen that in contrast to the non-optimized column the sensitivity of the system to changes in γ is very similar for all α values. However, the analysis time still increases with γ albeit not to the same extent that the analysis time increases with λ.

The general conclusion, not surprisingly, is that even when optimum particle diameters are employed, the quality of the packing must be high and both γ and λ made as small as possible. It is unfortunate that in practice it has been shown[16] that poor quality packing appears to increase λ to a greater extent than γ, and consequently as the analysis time is more sensitive to large values of λ than to large values of γ the effect of poor quality packing can be very significant.

The peak capacity of optimized chromatographic columns

The peak capacity of a chromatographic system is numerically equivalent to the total number of fully resolved solute peaks that can be fitted into a chromatogram between the dead volume peak and the peak for the last eluted solute. A number of equations have been developed to calculate the peak capacity of a chromatographic system, such as those of Giddings[17] and Scott[18]. More recently, Davis and Giddings[19] pointed out that the theoretical peak capacity is an exaggerated value of the true peak capacity due to the statistically irregular distribution of the individual k' values of each solute. Nevertheless, the theoretical value will be given here as a relative measure of the true peak capacity. The equation that will be used is that of Scott[18] namely

$$\varphi = \log (1 - \{(N)^{1/2} [k'/(1 + k') + 0.5] (1 - P)/4\})/\log P \qquad (12)$$

where φ is the theoretical peak capacity and $P = [N - 2(N)^{1/2}]/[N + 2(N)^{1/2}]$.

In Fig. 9 the upper curve shows the relationship between peak capacity and separation ratio for a column packed with particles of optimum diameter. The relationship is exactly that which one would expect for a non-optimized system; the peak capacity is greatest where the α value is least and consequently, the highest efficiency is available. It is seen clearly from eqn. 12 that the peak capacity increases as the efficiency increases. Furthermore, even for a column optimized for the separation of a solute pair having an α value of 1.12 the peak capacity is still quite significant and about 20.

Column radius

Explicit equations for the calculation of the column radius have been put forward by Reese and Scott[20] and Katz and Scott[1] and can be put in the following form

$$r = [\sigma_A k'(\alpha - 1)/800 d_p \lambda \theta (1 + k')]^{1/2} \qquad (13)$$

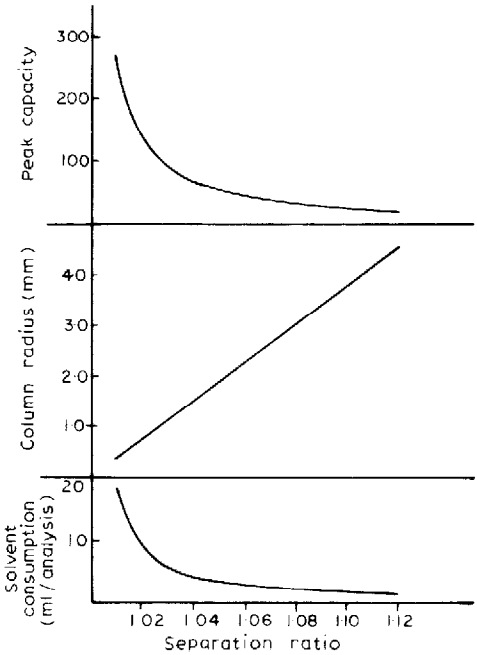

Fig. 9. Graphs of peak capacity, column radius and solvent consumption against separation ratio for columns packed with particles of optimum diameter. Inlet pressure: 3000 p.s.i.

where σ is the standard deviation of the dispersion due to the instrument and θ is the ratio of the volume of the mobile phase to the total column volume; the other symbols have the meanings previously ascribed to them.

In Fig. 9 the center curve relates column radius with separation ratio. It is seen that a linear relationship exists and that to cover the range of α values from 1.01 to 1.08, bearing in mind that a limit of 3 μm in practice is placed on the minimum particle diameter, then a range of column radii between about 0.2 to 3 mm needs to be available. This is very practical range of column diameters for the design of LC columns.

Solvent consumption

The solvent consumption can be simply calculated from the column volume and the capacity factor of the last eluted peak

$$V = \pi r^2 l\theta(1 + k)$$

where V is the volume per analysis and the other symbols have the meanings previously ascribed to them.

The lower graph in Fig. 9 shows the solvent consumption per analysis plotted against separation ratio. It is seen that the solvent consumption increases slowly with separation difficulty as α decreases to about 1.04. Below $\alpha = 1.04$ however, the solvent consumption increases very rapidly and it is therefore extremely important

that for difficult separations the minimum column radius is used otherwise solvent consumption could be extremely excessive. It is also seen that the optimum column diameter for a separation ratio of about 1.01 would be about 0.5 mm; a column that could be quite difficult to pack even with particles 10 μm in diameter (see Fig. 3B).

Practical use of the column design protocol

Explicit equations are now available to calculate all the necessary properties of the optimum column system and consequently a very simple computer program can be written to provide the chromatographer with the dimensions and properties of the column required and the performance that can be expected.

Information is requested in sequence starting with the separation ratio, α, of the critical pair and ending with the column volume factor as follows:

Enter separation ratio of the critical pair? 1.04
Enter capacity factor of first of the critical pair? 3
Enter separation ratio of last peak to the first of the critical pair? 2
Enter resolution required (a value of 4 is normal)? 4
Enter inlet pressure? 3000 p.s.i.
Enter solvent viscosity in poise (*n*-hexane, 0.0026 P; water, 0.01 P)? 0.0026 P
Enter solute diffusivity (benzyl acetate in *n*-hexane is $3.5 \cdot 10^{-5}$)? 0.000035 cm^2/sec
Enter multipath factor (0.5 for a well packed column)? 0.5
Enter longitudinal diffusion factor (0.6 for a well packed column)? 0.6
Enter instrument dispersion (standard deviation in millilitres)? 0.0015 ml
Enter column factor (mobile phase volume/total column volume, normally 0.65)? 0.65

Where appropriate, guidance is given as to the likely values of some of the basic parameters. For example solvent viscosity in poise is requested and the user is reminded that hexane has a value of 0.0026 P and water a value of 0.01 P. After the final entry, the calculations are commenced and the results are tabulated as follows.

First the operator is reminded of the performance criteria.

Performance criteria

Adequate resolution
Minimum analysis time
Maximum mass sensitivity
Minimum solvent consumption

The details of the instrument constraints are then reported.

Instrument constraints

Available column inlet pressure 3000 p.s.i.
Extra column dispersion 0.0015 ml
Multipath factor 0.5
Longitudinal diffusion factor 0.6

The instrument constraints shown are typical and the inlet pressure of 3000 p.s.i. is very common to many chromatographic analyses. The extra column dispersion defined as the standard deviation of the dispersion due to the instrument alone is set at 1.5 μl a level which is also now available commercially.

The elective variables are listed.

Elective variables

Separation ratio of the critical pair 1.04
Capacity factor of the first peak of the critical pair 3
Separation ratio of the last peak to the first of the pair 2
Solvent viscosity in poise (corrected for pressure) 0.002912 P
Solute diffusivity 0.000035 cm^2/sec

The separation ratio of a critical pair of 1.04 is an average to difficult separation; the capacity factor, 3, for the first peak is again general for a well chosen phase system and finally the separation ratio of the last peak to the first of the pair, 2, means the last peak is eluted at a k' value of 6. A solute viscosity of 0.0029 P would be equivalent to that of a 5% ethyl acetate solution in *n*-heptane and a solvent diffusivity of $3.5 \cdot 10^{-5}$ cm^2/sec would be equivalent to that of benzyl acetate in the heptane ethyl acetate solvent.

The computer then types out the column specifications.

Column specifications and operating conditions

Column length 10.6 cm
Column radius 0.17 cm
Optimum particle diameter 2.7 μm
Column flow-rate 0.88 ml/min
Linear mobile phase velocity 0.247 cm/sec

It is seen from the column specifications that the column length will be 10.6 cm and the column I.D. 3.4 mm. The optimum particle diameter would be 2.7 μm which would require a flow-rate of 0.88 ml/min.

Finally the analytical specifications are given.

Analytical specifications

Column efficiency in theoretical plates 17,800
Analysis time 301 sec
Solvent consumption per analysis 4.43 ml
Total peak capacity 65.4
Maximum sample volume $5.22 \cdot 10^{-6}$ μl

The optimized column would have an efficiency of 17,800 theoretical plates. The analysis would be completed in just over 5 min, the solvent consumption per analysis would be 4.4 ml and the total peak capacity would be 65. All these values are still in the range of general practical LC analysis but it should be noted that the separation of a fairly complex and reasonably difficult mixture could be completed in 5 min. This performance could only be achieved with complete optimization of the system where the column design and operating conditions are made completely compatible with the instrument specifications and consequently provide optimum performance.

CONCLUSIONS

Explicit equations can now be derived that permit all the pertinent parameters of a chromatographic system to be calculated including an explicit equation to determine the optimum particle diameter. Equations are also available that predict the performance that will be provided by the optimized column system. It follows that

a complete column design protocol can be developed that can be programmed in an easily usable form into a computer. Consequently, with the aid of such a program the chromatographer can simply and rapidly determine the optimum dimensions of the column, the particle size of the packing and all the pertinent performance data necessary to operate the chromatograph in the most efficient manner.

Certain interesting factors arise from the development of the protocol. There is an optimum particle diameter for any given separation and the more simple the separation the more critical become the particle diameter. Particles of small diameter (1–3 μm) are only suitable for optimum use in relatively short columns for very simple separations (α = 1.08–1.12). In contrast, large particles (10–20 μm) must be used in long columns to achieve difficult separations (α = 1.005–1.010). The optimized column must always be operated at the optimum mobile phase velocity to achieve the minimum analysis time. In the majority of liquid chromatography applications (α = 1.01–1.12) there is a relatively small reduction in analysis time (about 30%) if the inlet pressure is raised from 4000 to 6000 p.s.i. It follows that the strain on instrument design resulting from the need to operate at 6000 p.s.i. may be unnecessary and a maximum inlet pressure of 4000 p.s.i. may be quite adequate. As a result the design of pump seals, non-return valves, sample valves, etc., will be simpler, less costly and more reliable. The optimum k' value for the first solute of the critical pair is not particularly critical but should lie between 2 and 6. The solute diffusivity is compensated for in the relationship between optimum particle diameter and optimum column length and thus the analysis time is virtually independent of the magnitude of the solute diffusivity. The quality of the packing is just as important for an optimized column as for non-optimized columns and thus good packing techniques should always be employed to provide high quality packing.

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