Journal of Chromatography, 316 (1984) 81–93
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMSYMP. 487

EFFECT OF HIGH SPEED ON PEAK CAPACITY IN LIQUID CHROMATOGRAPHY

ELI GRUSHKA
Department of Inorganic and Analytical Chemistry, The Hebrew University of Jerusalem, Jerusalem 91904 (Israel)

SUMMARY

In order to decrease the analysis time of a given chromatographic system, many parameters may need to be changed. These changes can adversely affect the chromatographic performance: in particular, the peak capacity may decrease. The peak capacity is a measure of the number of components that can be resolved in a given time. Using this definition of the peak capacity, various options to obtain shorter analysis times were investigated. It was found that such parameters as particle size, column diameter, mobile phase velocity and column length can be changed so that the analysis time decreases without loss of peak capacity. Optimum procedures for that purpose are given. In the course of the analysis, a new efficiency criterion —the rate of peak capacity production— is defined and examined.

INTRODUCTION

High speed is one objective in the development of modern liquid chromatography (LC). This is apparent from current literature reports[1–5], as well as from trends in instrumentation. The advantages of high speed chromatography, in terms of cost efficiency, are obvious. Erni[5], in particular, has elaborated elegantly on this point. Speed, however, is not without its cost, and the old chromatographic dilemma of time *versus* performance and/or *versus* sample load must be kept in mind. The price paid for fast analysis time can be in terms of inlet pressure, of efficiency and thus resolution, or in terms of peak capacity. While these "prices" can be interrelated, it is, perhaps, prudent to examine each one individually, since they stress different aspects of the chromatographic system. The questions of pressure and plate height have been dealt with by DiCesare *et al.*[1,2], by Erni[5], and by Guiochon[6]. In this communicatioin we shall discuss the effect of decreasing the analysis time on the peak capacity.

The peak capacity, n, is a measure of the number of components that can be resolved, at any resolution level specified by the experimentalist, in a given analysis time, t_n. The concept of peak capacity was introduced by Giddings[7] and elaborated by Horváth and Lipsky[8] and by Grushka[9]. The peak capacity is defined as

$$dn = (4\sigma)^{-1}dt \tag{1}$$

0021-9673/84/$03.00 © 1984 Elsevier Science Publishers B.V.

Assuming gaussian peaks, a resolution of unity and no dependence of the plate number on the analysis time (or on the capacity ratio k'), the expression for n becomes

$$n = 1 + \frac{1}{4}\sqrt{N}\ln(t_n/t_m) \tag{2}$$

where N is the plate number and t_n and t_m are the retention times of the last solute and of the inert one, respectively.

The peak capacity is an important parameter in evaluating either a chromatographic system or a chromatogram. It is a kind of idealized upper limit of the number of solutes that the chromatograph can handle and still provide useful and unambiguous information. Based on statistical arguments, Davis and Giddings[10] have recently shown that the actual number of peaks in a chromatogram is less than n. However, the peak capacity should be known, if an analysis of the actual chromatogram is to be meaningful. The peak capacity is the ultimate (albeit perhaps an upper limit) index of chromatographic performance, since it takes into account the efficiency, the resolution, the analysis time, and the number of solutes separated. It depends, naturally, on all the experimental conditions that the analyst can control. Therefore, in the quest for shorter analysis time, the peak capacity is bound to be changed, unless special precautions are taken. There are two basic approaches to shortening the analysis time: (1) to decrease the column length; (2) to decrease the column diameter. It will be shown here that some of the variants of these two basic approaches change n in a constructive manner, whereas others diminish the peak capacity.

Improving the peak capacity may be wasteful, oddly enough, in terms of time. On the other hand, a decrease in n represents a loss of system efficiency as well as of information, which may render the chromatographic analysis meaningless. We thus need to establish a reasonable framework that will allow a decrease in time while at least keeping n constant.

Before we proceed with the discussion, a new criterion of system efficiency will be introduced here. One way to characterize column efficiency is by the use of plate production per unit time. A similar parameter can be established in the case of peak capacity. Eqn. 3 defines n', the "rate of peak capacity production".

$$n' = \frac{n}{t_n} = \frac{1}{t_n} + \frac{1}{4t_n}\sqrt{N}\ln(t_n/t_m) \tag{3}$$

Fig. 1 shows the behaviour of the rate of peak capacity production as a function of t_n at constant N. For the sake of generality, the time axis is in terms of a reduced parameter, t_n/t_m. It is seen that n' has a maximum, which occurs at $t_n = t_m e$, *i.e.* when the capacity ratio is *ca.* 1.7. Thus, while the peak capacity increases with time, the system becomes less efficient in so far as the rate of n production decreases with time (or k') at long retention times. In the context of the present work, however, the quantity of interest is n' at constant k'. Clearly, then, shorter analyses yield larger rate values, and the chromatographic system becomes more time-efficient.

The various approaches to shorter analysis times can now be discussed.

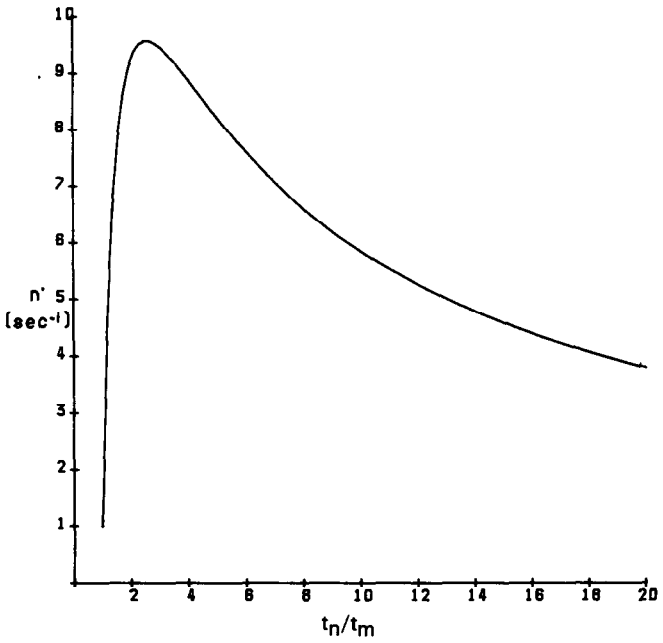

Fig. 1. The rate of peak capacity production *versus* t_n/t_m. In the calculation, N was taken to be 5000.

LENGTH-RELATED CHANGES

Eqn. 2 shows that, from a peak capacity point of view, a decrease in column length, L, alone is not a viable option for gaining analysis time. Since the resolution also suffers when the L is decreased, it is clear why this option of shortening the analysis time is not pursued. The column length, however, can be changed in conjunction with variation in other experimental parameters. When the peak capacity expression is rewritten in terms of column length, L, reduced plate height, h, and particle diameter, d_p,

$$n = 1 + \frac{1}{4}\sqrt{L/hd_p}\,\ln(t_n/t_m) \tag{4}$$

it is seen that the mobile phase velocity, u, and/or d_p can be manipulated, as L is decreased, in an attempt to maintain or improve n.

Changes in column length and mobile phase velocity

Eqn. 3 can be further extended by using the Knox[11] plate height equation

$$n = 1 + 0.25\sqrt{L/d_p\left(\frac{\gamma}{v} + Av^{0.33} + Cv\right)}\,\ln(t_n/t_m) \tag{5}$$

where v is the reduced velocity, and γ, A and C are constants. The plate height has a minimum with respect to the velocity and therefore these two parameters can vary, albeit within relatively narrow limits. If L is to decrease, h must decrease if n is to

remain constant. Under normal operating conditions, this means that v (or u) has to be decreased. The cardinal question in this mode of operation is: will the change required in mobile phase velocity offset the gain in analysis time obtained by reducing the column length? An example will best serve to illustrate the point.

Assume a 250 × 4.1 mm I.D. column, packed with 10-μm particles. If the column is well packed; *i.e.* $A = 1$ and $C = 0.02$, then, at a flow-rate of 2 ml/min (or linear velocity of *ca.* 0.33 cm/sec), the peak capacity for $k' = 5$ is 37. In the calculation, the diffusion coefficient was taken to be $1 \cdot 10^{-5}$ cm^2/sec and the viscosity 1 cP. For this column, the minimum reduced plate height is *ca.* 2 at reduced velocity of *ca.* 3. Under the stated conditions the reduced plate height is *ca.* 4. Therefore, the column length can be halved, with expectation of constant n. However, the change in the velocity needed to decrease h from 4 to 2, is by a factor of ten from $v = 33$ to 3. The net effect is that in this procedure of decreasing L and u the analysis time increases. Table I shows the chromatographic conditions as the column length is decreased from 25 to 20 cm and then to 15 cm. If the peak capacity is to remain at least constant, then the mobile phase velocity must decrease to improve h. In all cases shown in the Table, however, the decrease in u is greater than the decrease in L, and the analysis time increases. The same holds true for all columns that are packed reasonably well, and that one is most likely to encounter in the daily practice of LC.

TABLE I

THE EFFECTS OF CHANGING COLUMN LENGTH AND MOBILE PHASE VELOCITY AT CONSTANT PEAK CAPACITY OF 37

L (cm)	*u (cm/sec)*	*t_n (sec)**	*v***	*h****	*Δp (p.s.i.)§*	*n' (sec^{-1})*
25	0.33	454	33	3.9	957	0.081
20	0.19	631	19	3.1	441	0.058
15	0.08	1125	8	2.3	139	0.033

* The capacity ratio of the last solute was taken to be 5.

** The diffusion: coefficient was assumed to be $1 \cdot 10^{-5}$ cm^2/sec; d_p was taken as 10 μm.

*** The A and C values used in the calculations were 1 and 0.02, respectively.

§ A viscosity value of 1cP was used in the calculations. The permeability was assumed to be 1/800.

The behaviour shown in Table I can be explained in terms of the arguments advanced by Knox[12] over twenty years ago. He has shown that for a given separation the analysis time can be shortened by increasing the column length and the carrier velocity. This is so because the rate of increase in the plate number with L is greater than its loss due to increasing velocity. In the present analysis the situation is the same, even with the added constraint of at least a constant number of resolvable peaks. It is of interest to note that improving the analysis time by *increasing* the column length and the mobile phase velocity was not seriously pursued, at least not in LC. Perhaps this is due to the price one must pay in terms of pressure. For example, in the system described above, the column length can be increased to 53 cm and the velocity to 1.5 cm. The peak capacity is still 37 and the analysis time is now *ca.* 210 sec, *but* the inlet pressure will be close to 10,000 p.s.i. The gain in time is simply not sufficient to compensate for the rapid increase in pressure.

CHANGES RELATED TO PARTICLE DIAMETER

It has been known theoretically for some time that decreasing the particle size can shorten the analysis time[13,14]. Both Erni[5] and DiCesare *et al.*[1] have used small particles to achieve high speed separations. Since varying the particle diameter can affect several parameters, the improvement in the analysis time can be at the expense of such factors as peak capacity. Eqn. 2 can be rewritten in terms of the particle diameter

$$n = 1 + 0.25\sqrt{\frac{D_m v t_n}{h(1 + k)d_p^2}}\ln(t_n/t_m) \qquad (6)$$

and this expression can be used to study the effect of changing d_p on the peak capacity. D_m is the diffusion coefficient of the solute in the mobile phase.

Reducing d_p while keeping v constant

By the definition of v, a reduction of d_p requires an increase in the linear velocity. It is because of this reason that the analysis time decreases. Table II shows the effect of decreasing the particle diameter from an initial value of 10 μm on the retention time, velocity, peak capacity, rate of n production, and inlet pressure. In the calculations, the starting point was taken to be a 25-cm column, operated at a flow-rate of 2 ml/min (*ca.* 0.33 cm/sec). At constant v, and hence constant h, a decrease in d_p increases the peak capacity at shorter analysis time. The price in pressure, however, might be much too great for the small gain in analysis time. For example, changing d_p by a factor of *ca.* 3.3 causes a similar decrease in t_n. The pressure, on the other hand, is increased by a factor of 40 to 35,444(!) p.s.i., which is beyond the capabilities of most present-day instruments. Decreasing the particle size by itself is not, then, a viable approach to improving the analysis time.

TABLE II

THE EFFECT OF DECREASING THE PARTICLE SIZE ON THE ANALYSIS TIME AT CONSTANT COLUMN LENGTH AND REDUCED VELOCITY

d_p (μm)	u (cm/sec)	t_n (sec)*	Δp (p.s.i.)	n*	n' (sec^{-1})
10	0.33	454	957	37	0.080
8	0.41	363	1864	41	0.112
5	0.66	227	7656	52	0.23
3	1.10	136	35,444	66	0.49

* These values were used in the calculations L = 25 cm; v = 33. All other parameters were the same as in Table I.

Changing d_p and L while keeping constant v and n

To alleviate the pressure difficulties, there are several options, one of which is to decrease the column length. The rationale for this choice of approach is that the increase in n, described in the previous section, can actually be wasteful in terms of analysis time. We need, however, a framework to decide on the required decrease in L, so that the effect on performance is minimal. A possible approach is to develop

the separation on a conventional column. Once the resolution is acceptable, then the particle size and the column length are decreased in such a manner that the reduced velocity and the peak capacity remain constant. For that purpose, use is made of the following equation:

$$n = 1 + 0.25[D_m \, vL/(hud_p^2)]^{1/2} \ln(t_n/t_m) \quad (7)$$

It shows the relationship between the peak capacity, the velocities, the particle diameter, and the column length. Table III shows the behaviour of the pertinent parameters when d_p and L change. It is seen that the consequent gain in the analysis time is quite substantial compared with that when just a decrease in d_p is used. The pressures are quite manageable even with 3-μm particles. Perhaps the most important difference between the two approaches is the rate of peak capacity production, n', as shown in Fig. 2. Because of the much shorter t_n values in the case of changing both L and d_p, n' is larger, even though the plate number is smaller. The conclusion that can be drawn here is that decreassing d_p and L, while keeping a constant reduced velocity (or reduced plate height) and a constant peak capacity, is one method of achieving shorter analysis time. The price paid here in terms of higher inlet pressures, and in terms of the need for a better chromatographic system, *i.e.*, fewer extra-column contributions of all kinds, is not too demanding, even for today's 3-μm technology. The 1-μm column, shown in Table III, is a future goal.

TABLE III

THE EFFECT OF DECREASING d_p AND L WHILE KEEPING n CONSTANT AT 37

The reduced velocity is held constant at 33. All other values are the same as in Table I.

d_p (μm)	L (cm)	u (cm/sec)	t_n (sec)	Δp (p.s.i.)	n' (sec^{-1})
10	25	0.33	454	957	0.081
8	20	0.41	291	1495	0.13
5	12.5	0.66	114	3828	0.32
3	7.5	1.10	41	10,633	0.90
1*	2.5	3.30	4.5	95,700	8.10

* Goal for future development.

Decreasing d_p and L while keeping u and n constant

Instead of keeping v, and therefore h, constant, one can keep the linear velocity constant. Thus, as d_p decreases, so does v and the reduced plate height. The net effect is that the plate number increases drastically, causing an increase in the peak capacity. However, when the mobile phase velocity and column length are constant, the analysis time does not change with decreasing particle size. To change t_n, the column length needs to be changed, as seen from eqn. 7.

If the peak capacity is to remain constant, then L must decrease with d_p. Table IV shows the effect of reducing the particle size and the column length at constant n and u on the pertinent parameters. It is seen that in this procedure the analysis time decreases with decreasing d_p. Because of the decrease in the column length, the pressures required to achieve the faster analyses are easily within current instrumental

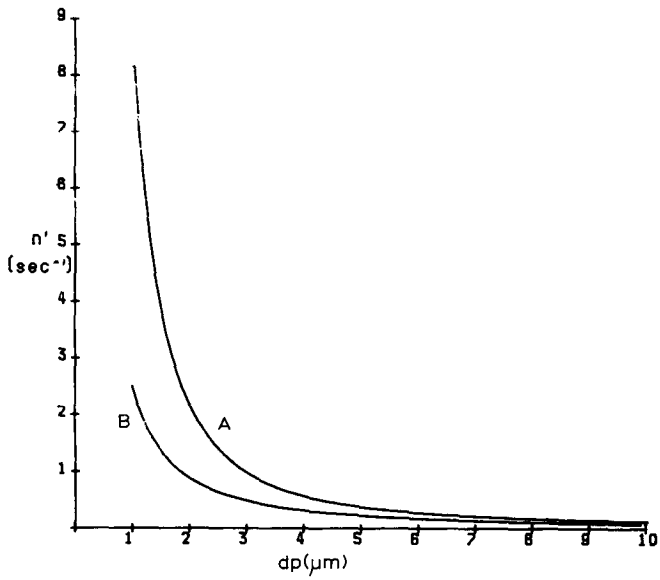

Fig. 2. The rate of peak capacity production *versus* the particle diameter. Curve A is for the case where d_p, L, and u change. Curve B is for the case where only d_p and u change.

capabilities. The column length decrease can be quite drastic, and, in fact, it might be the limiting factor, as extra-column effects can destroy the efficiency. Present packing technology also limits the use of 1.28-cm column with 1-μm particles operated at 0.33 cm/sec.

TABLE IV

THE EFFECT OF DECREASING d_p AND L WHILE KEEPING n CONSTANT AT 37

The mobile phase velocity is held constant at 0.33 cm/sec. All other values used in the calculations are the same as in Table I.

d_p (μm)	L (cm)	t_n (sec)	v	h	Δp (p.s.i.)	n' (sec^{-1})
10	25	454	33	3.9	957	0.081
8	18.7	331	26	3.5	1089	0.11
5	9.5	172	16.5	2.9	1451	0.21
3	4.8	87	9.9	2.4	2035	0.42
1*	1.28	23	3.3	2.0	4919	1.56

* Goal for future development.

Fig. 3 compares n' in the case where u is constant, but d_p and L decrease with the case where only d_p changes (constant t_n). Although the peak capacity increases in the latter case, and remains constant in the former, the rate of n production is greater when both d_p and L decrease. This is because of the decrease in the analysis time. Thus, the system is more efficient in producing separations when the peak capacity is constant and the analysis time drops.

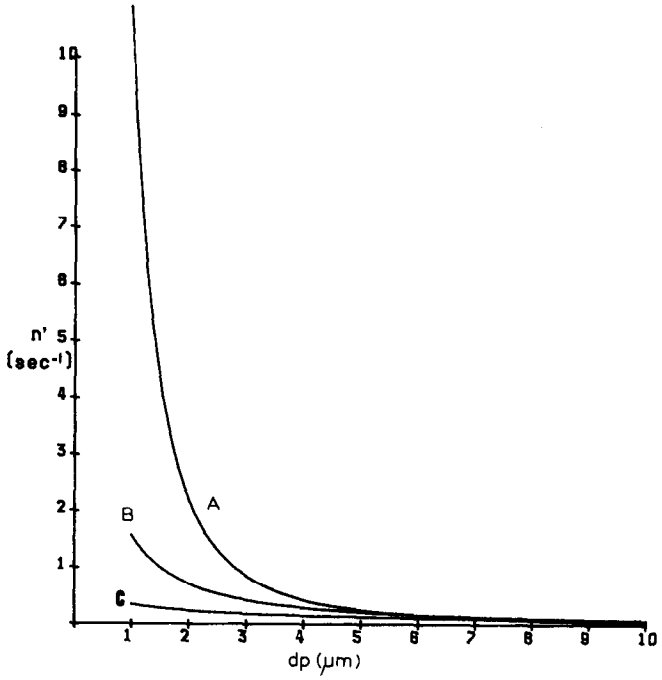

Fig. 3. The rate of peak capacity production *versus* the particle diameter. Curve A shows the change in n' when d_c, d_p, L, and u change. Curve B is for the case where d_p, L, and v change. Curve C is for the case where only d_p and v change.

It is of interest to compare the constant u approach (Table IV) with the constant v approach (Table III), since in both cases the peak capacity is kept constant while d_p changes. Moreover, both approaches seem viable. In terms of analysis time alone, decreasing the particle size at constant reduced velocity seems to be more advantageous. Also, in this mode of operation, the column length for each particle size is longer than in the constant u procedure. Thus, the instrumental requirements are much less stringent. The pressures required are more demanding, although not necessarily prohibitive, in the constant reduced velocity method. In the example given in Table III, the pressure of 10,000 p.s.i. needed to obtain an analysis time of 41 sec (for $k' = 5$) with 3-μm particles is achievable even with present-day equipment. It is felt that the constant v approach is preferable for the attainment of high-speed liquid chromatography via changes in the particle diameter and the column length.

The examples shown in Tables III and IV represent two practical limiting conditions for constant peak capacity operations. Eqn. 7 points to the fact that d_p L, v, and u can change in an infinite variety of ways, even with the requirement for shorter analysis time. Fig. 4 shows a surface of constant n in a L–u–d_p space. It was generated by assuming that the initial separation was maximized on a 25-cm column, packed with 10-μm particles and operated at a mobile phase velocity of 0.33 cm/sec. The capacity ratio of the last solute was taken to equal 5. The highest reduced velocity allowed in the calculations was equal to the original one, *e.g.* 33. This is an arbitrary restriction but, it is felt, a practical one owing to inlet pressure limitations, and to

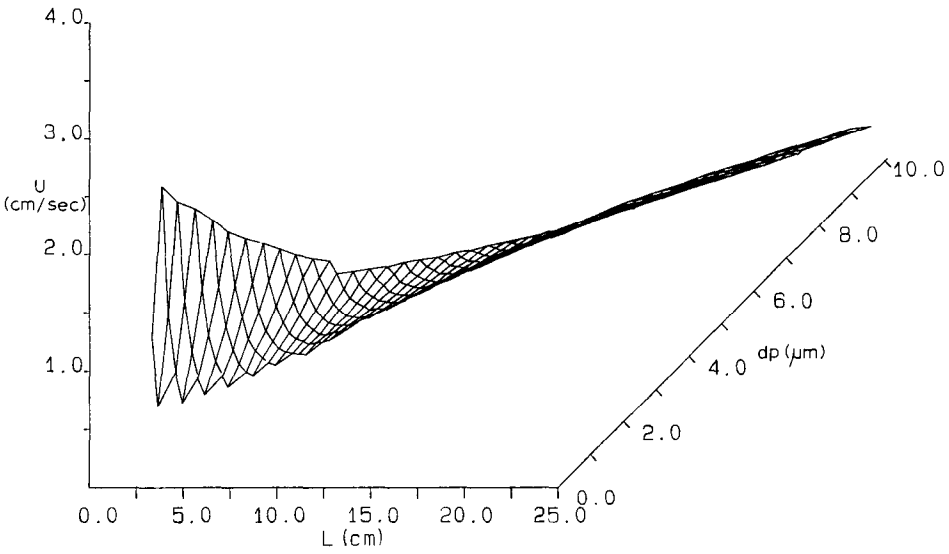

Fig. 4. The surface of constant peak capacity as a function of u, L, and d_p.

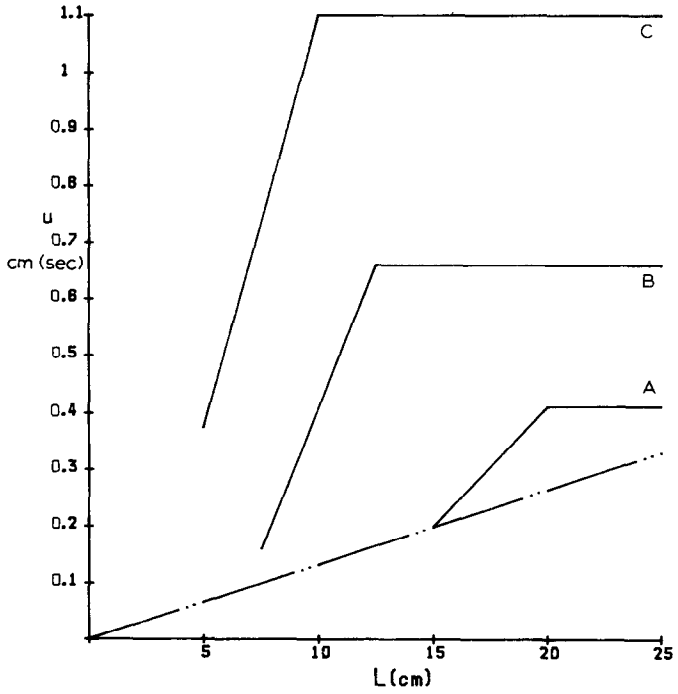

Fig. 5. Velocity regions, for several d_p values, as a function of column length. The broken line, common to all regions, is the minimum allowed velocity, as determined from the original analysis time. Continuous lines are high-velocity limits for the following d_p values: (A) 8 μm; (B) 5 μm; (C) 3 μm.

the assumption made here that the initial method development was carried out at reasonable times. The low-velocity end of the surface can be below the values shown in Table IV. However, in such cases the gain in analysis time is not too impressive. Thus, Tables III and IV represent easily calculated practical limits of approach to high-speed liquid chromatography.

For the sake of generality, it should be pointed out that Fig. 4 is a special case — that of constant peak capacity. When this restriction is removed, the possible choices of u, L, and d_p are even greater, and the computation more laborious. Fig. 5 shows schematically velocity regions as a function of column length, for several d_p values. Within each region the analysis time is shorter than, and the peak capacity at least equal to, the original conditions. The low-velocity line (the dashed line) represents no improvement in the analysis time. It is a function of the length and the velocity alone. Thus, that line is common to all particle sizes. The upper boundary of each region depends on experimental factors, such as ΔP. In the present case, it was limited by the choice of upper $v = 33$. As the particles decrease, the regions become wider. Overlapping points from different regions have equal retention times, but the inlet pressure increases as d_p becomes smaller. The peak capacity, and thus n', improve for lower d_p values.

IMPROVING ANALYSIS TIME BY REDUCING COLUMN DIAMETER

Narrow-bore columns (*viz.* refs. 15–20) have gained in popularity over in recent years, mainly because of their low consumption of mobile phase. The applicability of such columns to high-speed liquid chromatography is self-evident, and Erni[5] has advocated that approach. Is it more advantageous to use narrow-bore columns? To answer this question, the peak capacity expression is rewritten as a function of the column diameter, d_c

$$n = 1 + 0.125[\varepsilon\pi D_m \, vL \, d_c^2/(hFd_p^2)]^{1/2}\ln(t_n/t_m) \tag{8}$$

where ε is the column porosity and F is the volumetric flow-rate. Following the analysis carried out before, it is easy to show that when the flow-rate and the reduced velocity are kept constant, to decrease the particle size requires an increase in the linear velocity. Such an increase can be accomplished by reducing the column radius. The required inlet pressures dictated by changes in d_p and d_c are the same as described in Table II. There, the column radius remained constant but the flow-rate changed, whereas in the present situation the opposite is true. The net effect is that, from an analysis time point of view, the two situations are identical. However, solvent consumption, is far less with the narrow-bore columns. The same conclusions are reached for the constant v and n approach.

Changes in d_p, d_c, L and u when only n is kept constant

The column diameter, then, is another parameter that can be manipulated to improve the speed of the analysis. From eqn. 8 it is clear that many options are available to attain shorter retention times, not all of which are attractive. Perhaps the best approach is to keep the ratio d_p/d_c and the peak capacity constant. An example is shown in Table V, where the same initial conditions (the first row in the

TABLE V

THE EFFECT OF CHANGING THE COLUMN DIAMETER AS WELL AS d_p, L, AND u, WHILE KEEPING n CONSTANT AT 37

All other parameters are the same as in Table I.

d_p (μm)	L (cm)	u (cm/sec)	t_n (sec)	v	h	Δp (p.s.i.)	n' (sec^{-1})	d_c (cm)
10	25	0.33	454	33	3.9	957	0.081	0.41
8	22.1	0.51	257	41.2	4.3	2061	0.14	0.328
5	17.2	1.32	78	66	5.3	10,634	0.47	0.205
3	13.4	3.67	22	110	7.0	63,452	1.67	0.123

table) are used as before. When both d_p and d_c decrease, the velocity increases rapidly owing to the square dependence on the column radius. Thus, the pressure increase is very rapid. The reduced velocity increases linearly with the decrease in d_p and d_c as a result of the opposing effects of u and d_p. This means that the increase in the reduced plate height is relatively slow, so that the product hd_p decreases with decreasing particle size. To maintain constant peak capacity, the column length must be decreased, a course of action that diminishes the required inlet pressure. This explains why, in Table V, the inlet pressure does not increase by a factor of $(d_{p_1}/d_{p_2})^8$, where subscripts 1 and 2 represent two different sizes.

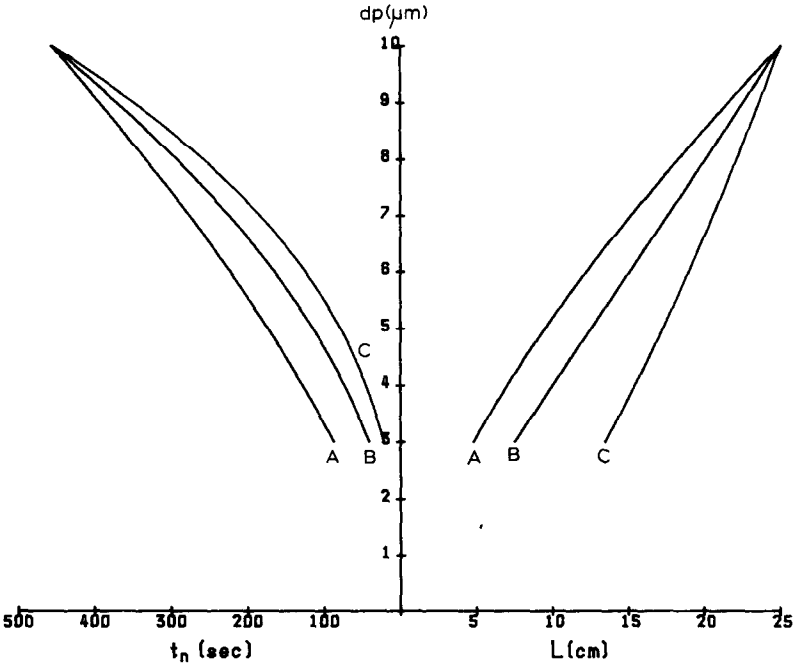

Fig. 6. Column length and retention times as a function of d_p. Curves A are for the case where d_p, L, and v vary. Curves B are for the case where d_p, L, and u change. Curves C are for the case where d_p, L, u, and d_c vary.

A comparison is in order between this procedure and those in Tables III and IV, because all three approaches start with the same initial conditions, and the improvement in time is not at the expense of the peak capacity. The demands, as far as the column length is concerned, are the least stringent when the column diameter is allowed to change. Packing such columns should not present any difficulties, at least down to 3-μm particles. When the column diameter is allowed to vary at constant flow-rate, the changes in velocities and inlet pressures can be quite severe. This might lead to velocity-induced temperature gradients, which can destroy efficiencies and, hence, lower the peak capacity. The temperature limitations of high-speed liquid chromatography are all to often neglected. It is an important point, which will be dealt with in a separate publication. For the purpose of the present discussion, it is sufficient to indicate that narrow-bore columns in the right experimental configuration can reduce the deleterious effects of temperature gradients.

At present, the pressure cost of the narrow-column approach is the limiting factor. In the example discussed here, 3-μm columns could be operated, in present instruments with minimal modification, only in the cases where the column diameter did not change (see Tables III and IV). In fact, the theoretical treatment leading to the Tables is an approximation, because pressure effects on the diffusion coefficients, and therefore on h[21], have been ignored. Bearing this in mind, it is still safe to state that changing the column diameter, along with other parameters, is the most beneficial way to shorten the analysis time. This is amply demonstrated in the n' values, which are plotted in Fig. 3. Moreover, mobile phase consumption is the smallest in the approach in Table V. On the other hand, the instrumental demands concerning extra-column effects are the least flexible with the narrow-column procedure.

Comparison of Tables III, IV, and V shows that the decrease in time is greatest in the case where the column diameter is allowed to change. On the other hand, the decrease in the column length is the least in that case. Fig. 6 shows the point graphically. In none of the cases was the decrease in t_n linearity dependent on the decrease in d_p. With the exception of the case where v was held constant, the same is true for the decrease in column length.

CONCLUSIONS

It is clear from the tables and Figs. 3 and 6 that, with present instrumental limitations, the best way to gain analysis time is by changing d_p, d_c, L, and u together. If pressure limitation poses a problem, then it is best with present technology to adopt the approach of constant u (Table IV). Once the packing technologies of very short columns (less than 1 cm) have been perfected, and the extra-column effects, including time constants, have been minimized by instrumental redesign, then the best approach is probably that of keeping v constant (Table III). It is certainly the most appealing. Mobile phase consumption can be controlled and reduced by changing d_c and the flow-rate, so that the reduced velocity is constant. It should be kept in mind that in all cases the system is changed in such a way that the peak capacity is kept constant *i.e.* the rate of n production is increasing.

There are other algorithms for achieving short analysis time. However, it is felt, that the procedures and options given here present a compromise between practicality, and *a priori* calculations. They represent, as mentioned before, limits of

operation that can be grasped conceptually. In any event, the improvement in analysis time is a desirable goal, which can be achieved without sacrificing chromatographic performance and resolution.

Future developments hinge on finding solutions, theoretical and practical, to temperature gradients at high mobile phase velocities, and the pressure dependence of the plate height.

REFERENCES

1 J. L. DiCesare, M. W. Dong and L. S. Ettre, *Chromatographia,* 14 (1981) 257.
2 J. L. DiCesare, M. W. Dong and J. G. Atwood, *J. Chromatogr.*, 217 (1981) 369.
3 M. W. Dong and J. L. DiCesare, *J. Chromatogr. Sci.*, 20 (1982) 49.
4 M. W. Dong and J. L. DiCesare, *J. Chromatogr. Sci.*, 20 (1982) 517.
5 F. Erni, *J. Chromatogr.*, 282 (1983) 371.
6 G. Guiochon, in Cs. Horváth (Editor), *High Performance Liquid Chromatography,* Academic Press, New York, 1980, vol. 2, pp. 1–56.
7 J. C. Giddings, *Anal. Chem.*, 39 (1967) 1027.
8 C. G. Horvath and S. R. Lipsky, *Anal. Chem.*, 39 (1967) 1893.
9 E. Grushka, *Anal. Chem.*, 42 (1970) 1142.
10 J. M. Davis and J. C. Giddings, *Anal. Chem.*, 55 (1983) 418.
11 J. H. Knox and M. J. Saleen, *J. Chromatogr. Sci.*, 7 (1969) 614.
12 J. H. Knox, *J. Chem. Soc.*, (1961) 433.
13 J. C. Giddings, *Dynamics of Chromatography,* Marcel Dekker, New York, 1965.
14 G. Guiochon, *Anal. Chem.*, 52 (1980) 2002.
15 R. P. W. Scott and P. Kucera, *J. Chromatogr.*, 169 (1979) 51.
16 R. P. W. Scott, P. Kucera and M. Munroe, *J. Chromatogr.*, 186 (1979) 475.
17 P. Kucera, *J. Chromatogr.*, 198 (1980) 93.
18 F. J. Yang, *J. Chromatogr.*, 236 (1982) 265.
19 M. Novotny, *Anal. Chem.*, 53 (1981) 1294a.
20 T. Takeuchi and D. Ishii, *J. Chromatogr.*, 238 (1982) 409.
21 M. Martin and G. Guiochon, *Anal. Chem.*, 55 (1983) 2302.