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PREPARATION AND OPERATION OF LIQUID CHROMATOGRAPHIC COLUMNS OF VERY HIGH EFFICIENCY

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SUMMARY

On the basis of theory, the optimization of the various parameters involved in the preparation and operation of liquid chromatographic (LC) columns is discussed. The different approaches to building columns of extremely high efficiencies meet different technological difficulties, mainly the use of very high inlet pressures or very long analysis times. Narrow-bore packed columns have some advantages, but the contribution of extra-column sources of band broadening is very difficult to reduce below a reasonable limit.

A good compromise seems to lie in the use of 10-μm particles, which could permit the achievement of a million-plate efficiency in 1.5 days for a compound with $k = 3$, if the solvent is moderately viscous and the diffusion coefficient reasonable. The number of peaks resolved with resolution 1 would be 500 in 2.85 days ($k = 6.4$) at a pressure of 600 atm. Faster analyses are possible with smaller particles and higher pressures.

Formidable technological difficulties have to be resolved before capillary or packed capillary columns could compete with standard packed columns. It is unlikely that we can go beyond this million-plate barrier unless some major breakthrough is made.

Although gas chromatography (GC) is potentially less efficient than LC, it is also much faster. In spite of the use of larger diameter open tubes or particles and of the availability of lower pressures, it remains possible to achieve by GC efficiencies that largely exceed those which can be obtained by LC.

INTRODUCTION

The analysis of some very complex mixtures requires both high efficiency and high selectivity. Even with a column of high efficiency no satisfactory result is obtained if the chromatographic system does not offer the kind of interactions with the compounds of the studied mixture which will spread their elution over a large span of retention times. If several such systems are available, however, it is likely that interferences between some compounds on one system will be replaced by interferences between other compounds on the second system.

Environmental analysis, food analysis, geochemistry, biochemistry and clinical

chemistry, among other areas, offer some very challenging mixtures to the analyst. Some problems can be solved by the combination of sample clean-up and preparation, a selective chromatographic system and selective detection: they are related to the quest for one or a few well defined compounds. Other such problems can be solved only by the use of multi-dimensional chromatography and/or the use of columns of very high efficiency. Gas chromatography has offered, for the last 20 years, the possibility of achieving easily separations that require several hundred thousands and probably up to a million plates without much difficulty, using commercially available equipment. Hundreds of such analyses have been published already, making common any analysis that requires less than half a million plates.

On the other hand, although the technique is potentially more powerful, as was demonstrated long ago by Giddings[1], analyses with more than $5 \cdot 10^4$ plates are still rare in liquid chromatography (LC) and considerable controversy exists regarding the most convenient and promising technique for achieving a 10- to 20-fold increase in overall efficiency[2–4].

The aim of this paper is to discuss, from a theoretical standpoint, the experimental conditions that would allow the achievement of columns of very high efficiency, while taking the analysis time into account. The analysis time is important for economic reasons and because dilution takes place continuously during separation, in spite of the efficiency, and ultimately detection becomes impossible: a high separation efficiency and low detection limits may have opposite requirements. Various experimental data are discussed in respect of the results of the theoretical approach and some suggestions for further work are presented.

EQUATIONS FOR FLOW AND EFFICIENCY

All discussions regarding the optimization of a separation process need equations relating the separation time and the efficiency to the experimental parameters. In liquid chromatography these equations are simple and well known, so their discussion here will be minimal and centred on their range of validity at high pressures and large column lengths. The reader is referred to previous discussions[5–8].

Flow velocity and retention time

In a porous medium, the flow velocity, u, is given by the Darcy law:

$$u = -\frac{K}{\eta} \cdot \frac{dp}{dx} \tag{1}$$

where dp/dx is the pressure gradient along the column (negative), η the mobile phase viscosity and K the column permeability[5–8]. This permeability depends almost only on the particle size of the packing for conventional columns, as the mobile phase flows only around the particles, even if porous, not across them, and the techniques used to prepare the highly homogeneous packings necessary to achieve high efficiencies tend to produce packings of large, constant density. In practice we have

$$K = K_0 d_p^2 \tag{2}$$

K_0 is approximatively 10^{-3} for porous silica. For particles with a lower internal

porosity, and especially for porous layer beads, and because of the way u, and hence K, are measured, K_0 appears to be somewhat larger.

It has been shown[7] that eqn. 1 can be solved by neglecting the variation of the viscosity with pressure as well as the compressibility of the liquid. This is valid at pressures up to 600 atm. The correction becomes important above 1000 atm. Hence

$$\bar{u} = \frac{K_0 d_p^2 \Delta P}{\eta L} \tag{3}$$

where ΔP is the difference between the pressures at the column inlet and outlet and, as the outlet pressure is atmospheric, ΔP is the reading of the inlet pressure gauge; L is the column length.

The hold-up time of the column is

$$t_m = \frac{L}{\bar{u}} = \frac{\eta L^2}{K_0 d_p^2 \Delta P} \tag{4}$$

This is the elution time of a non-retained compound[9]. The retention time of a retained compound is

$$t_R = (1 + k)\, t_m = \frac{L}{\bar{u}}(1 + k) \tag{5}$$

where k is the column capacity factor, proportional to the equilibrium constant for the distribution of this compound between the two phases and to the ratio of the amounts of the two phases in the column.

Eqns. 4 and 5 permit the prediction of analysis time as a function of experimental parameters. K_0, k and η are determined by the selection of the chromatographic system. With silica to a certain extent, and with chemically bonded silica to a large extent, k is proportional to the specific surface area of the packing. Eqn. 4 shows that if $\bar{u}$ is kept constant, the analysis time increases in proportion to the column length. This in turn means that the pressure increases in proportion to the column length (*cf.* eqn. 3).

Column efficiency and resolution

The resolution between two peaks is classically defined as

$$R = 2\left(\frac{t_{R,2} - t_{R,1}}{w_1 + w_2}\right) \tag{6}$$

where $t_{R,1}$ and $t_{R,2}$ are the retention times of the two peaks and W_1, W_2 their respective base widths, assuming that the peaks are nearly symmetrical. We can assume that two peaks which are close are Gaussian, an assumption which is valid as a first approximation provided that exchanges are fast between the two phases, a prerequisite for designing and building columns of high efficiency. Eqn. 6 is then rewritten as

$$R = \frac{\sqrt{N}}{4} \cdot \frac{\alpha - 1}{\alpha} \cdot \frac{k}{1 + k} \tag{7}$$

where α is the ratio of the column capacity factors for the two compounds ($\alpha = k_2/k_1 > 1$) and N the number of theoretical plates for the second compound:

$$N = 16\left(\frac{t_R}{W}\right)^2 \tag{8}$$

Eqn. 7 emphasizes the importance of achieving large plate numbers for resolving closely eluted compounds ($\alpha \approx 1$) and also of having a sufficient retention: if k is small, resolution can be obtained only with a very large plate number.

Experiments suggest that over a wide range, the efficiency of a column, as given by the number of theoretical plates, is proportional to column length, provided that the columns of different lengths are packed by the same technique using the same material and are operated at the same flow velocity. Hence

$$H = \frac{L}{N} = f(u) \tag{9}$$

where H is the height equivalent to a theoretical plate (HETP). In fact, we have reason to assume that deviations from this law at large column lengths result from our inability to pack long columns as efficiently as short columns or to connect several columns without losing part of the efficiency. This is discussed in the last section.

As shown by Knox[10] and Unger *et al.*[11], columns packed with particles of different average diameter have HETPs that are given by the equation

$$h = \frac{B}{\nu} + A\nu^{1/3} + C\nu \tag{10}$$

with

$$h = \frac{H}{d_p} \tag{11a}$$

and

$$\nu = \frac{ud_p}{D_m} \tag{11b}$$

where h and ν are the reduced HETP and the reduced velocity, respectively, D_m is the diffusion coefficient of the studied compound and B, A and C are dimensionless coefficients[6,8,10]. B is usually around 1.5 and varies only slightly for most commonly used packings. To some extent it may be a function of k (ref. 8). The term $1.5/\nu$ accounts for axial diffusion. The term $A\nu^{1/3}$ accounts for the contribution of the lack of homogeneity of the packing. The more homogeneous the packing, the lower is the value of A. Good columns have a value of A below 2 and it is possible to produce columns with A lower than 1. The term $C\nu$ accounts for the resistance to mass transfer by diffusion inside the particles and because of the finite rate of the adsorption–desorption process. For good silica particles (pure silica or chemically bonded silica) C is assumed to be around 0.03. There is considerable uncertainty about the exact value of C in most instances. As shown in Fig. 1, even with a good packing technique, the influence of the $C\nu$ term is small unless the reduced velocity

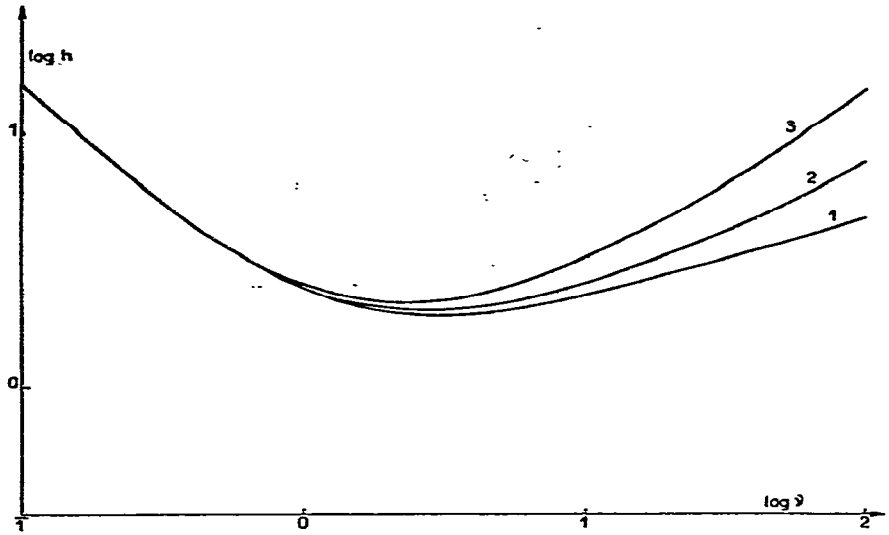

Fig. 1. Column efficiency plot of reduced plate height *versus* reduced velocity (logarithmic scales) $A = 1$. 1, $C = 0$; 2, $C = 0.03$; 3, $C = 0.10$. Coordinates of minima in Table I.

TABLE I

OPTIMAL EFFICIENCY OF PACKED LC COLUMNS

A	$C = 0$		$C = 0.03$		$C = 0.10$	
	h_m	v_0	h_m	v_0	h_m	v_0
1.2	2.23	2.7	2.30	2.4	2.46	2.1
1.0	1.94	3.1	2.03	2.7	2.20	2.3
0.8	1.64	3.7	1.74	3.1	1.94	2.5
0.6	1.32	4.5	1.44	3.6	1.66	2.6

is very large, so because of experimental errors most values of C found in the literature are unreliable[12].

If we neglect the Cv term in eqn. 10, as a first approximation, it is easy to show by differentiation that the reduced flow velocity, v_0, corresponding to the minimal plate height is:

$$v_0 = \left(\frac{3B}{A}\right)^{3/4} \simeq \frac{3.09}{A^{3/4}} \tag{12}$$

and the minimum reduced plate height is

$$h_m = 1.75\,(A^3B)^{1/4} = 1.94\,A^{3/4} \tag{13}$$

As shown in Fig. 1, the use of eqns. 12 and 13 gives a good approximation

for the coordinates of the minimum. Even with $C = 0.1$, which corresponds to a poor chromatographic system, the error in the minimum value of the plate height is only 13%. It is obvious that the quality of the packing is very important for the overall performance of the column.

Finally, we observe that if the packing technique is improved, h_m decreases while ν_0 increases, so the column has to be operated at a larger flow velocity.

Relationships between column characteristics and performances

We are interested in achieving a given separation or in providing a given level of efficiency to resolve as many compounds in a complex mixture as required. This means that the column should provide a given number of theoretical plates. We need to relate that to the column characteristics. Combining eqns. 9 and 11 gives the necessary column length:

$$L = N h d_p \tag{14}$$

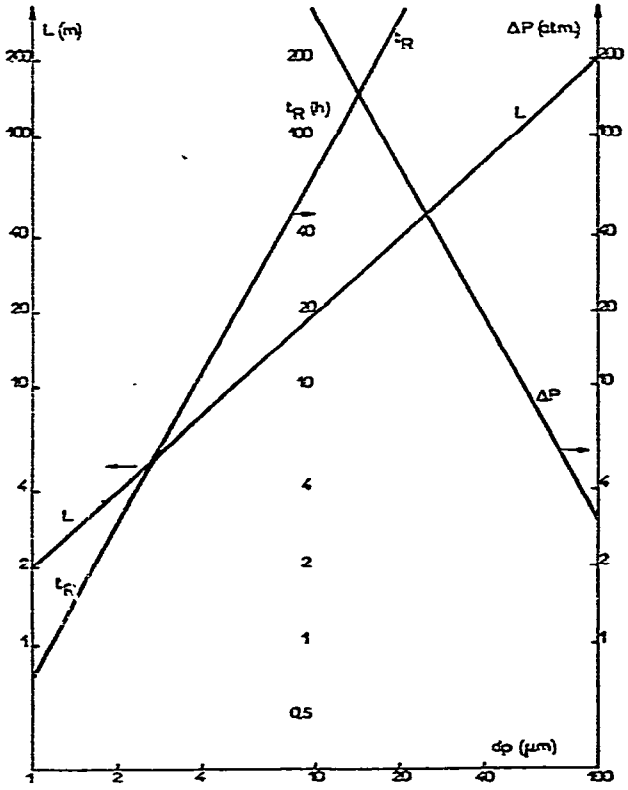

Fig. 2. The achievement of 10^5 plates. Plot *versus* the particle diameter of the column length (L), the analysis time (t_R for $k = 3$) and the pressure (ΔP). $K_0 = 1 \cdot 10^{-3}$; $D_m = 1 \cdot 10^{-5}$ cm²/sec; $\eta = 0.5$ cP; $h = 2$; $\nu = 3$; $k = 3$.

The analysis time is obtained by combining eqns. 5, 11 and 14:

$$t_R = \frac{Nd_p^2}{D_m} \cdot \frac{h}{v}(1 + k) \tag{15}$$

The necessary pressure is obtained by combining eqns. 3, 11 and 14:

$$\Delta P = N \cdot \frac{hv}{d_p^2} \cdot \frac{\eta D_m}{K_0} \tag{16}$$

Eqns. 14–16 permit the determination of the column characteristics necessary for achieving given performances. For example, Fig. 2 gives the variation with particle diameter of the column length, analysis time and pressure corresponding to an efficiency of 10^6 plates, the column being operated at minimal plate height ($h = 2$, $v = 3$). This appears to be feasible, using particles with diameters between 5 and 10 μm, a column length between 10 and 20 m, an analysis time between 1 and 3 days and a pressure between 1000 and 300 atm. If necessary, the analysis time can be reduced by operating the column at a larger flow velocity. The HETP increases with flow velocity, however, so the pressure must be increased considerably, and in the present case this possibility is merely theoretical.

Eqns. 14–16 also permit the selection of optimal conditions when the relationship between h and v is known[6–8].

Critical pressure

This concept was introduced by Giddings[13]. When the pressure necessary to operate a long column at the optimal flow-rate is larger than the maximum possible with a given equipment, we can elect to operate it at a lower flow-rate. Then the plate height is larger and the column length needed to achieve a given plate number becomes longer. When we decrease the flow-velocity to a very low value, the column length increases to infinity but the inlet pressure necessary to achieve a given plate number does not decrease to zero but tends towards a limit, the critical inlet pressure.

Combination of eqns. 10 and 16 gives

$$\Delta P = \frac{N}{d_p^2} \cdot \frac{\eta D_m}{K_0}(B + Av^{4/3} + Cv^2) \tag{17}$$

It is obvious from this equation that it will be impossible to achieve the required plate number with the available packing if the equipment cannot deliver the solvent under a pressure larger than the critical pressure ΔP_c:

$$\Delta P_c = \frac{N}{d_p^2} \cdot \frac{\eta D_m}{K_0} \cdot B \tag{18}$$

The corresponding column length is infinite, of course, as it is the only way to operate a column at zero flow-rate with a finite inlet pressure.

If the column is to be operated at the flow velocity giving the minimum plate height, assuming $C \approx 0$, we can combine eqns. 12 and 17 and obtain

$$\Delta P = 4\,\Delta P_c \tag{19}$$

The pressure must be four times larger than the critical pressure. When the column is operated at an inlet pressure lower than $4\Delta P_c$, the analysis time becomes very large as we have to use a longer and longer column to achieve the required plate number, as shown by eqn. 17 and time becomes infinite for $\Delta P = \Delta P_c$.

Number of effective peaks resolved

To qualify column performance, Giddings[14] and Grushka[15] introduced the effective peak number or number of peaks eluted from a column between $k = 0$ and k and all resolved from their neighbours with a resolution of unity. Grushka[15] showed that with an excellent approximation this number, n, is given by the equation

$$n = \frac{\sqrt{N}}{4} \cdot Ln(1 + k) \tag{20}$$

This parameter is very useful for describing the performances of columns of high efficiency used to analyse complex mixtures.

We can increase the effective peak number either by increasing the plate number or by increasing the range of k during which the analysis is carried out. As can be seen from eqn. 20, n increases with the square root of the plate number and only as the logarithm of $(1 + k)$. In both instances the analysis time increases faster.

It is therefore interesting to determine which is the optimal range of k to consider. In other words, given a certain analysis time, t_R, what is the best way of maximizing n, a long column and hence a large N and a small range of k, or a short column and a large range of k? Combining eqns. 4 and 20 we obtain

$$n = \frac{\sqrt{t_m}}{4}\sqrt{\frac{u}{H}} \cdot Ln \cdot \frac{t_R}{t_m} \tag{21}$$

since $L = NH = u\,t_0$. If we want to maximize the effective peak number achieved in a given analysis time, we search for the extreme value of this function of t_m. Differentiation of eqn. 21 with respect to t_m shows that the optimum is achieved when

$$Ln \cdot \frac{t_R}{t_m} = 2 \tag{22a}$$

and

$$1 + k = e^2 \tag{22b}$$

Then:

$$n = \frac{\sqrt{N}}{2} \tag{23}$$

assuming N is constant.

The largest value of k is $e^2 - 1 = 6.39$. If the mixture analysed is not eluted completely within the range of capacity factors 0–6.4, it means that better results, *i.e.*, a larger number of resolved peaks, would be obtained in the same time by using

a longer column and a stronger solvent. Conversely, if the last peak is eluted with $t_R < 7.4t_0$, more peaks could be resolved in the same time using a shorter column and a weaker solvent.

Selection of optimum conditions

In the following discussion we assume that all columns are used at maximum efficiency, *i.e.*, at the flow-rate at which the plate height is minimal. It has been shown that this also corresponds to the minimal pressure. As we are interested in very large efficiencies, we can anticipate that pressure will be a limiting factor in the possible performances.

Further, in order to compare various technological solutions, we shall use the effective peak number in the range $k = 0$–6.39. The corresponding time will be called the analysis time, t_A.

Relationship between viscosity and diffusion coefficient

The diffusion coefficient of a dilute solute in a solvent is given by the empirical Wilke and Chang equation, which is usually approximate:

$$D_m = 7.4 \cdot 10^{-10} \cdot \frac{(\psi_2 M_2)^{0.5} T}{\eta_2 V_1^{0.6}} \tag{24}$$

where η_2 and M_2 are the viscosity and molecular weight of the solvent, respectively, T the absolute temperature and V_1 the molar volume of the solute; ψ is a constant equal to 1.0 for non-associated liquids, to 2.6 for water, 1.9 for methanol and 1.5 for ethanol.

The product ηD_m appears in eqns. 16 and 18, which are of critical importance for the following. It is therefore necessary to have a good estimate of the practical range of this product.

In normal adsorption chromatography, ψ_2 is 1.0, T ambient temperature, M_2 is between 72 (*n*-pentane) and 154 (carbon tetrachloride), and V_1 is usually between 100 and 400, few high-molecular-weight compounds being analysed by this technique. The largest range of ηD_m is thus between $5 \cdot 10^{-8}$ and $1.7 \cdot 10^{-7}$, a ratio of only 3. In reversed-phase chromatography, the lightest solvent is water with $\psi_2 M_2 = 46$ and the heaviest in practice is acetonitrile with $\psi_2 M_2 = 70$. The molecular weight of solute is usually between 100 and 1000 and temperature, although usually ambient can be as high as 60°. The largest range of ηD_m is thus between $2.4 \cdot 10^{-8}$ and $1.3 \cdot 10^{-7}$.

In gel permeation chromatography, assuming this equation is valid for polymers with molecular weights between 5000 and 100,000, analysed at ambient temperature, ηD_m is between $7 \cdot 10^{-9}$ and $3 \cdot 10^{-8}$. These values will be used below.

DIFFERENT THEORETICAL APPROACH TO THE ACHIEVEMENT OF VERY HIGH EFFICIENCIES

In the following we consider either the elution time of an inert compound, t_0, or the analysis time $t_A = 7.4 \cdot t_0$, as explained above.

Depending on the relative importance given to pressure and time, different approaches are possible.

Critical pressure and maximal efficiency

Assuming we are first interested in the highest possible efficiency, disregarding the importance of analysis time in the overall performances, we can use eqn. 18. We can see that given the maximal pressure at which the equipment can work, the maximal number of plates we can generate in an infinite time is given by

$$N_M = K_0 \Delta P_c d_p^2 / \eta D_m B \tag{25}$$

N_M is proportional to the square of the particle diameter. The optimization or even the adjustment of the other parameters, ηD_m, K_0 and B, is not possible at present. As we suspect that the particle size has a critical effect on analysis time, we show in Fig. 3 a plot of maximal plate number *versus* pressure, assuming values of the particle size between 5 and 100 μm. ηD_m has been given the rather favourable value of $4 \cdot 10^{-8}$. It can be seen that extremely large plate numbers can be achieved with moderate pressures.

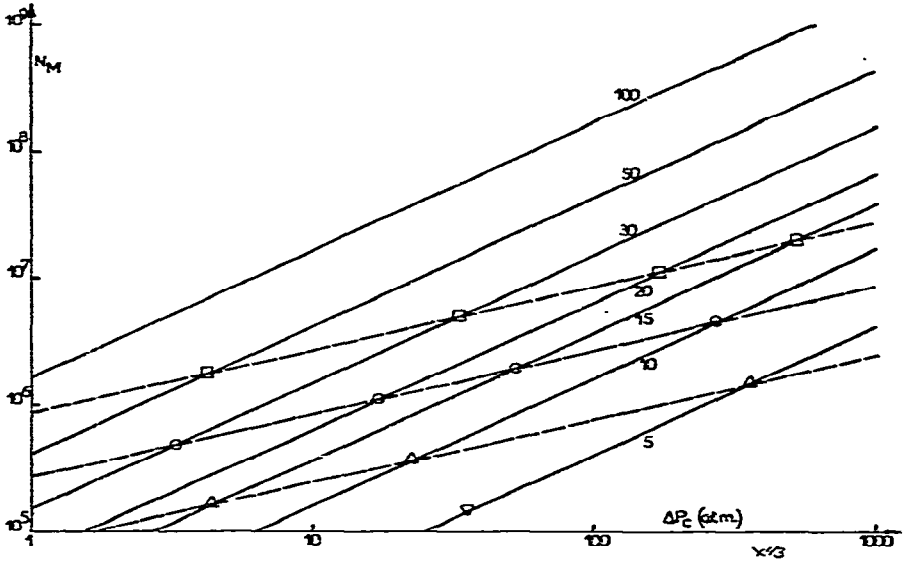

Fig. 3. The maximum plate number. Plot of maximum plate number achieved at zero flow-rate *versus* the critical pressure (*cf.*, eqn. 18). $\eta = 0.5$ cP; $D_m = 1 \cdot 10^{-5}$ cm²/sec; $B = 1.5$; $k_0 = 1 \cdot 10^{-3}$. The particle size in micrometres is given on each line. The dotted lines correspond to constant breakthrough time. ($k = 0$): □ 10 years; ○ 1 year; △ 1 month; △ 3 days.

To check how large an infinite time as understood in the discussion of eqn. 18 is, let us assume than we want to achieve only 95% of the maximal efficiency. Combination of eqns. 17 and 22 shows that the corresponding velocity is given by

$$0.95 (B + A \nu^{4/3}) = B \tag{26}$$

Assuming a well packed column ($A = 1$), this gives $\nu = 0.15$, well below the optimal

velocity, and accordingly $h = 10.6$ ($B = 1.5$). In favourable conditions ($D_m = 1 \cdot 10^{-5}$ cm²/sec and $\eta = 0.4$ cP), eqns. 14–16 show that the corresponding column characteristics for an efficiency of 10^7 plates are an inlet pressure of 70 atm, a column length of 3180 m and an elution time of an inert compound of 20 years. The last two values are unrealistic, especially the last one.

This means that any comparison between column performances or column types based on the sole value of eqn. 18 is practically meaningless. Actually, we need to take time into account and accordingly use eqns. 14–16.

Fig. 3 also shows, for the different particle sizes, the plate numbers that can be achieved in 3 days, 1 month, 1 year and 10 years when working at $v = 0.15$, which permits the achievement of 95% of the maximum plate number at the critical pressure (*cf.*, eqn. 17). From Fig. 3 it follows that large particle sizes have no future in high-efficiency LC.

Columns working at optimal flow-rate

Combination of eqns. 17–19 shows that the efficiency is now given by

$$N = \frac{K_0 d_p^2 \Delta P}{4B\eta D_m} \tag{27}$$

For a given pressure the efficiency is four times smaller than it was at zero flow-rate, but this loss in efficiency is compensated for by a finite analysis time.

Fig. 4 shows a diagram similar to Fig. 3, using the same values for the parameters. Assuming a well packed column ($A = 1$; *cf.*, Table I) the values of v

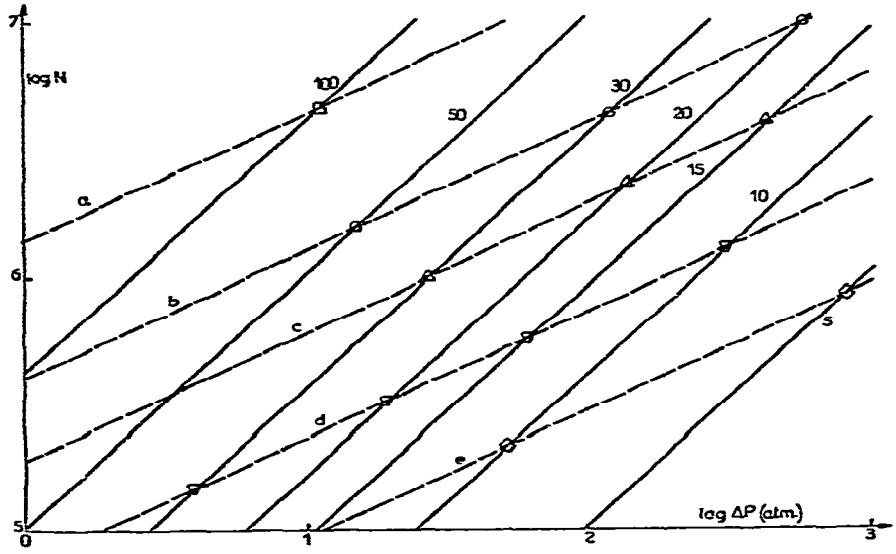

Fig. 4. Plate numbers achieved at minimum plate height. Plot of plate number versus pressure. $\eta = 0.5$ cP; $D_m = 1 \times 10^{-5}$ cm²/sec; $h = 2$; $v = 3$. The particle size (μm) is given on each line. The broken lines correspond to constant breakthrough time ($k = 0$). □, 1 year; ○, 1 month; △, 1 week; ▽, 1 day; ◇, 4 h.

and h are now 3 and 2, respectively, so the analysis times have become much shorter. Note that in both Fig. 4 and Fig. 3 the breakthrough time, or the elution time of a non-retained compound, is given. As explained above, for optimal analysis of complex mixtures, the analysis time is 7.4 times longer, *i.e.*, about 1 week for $t_0 = 1$ day, which thus appears as a practical maximum.

Fig. 4 shows that the highest efficiency we can achieve in practice within such a time would be about $2.5 \cdot 10^6$ plates using 7-μm particles, which demands an inlet pressure of about 1300 atm. About $1.3 \cdot 10^6$ plates could be achieved with 10-μm particles at an inlet pressure of 300 atm. The corresponding column lengths are 35 and 26 m, respectively, and 670 and 790 peaks, respectively, would be resolved in 1 week.

Performances at maximal inlet pressure

Alternatively, we can place the greatest emphasis on analysis time and examine the performances achieved by columns packed with particles of different sizes when columns are operated at the largest possible inlet pressure, *i.e.*, the maximal pressure allowed by the equipment.

Multiplying eqns. 15 and 16 gives

$$t_R \Delta P = \frac{N^2 h^2 \eta}{K_0} \cdot (1 + k) \tag{28}$$

This relationship shows that at a constant inlet pressure the analysis time is proportional to the square of the plate number and of the reduced HETP and proportional to the mobile phase viscosity. Fig. 5 gives numerical results for different values of this viscosity; 2 cP is very large in LC, while 0.4 cP is more standard. Lower values are possible. The particle diameter that permits the achievement of a given number of plates is calculated from eqn. 16. We see again that under rather extreme conditions ($\Delta P = 1300$ atm) and with a favourable system ($\eta = 0.4$ cP, $D_m = 1 \cdot 10^{-5}$ cm^2/sec), it is almost easy to achieve an analysis of about $1.5 \cdot 10^6$ plates ($t_0 = 6.5$ h, $t_A = 42$ h) using 5-μm particles and almost impossible to achieve an analysis requiring 10^7 plates ($d_p = 13.6$ μm, $t_m = 14$ days, $t_A = 91$ days). Intermediate performances rapidly become very difficult.

It is interesting that the column length is 14 m in the first instance and 272 in the second. Owing to the technological difficulties encountered in column packing, the first appears difficult but possible to prepare, and the second needs either some unexpected breakthrough or the development of a special workshop or plant.

If only a smaller pressure is available, the analysis time for a given separation power increases in proportion to the inverse of this pressure (*cf.* eqn. 28).

If we operate columns packed with particles of constant diameter but of different lengths at the maximal pressure, so as to achieve the fastest possible analysis with each of them, we see in Fig. 6 that the analysis time becomes infinite when the maximal pressure becomes equal to the critical pressure.

Fig. 6 also illustrates the fact that although the plate number limit increases with increasing particle size, the use of large particles is associated with very long columns and large analysis times. This demonstrates again that column performances should be compared for columns working at minimum HETP. Marked deviations

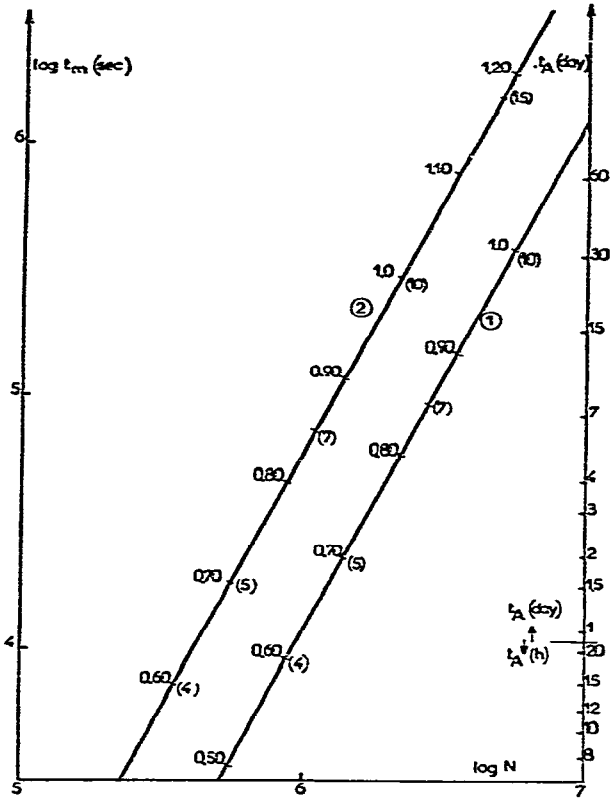

Fig. 5. Plot of breakthrough time ($k = 0$) and analysis time (right, $k = 6.4$) *versus* plate number achieved at a constant inlet pressure of 1300 atm (*cf.*, eqn. 28), using optimal columns (i.e., the particle size and column length are chosen so that $h = 2$ and $v = 3$ with $\Delta P = 1300$ atm). The numbers on each line are the logarithm of particle size, with the particle size in parentheses. 1, $\eta = 0.4$ cP, $D_m = 1 \cdot 10^{-5}$ cm²/sec; 2, $\eta = 2$ cP, $D_m = 5 \cdot 10^{-6}$ cm²/sec.

from the optimal flow velocity allow only marginal improvements in the practical speed of analysis. Table II gives some comparative figures of performances which can be obtained with an inlet pressure of 1000 atm.

TABLE II

PERFORMANCES OF AN LC COLUMN

$\eta = 0.5$ cP, $D_m = 2 \cdot 10^{-5}$ cm²/sec.

ΔP (atm)	d_p (μm)	v	N	t_A (min)	L (m)	ω^* (sec)
1000	5	6	$185 \cdot 10^3$	107	2	8
1000	5	3	$420 \cdot 10^3$	420	4.2	21
1000	10	3	$2200 \cdot 10^3$	2227	22	49
1000	3	3	$150 \cdot 10^3$	55.5	0.9	4.6

* Non-retained peak.

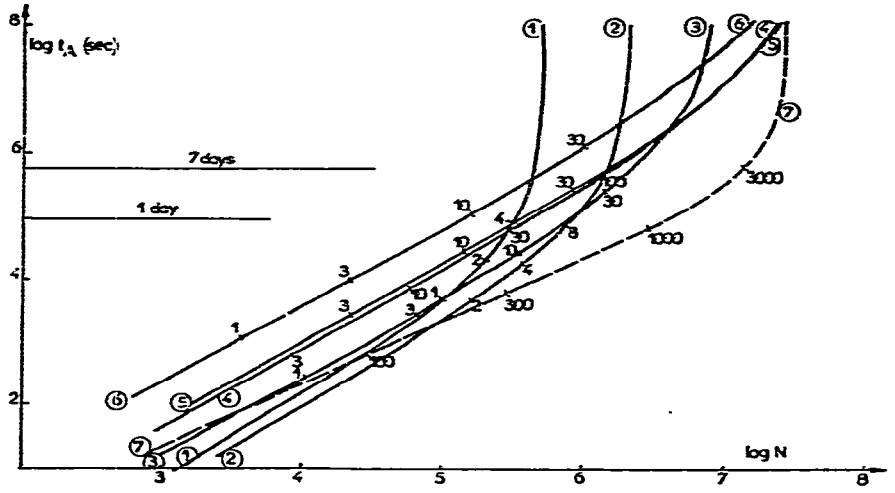

Fig. 6. Plot of analysis time *versus* plate number at constant inlet pressure. Particle size is constant along each curve and the column length is increased, hence the flow velocity decreases steadily with increasing L and N. The curves are derived as follows. From the choice of D_m and η, eqn. 16 gives $Nh\nu$ for a given ΔP, and hence ν as a function of L ($Nh = L/d_p$). For increasing values of L the corresponding values of ν are calculated, hence h, plate number and analysis time. The figures on each curve give corresponding column lengths. The data used were the following:

Curve No.	*ΔP (atm)*	*d_p (μm)*	*η (cP)*	*D_m (cm²/sec)*
1	300	5	0.5	$2 \cdot 10^{-5}$
2	1300	5	—	—
3	—	10	—	—
4	—	20	—	—
5	—	10	—	$4 \cdot 10^{-6}$
6	—	10	—	$1 \cdot 10^{-6}$
7	GC capillary column, $\Delta P = 10$ atm, I.D. = 0.25 mm, $h = 1$, $\nu = 5$ at optimum, $\eta = 1 \cdot 10^{-4}$ cP, $D_m = 0.1$ cm²/sec (hydrogen)			

Pressure gradient and number of plates per unit time

Eqns. 14–16 can be rearranged to give the pressure gradient and the number of plates per unit time, both as a function of the particle size:

$$\frac{\Delta P}{L} = \frac{\nu}{d_p^3} \cdot \frac{\eta D_m}{K_0} \tag{29}$$

and

$$\frac{N}{t_R} = \frac{D_m}{d_p^2} \cdot \frac{\nu}{h} \cdot \frac{1}{1+k} \tag{30}$$

Fig. 7 gives a plot of $\Delta P/L$ *versus* the particle diameter for four different sets of experimental conditions, one very favourable, one typical of standard LC conditions and one rather unfavourable, and a last one, typical of the conditions encountered in gel permeation chromatography (GPC). Fig. 8 shows the variation of the effective peak number as a function of analysis time in the four instances. The variation is very slow.

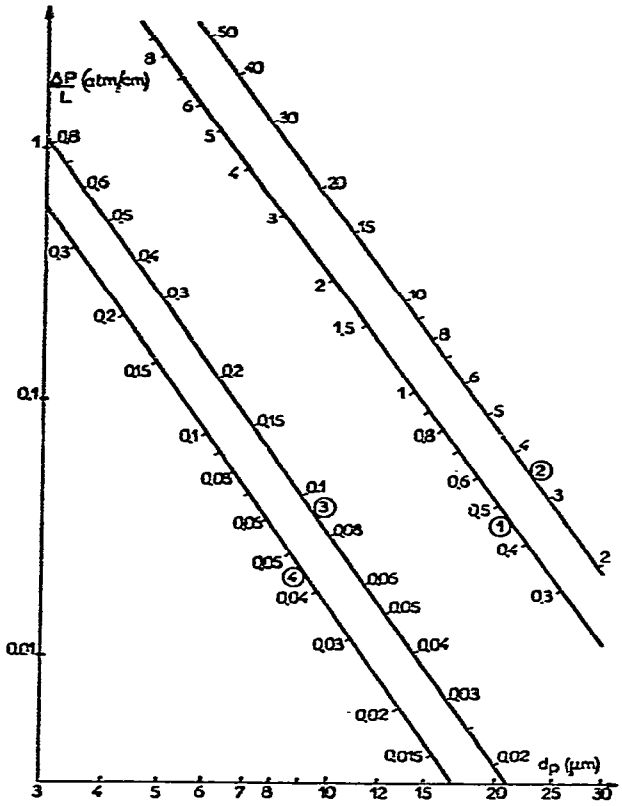

Fig. 7. Plot of pressure gradient *versus* particle diameter. The number on each line gives the number of plates generated per second ($k = 6.4$).

Curve No.	*v*	*h*	*η (cP)*	$D_m \times 10^5$ *(cm²/sec)*
1	3	2	1	1
2	4	1.5	0.3	5
3	1	5	1	0.3
4	2	3	2	0.04

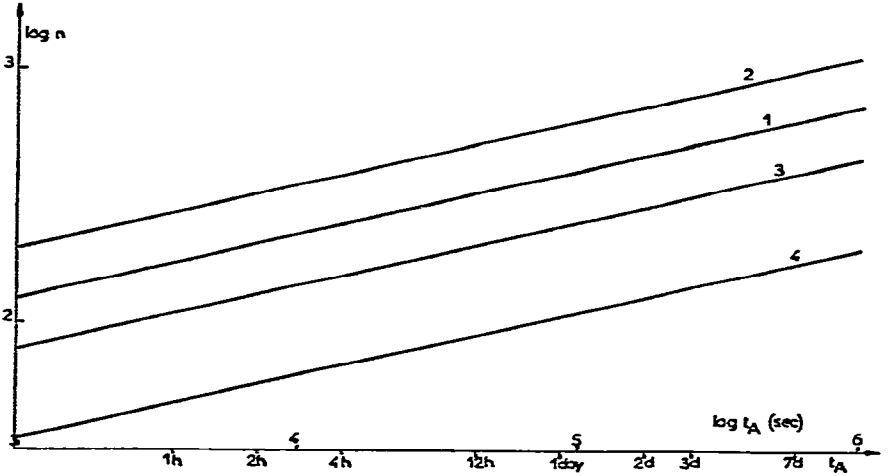

Fig. 8. Plot of the number of peaks resolved *versus* analysis time. Same conditions as for Fig. 7 for corresponding lines.

The effective peak number is calculated by combination of eqns. 15 and 23, except for GPC, where we write

$$n = \frac{\sqrt{N}}{4} \cdot Ln2 \tag{31}$$

An increase in the constant maximal pressure from 300 to 1300 atm results in an increase in the peak number by a factor 1.44, which is not substantial.

EXPERIMENTAL PROBLEMS

The use of very long columns and large analysis times is possible only if several difficult experimental problems, mainly relating to detection, are solved.

If standard columns are used, there will not be too serious problems associated with sample volume, detector cell volume and response time.

Standard commercial detectors have a cell volume of about 10 μl and a response time of about 0.5 sec. This permits the accurate recording of elution bands having more than 200-μl and 10-sec band widths in volume and time units, respectively. If the band has an efficiency of $1 \cdot 10^5$ plates, the detector performances are correct provided that the retention times and volumes exceed 800 sec and 16 cm^3, respectively. The first specification will certainly be met in all circumstances as shown above, but the second is slightly more difficult to meet. With 4-mm I.D. columns, $\nu = 3$, $d_p = 10\,\mu$m and $D_m = 3 \cdot 10^{-6}$ cm^2/sec, the flow velocity is $9 \cdot 10^{-3}$ cm/sec and the flow-rate 68 μl/min. This value of the flow-rate is low in relation to current practice, but all figures have been chosen to give a value in the lower range of possible flow-rates. In this instance, however, the detector cell is too large if the retention time is less than 4 h. Other figures are given in Table II.

The problem can be solved by using a smaller cell, but careful attention must be paid to it because, as shown by the above figures, the analysis can be performed in a shorter time. Although difficult, the problem appears to be solvable.

It is shown in a companion paper[16] that specifications for sampling time and volume are very close to those for detector response time and cell volume, so this problem does not need to be discussed further here.

The detection limits depend largely on the specific problem as they are properties of detectors, and the detectors available have widely different response factors for different compounds. A detection limit of 10^{-6} g/ml is typical, however. Assuming a Gaussian peak profile, the maximal concentration is given by

$$C_M = \frac{m_s \sqrt{N}}{V_R \sqrt{2\pi}} \tag{32}$$

where m_s is the mass of compound of interest contained in the sample volume and V_R its retention volume. C_M should be larger than the detection limit. Eqn. 32 can be rewritten as

$$C_M = \frac{4m_s}{\sqrt{N} h d_p (1 + k') \pi d_c^2 \varepsilon_T \sqrt{2\pi}} \tag{33}$$

where d_c is the column diameter and ε_T the total porosity of the packing. It is commonly accepted that the sample size is proportional to cross-sectional area of the column, so unless we are limited by the available sample mass (as in some biochemical or clinical analyses, for example) the inverse dependence of C_M on the square of the column diameter is only formal. In a companion paper[16] we show that the maximal sample volume that can be injected without increasing the band width markedly is proportional to the square root of the plate number; hence

$$m_s = C_s \mu \cdot \frac{\pi d_c^2}{4} \sqrt{N} \tag{34}$$

where μ is a length characteristic of the system and of the figure accepted for column loadability. It follows that the usual values in grams of sample per gram of stationary phase should be used cautiously.

The sample size should be such that the column is not overloaded. This, however, is difficult to assess exactly, as we accept a serious overload at the column inlet, at the end of sampling, under the condition that the decrease in maximal concentration during elution results in the column not being overloaded during most of the development (overloading means that the concentration is large and the isotherm can no longer be considered as linear). Eqn. 32 shows that during elution the concentration at the peak maximum decreases in proportion to the square root of migration distance, z. If the sample size is increased in proportion to the square root of column length, L, the plot of maximal concentration *versus* relative distance, z/L, remains identical and the peak profile will be the same. Thus, if we select μ so as to achieve maximal loadability on a given column, eqn. 34 permits the calculation of sample size on any column.

Combination of eqns. 33 and 34 gives

$$C_M = \frac{\mu C_s}{h d_p (1 + k') \varepsilon_T \sqrt{2\pi}} \tag{35}$$

In such a case the minimal detectable concentration of a compound in the sample is independent of column length and rises slightly from the non-retained compound to the last compound (with our conventions by a factor of 7.4). It is mainly characterized by the reduced plate height, the particle diameter and μ. Although we lack experimental data at present, we can expect that μ is proportional to d_p or at least increases with increasing particle diameter, with the consequence that C_M would be essentially a function of C_s. The result is different from the conclusion of the discussion between Scott and Snyder[17–19], but the approach is too. We consider only a limited range in k values (0–6.4).

DISCUSSION OF EXPERIMENTAL RESULTS

There are few such results available in this field yet, except in the recent publication by Scott and Kucera[21], who reported the achievement of extremely high efficiencies and outstanding separations.

They selected 1-mm I.D. columns and prepared 1-m long columns for which they designed special equipment by suitable modifications of available commercial

TABLE III

PERFORMANCES OF THE LONG COLUMNS REPORTED BY SCOTT AND KUCERA[21]

L (l)	d_p (μm)	N	t_m (min)	t_A (min and days)	ΔP (atm)	h	ν	t_A (calc)* (days)	ΔP (calc)*
10	20	160,000	[165]	1221 (0.85)	54	3.13	[10]	0.86	125
10	20	250,000	[250]	9300 (6.4)	7.1	2	[1.32]	6.5	16.5
14	5	650,000	[352]	2600 (1.81)	—	4.3	[1.66]	1.80	1800
14	5	510,000	780	5772 (4.0)	—	5.5	0.75	4.0	841

* From eqns. 14–16. $t_A = 7.4\, t_m$.

detectors, pumps and valves. This part of their work is discussed later. Two columns were made by coupling ten 1-m long columns packed with 20-μm particles and fourteen 1-m long columns packed with 5-μm particles. The performances of these columns are summarized in Table III. The values in brackets are interpolated from other available data or from the text. In most instances the inert peak retention time was not given and was calculated from the flow-rate. The pressure was derived from the corresponding velocity, and is not very accurate. The volume of a 1 m × 1 mm I.D. tube is 785 μl while Table II in ref. 21 gives only 700 μl. A packing porosity of 0.80 has been assumed. All this may result in an error in the pressure as large as 25%. It seems, though, that the column permeability is somewhat larger than $d_p^2/1000$. The performances that could be expected from these columns are given in Table IV.

TABLE IV

THEORETICAL PERFORMANCES OF LONG COLUMNS

L (m)	d_p (μm)	N	ΔP (atm)	t_A (days)
10	20	250,000	37.5	2.85 ($h = 2$, $\nu = 3$)
10	20	273,000	34.4	3.06 ($h = 1.83$, $\nu = 2.8$)
14	5	1,400,000	3360	1.00 ($h = 2$, $\nu = 3$)
14	5	1,100,000	1120	3.00 ($h = 2.53$, $\nu = 1$)

A comparison of Tables III and IV shows that the 20-μm particle column gives the expected efficiency but is too slow, hence giving too long an analysis time. From the two points on the HETP curve given ($h = 2$, $\nu = 1.32$; $h = 3.13$, $\nu = 10$) we tentatively calculated $A = 0.79$ and $C = 0.06$, which demonstrate an execellent packing method. We also derived an optimal velocity of $\nu = 2.8$ and a minimal reduced plate height of 1.83 (*cf.*, Table IV).

The 14-m long column packed with 5-μm particles is much less efficient than predicted. This is probably due partly to the still excessive equipment contribution (*cf.*, Table V), and partly to packing problems, as illustrated by Scott and Kucera[21] and discussed below. The efficiency is still very good and the compromise between various opposing technical requirements appears to be very difficult to improve.

Another comparison between achievements and expectations is provided in Fig. 9. The open points show the analysis time achieved and pressure used. The solid points show what analysis time should have been obtained and what pressure used to achieve the performances obtained using the particles selected. For the 5-μm col-

TABLE V

BASE WIDTHS OF INERT PEAKS ELUTED FROM 1-mm I.D. COLUMNS

d_p (μm)	L (m)	N	ω_t* (cm)	ω_v** (μl)
5	1	100,000	1.26	9.9
5	14	$1.4 \cdot 10^6$	4.73	37.2
10	1	50,000	1.79	14.0
10	10	500,000	5.66	44.4
20	1	25,000	2.53	19.9
20	10	250,000	8.0	62.8

* $\omega_t = 4L/\sqrt{N}$.

** $\omega_v = \omega_t \cdot \frac{\pi d_c^2}{4}$.

umn, the analysis time is too long in comparison with the efficiency achieved. For the 20-μm column the reduction in analysis time is explained by the increase in pressure and flow velocity. For the 5-μm column the analysis time at a given pressure

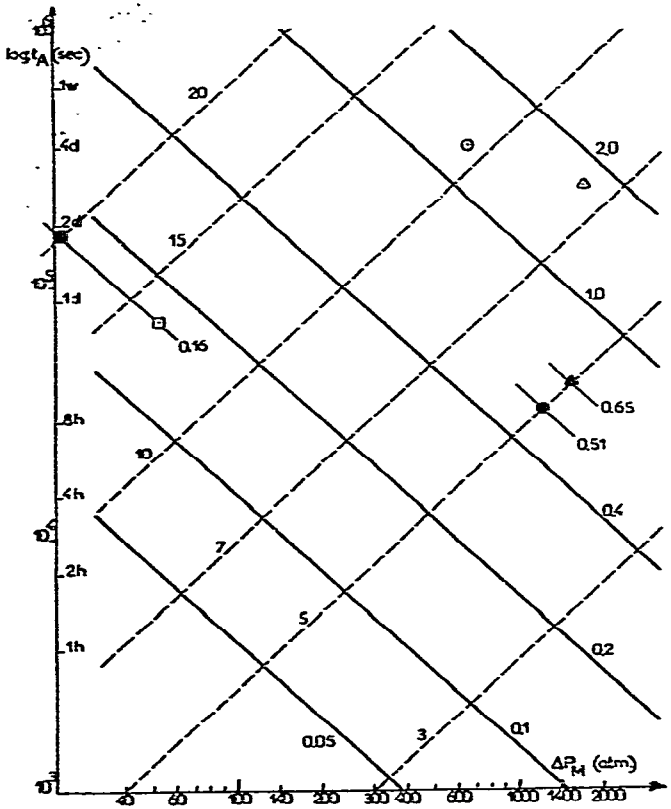

Fig. 9. Plot of analysis time *versus* pressure. Solid lines: constant number of plates, given by the number on each line (in millions). Broken lines: constant particle size, given on each line (in micrometres). Solid points: plate number achieved by Scott and Kucera using the corresponding particle size. Open points: retention time and pressure corresponding to these experiments (hence number of plates expected).

is about six times larger than predicted. This is not much, but may be the difference between what is possible and what remains an impossible dream.

One of the reasons for the lack of experimental data is the difficulties that are encountered in the packing of long columns and in the connection of packed columns in series.

Long columns are difficult to pack. All packing techniques evolved today use the autofiltration of a slurry of the packing material in a suitable solvent. The particles are forced into place by the drag force generated by the viscous flow. This force is given by Stokes law. The ratio of this force to the actual particle weight is

$$\frac{F}{P} = \frac{18\eta u}{d_p^2 \varrho g} \tag{36}$$

where ϱ is the density of the particles and g the gravity. For a solvent velocity of 1 cm/sec, which is largely exceeded during most of the packing operation, this ratio is about 740 for 5-μm particles ($\eta = 1$ cP, $\varrho \approx 1$), which explains why slurry packing is so much more efficient than dry packing. Further, the force applies on each individual particle, forcing it to set in the most stable position above the bed. If this picture is correct it follows that reducing the density of the slurry could improve the efficiency of the column (*i.e.*, fewer particles per unit volume).

Obviously, the flow-rate of the solvent falls while the column is being packed and the end section is not packed as densely as the beginning. It is easy to observe that this last part is very hard while for long columns the end is soft. For example, Scott and Kucera[21] packed their columns with a Haskell pump at about 1750 atm. At that pressure, the flow velocity through a 1-m long column is 0.44 cm/sec with 5-μm particles and 7 cm/sec with 20-μm particles, making the ratio given by eqn. 36 320 in both instances. This is not much, probably one order of magnitude smaller than that which is necessary, and may explain why in several instances the permeability of the columns packed by Scott and Kucera[21] appears larger than usual: the velocity achieved at the end of the packing corresponds to about three times the optimal velocity ($\nu = 3$, $D_m = 2 \cdot 10^{-5}$ cm^2/sec) for 5-μm particles. Also, the ratio given in eqn. 36 is not the only parameter to consider; the pressure sticking one particle against the bed ($12\,\eta u/d_p$) is also important. The packing velocity should be larger with smaller particles. This is in agreement with the data in Fig. 9 in ref. 21, which illustrate that 5- and 10-μm particles are more difficult to pack than 20-μm particles.

For a non-retained peak, the 20-μm particle, 1-mm I.D. column gives $h = 2.7$ and $\nu = 2$ at the optimum. This probably results from the still excessive contribution of detector cell volume (1 μl) and injector (0.5 μl). For a 10-μm particle, 1-m long column, the zone width (4 σ) for $h = 2$, for an inert peak, would be 12.5 μl (Table II in ref. 21). With the modified detector and cell the observed band width is 21 μl, accounting for a reduced plate height of 5.7 at 0.11 cm/sec ($\nu = 5.3$) (*cf.*, Table III). The optimal performances of this column are $h = 3.6$ and $\nu = 1.25$. Because of the lack of data, it is difficult, however, to assess the relative contributions of equipment and packing difficulties to this loss of performance. The fact that the 10-m long, 20-μm particle column performs better than expected ($h = 2$ instead of 2.7 for a 1-m long column) demonstrates, however, that the equipment contribution is signif-

icant, but the fact that the *h versus v* curves have a slope of about unity at large reduced velocities shows that this contribution cannot be very important at low flow-rates.

Probably the fact that very good efficiencies were still obtained by Scott and Kucera, better with 1-mm I.D. than with 2- or 4-mm I.D. columns, is due to the smaller scale over which the fluctuations of packing permeability occurs. Giddings[22] has shown that such fluctuations can be accounted for by an HETP contribution proportional to the square of the ratio of the column diameter to the particle diameter and proportional to the mobile phase velocity. Such a term would be 16 times smaller for the columns used by Scott and Kucera than for 4-mm I.D. columns.

In addition, the influence of the heat effect on column efficiency, which has been neglected here, decreases sharply with decreasing column diameter, firstly because the radial temperature gradient becomes smaller and secondly because its consequences, through the appearance of a trans-column fluctuation of the mobile phase velocity, again decrease with the square of the column diameter.

For all of these reasons, and because the otherwise formidable requirements on the performance of the equipment are reduced by the large column length, we feel that the approach suggested by Scott and Kucera, the use of small-bore packed columns, is probably the most valid for the achievement of extremely high efficiencies.

CONDITIONS FOR A BREAKTHROUGH BEYOND THE MILLION-PLATE WALL

All the results discussed here, theoretical and experimental, demonstrate that, unless we are prepared to work at enormous pressures and to accept considerable analysis times we shall be limited to analyses that require not more than 10^6 plates (*i.e.*, 500 resolved peaks) in about a day. This already needs pressures in the order of 1300 atm. Is it possible to do much better? We are of the opinion that it is not, unless some major breakthrough in column technology is made.

We shall first discuss the possibilities offered by either working at high temperature, to reduce viscosity, or using the recycling technique, without recycling, just using pumps on-line, between column segments, to achieve high effective pressure drops. Finally, we discuss briefly the use of capillary columns as far as they can be studied at present because of the lack of data.

Effect of increasing temperature

As shown by eqn. 24, the product ηD_m increases very slowly with increasing temperature, about 30% for an increase in temperature by 100° above ambient. Accordingly, eqn. 16 shows that we can achieve 30% more plates with a given pressure. The viscosity decreases markedly with increasing temperature, however, by a factor of 2–2.5 for an increase in temperature of 50° for water, methanol, acetonitrile and their mixtures[20]. We can expect a decrease by a factor of 5 for a temperature increase of 100° (which now makes necessary the use of a pressurized detector cell) and consequently an increase in D_m by a factor of 6.5. Eqn. 15 shows the analysis time has become five times shorter. This is a modest improvement, although not negligible: instead of generating 10^6 plates in 1 day (up to $k = 6.4$) we can generate $1.3 \cdot 10^5$ plates in 5 h, in both instances with an inlet pressure of 1300 atm and in 1 day we can generate $2 \cdot 10^6$ plates. Clearly we need something else in order to go beyond these limits.

Pump series

We have seen above that because the maximal pressure is limited, the time necessary to achieve a given plate number increases more rapidly than this number, as we cannot keep the velocity constant.

Assuming an inlet pressure of 1000 atm, and using 5-μm packed columns, we can operate them at twice the optimal velocity ($\nu = 6$) and still obtain a reduced HETP of 2.24. Eqn. 16 shows that for a system with $\eta = 0.5$ cP and $D_m = 2 \cdot 10^{-5}$ cm^2/sec, we can generate 185,000 plates in 1.75 h using a 2-m long column ($k = 6.4$). Ten such systems in series could generate $2 \cdot 10^6$ plates in 18 h. Other such combinations can be derived from the data in Table II. Five pumps and five columns, each 22-m long and packed with 10-μm particles, could generate 10^7 plates in about 1 week.

Combined with some temperature increase this solution could prove attractive, although a large oven would be necessary. On the other hand, the data in Table II show that the loss in efficiency inside the pump could be significant, especially with 3- and 5-μm columns. The requirement would be that the pump gives more than one stroke per second, which is not very demanding, except the stroke volume could be only a few microlitres.

Clearly a marked increase in column permeability is required.

Capillary columns

For obvious reasons capillary columns are attractive and recently have generated considerable interest and serious hopes. Work is more advanced with packed capillaries as they are easier to prepare. We still lack data on their performances, however, especially as far as the efficiency for retained peaks is concerned. The efficiency for inert peaks appears excellent but this is unfortunately not sufficient for chromatographic separations. The scant information available is derived from examination of chromatograms and from a few publications. It points towards a disappointingly rapid increase in plate height with increasing retention, especially at large velocities. This is normal and expected: the resistance to mass transfer in the stationary phase becomes important at large velocities and also, as the literature of 10 years ago illustrates[25,26], the resistance to mass transfer and the plate height increase markedly with increasing column capacity factor for large particle diameters. Even packed capillaries prepared with 10-μm particles exhibit this phenomenon, however[23].

Assuming that we can use a column with $h = 20$ and $\nu = 10$ for $k = 6.4$ and $k_0 = 10^{-2}$, which is optimistic at that stage[23], we calculate that the performances are much poorer than with standard packed columns.

The glass tube cannot withstand an inlet pressure much larger than 200 atm and, in spite of the ten times larger permeability, the analysis time remains very large[24]. Even assuming that a considerable improvement in performance is possible, we still fall very short of the present expectations (*cf.*, Table VI).

Theoretical calculations give little hope that true capillary columns can ever compete, as illustrated by the data in Table VII[24,27]. It is difficult to see how the various problems can be solved simultaneously.

In a capillary column the permeability coefficient is 1/32 and eqns. 14–16 apply as well as for packed columns. Assuming a simple system, with $\eta = 0.5$ cP and $D_m = 2 \cdot 10^{-5}$ cm^2/sec, we see that if the maximum pressure is 200 atm, which is

TABLE VI

PERFORMANCES OF PACKED CAPILLARY LC COLUMNS
$K_0 = 10^{-2}$, $\eta = 0.5$ cP, $D_m = 2 \cdot 10^{-5}$ cm²/sec.

ΔP (atm)	d_p (μm)	N	t_A (h)	L (m)
200	10	$100 \cdot 10^3$	20.6	20 ($v = 10, h = 20$)
200	10	$1330 \cdot 10^3$	228 (9.5 days)	67 ($v = 3, h = 5$)
1000	5	$1670 \cdot 10^3$	71 (3 days)	42 ($v = 3, h = 5$)
1000	10	$500 \cdot 10^3$	103 (4.3 days)	100 ($v = 10, h = 20$)

very difficult to exceed with a glass tube, even when very narrow, the maximum plate number depends only on hv/d_p^2. If we take a 20-μm I.D. tube, and assume $h = 1$ and $v = 10$, which corresponds to an excellent column, better than is achieved in gas chromatography, we find that we can generate $12.5 \cdot 10^6$ plates with a 250-m long column and the analysis time ($k = 6.4$) is 18.5 days. This still corresponds to about 1.5 days for 10^6 plate analysis. A 10-μm I.D. capillary column would be four times faster.

The problem becomes the preparation and use of such a column. The resistance to mass transfer in the stationary phase, which has to be a porous layer prepared by attacking the column wall, should be small, and hence this layer thin, but a 2-μm thick layer at the surface of a 20-μm I.D. tube provides a phase ratio only four times larger than in a column packed with silica particles having the same specific surface area, which is certainly acceptable. The inert peak has a $9 \cdot 10^{-2}$-μl band volume at the column exit (282 sec). While the response time is not a problem, a $4 \cdot 10^{-3}$-μl detector cell has to be designed and a similar volume only is available for injection. A 10-μm I.D. column, being twice as short, will demand an eight times smaller detector cell to provide the same efficiency.

The gain, compared with the performances of packed columns, is small and the technological problems formidable, which make the approach unattractive.

TABLE VII

THEORETICAL AND PRACTICAL PERFORMANCES OF A CAPILLARY COLUMN
Column characteristics: $d_c = 60$ μm, $L = 25$ m, $u = 8.7 \cdot 10^{-2}$ cm/sec, $\eta = 0.4$ cP, $D_L = 3 \cdot 10^{-5}$ cm²/sec, $\Delta P = 0.77$ atm, $t_0 = 8$ h.

k	N (exptl)[27]	N (calc.)*	C_S (sec)
0	1,250,000	1,407,000	—
0.5	100,000	663,000	0.24
1	60,000	239,000	0.36

* From the Golay equation.

CONCLUSION

It seems easy to prepare LC columns that provide efficiencies of a few hundred thousand plates and that permit the elution of 250–400 well resolved peaks in a reasonable time. Available column packings and LC instrument technology exist in many different laboratories; only analytical problems and the will to solve them seem to be missing.

Analytical problems that can be solved only through this approach do exist and, as Scott and Kucera[21] have demonstrated the feasibility of the method, there is little doubt that we shall rapidly see some new developments and applications in this field. Chromatograms similar to those obtained with capillary columns in gas chromatography will be used to analyse complex mixtures.

It is still doubtful whether many chromatograms exhibiting more than a million plates will ever be published. Although this is well within the reach of present GC technology it does not seem that there is still a real need for it or that it is worth the analysis time. In LC the analysis time for that kind of performance will remain longer than in GC, as shown in Fig. 8, and will be several days.

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