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Simple Considerations on Column Design in Preparative-Scale Liquid Chromatography

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Abstract

This paper discusses some simple considerations on the design of preparative columns (particle size and column length and diameter). It is shown that the loadability of a column does not depend on the size of the particles for a given efficiency. In order to obtain the maximum efficacity from the pumping system, the design of the column must take into account the characteristics of the pumping system, particularly the flow rate. It is shown that a convenient particle size is in the range 10 to 20 μm. Small particles are associated with shorter and bulkier columns than large particles, while at the same time giving less back pressure (in order to generate a given plate number at a given flow rate). The larger the flow rate generated by the pumping system, the smaller must be the particles.

INTRODUCTION

The design of the column is a very important aspect in preparative liquid chromatography (PLC). Several studies have been published on that subject. They can be divided into two categories, depending on the mode of operation of the column. One group deals with volume overload (*1-5*), the other group with mass overload (*5-12*). Volume overload is a kinetic effect and is related to zone spreading. When it takes place, the contribution of the injected volume to bandspreading is comparable to dispersion due to the column. The sample concentration, however, is sufficiently low to avoid departure from linearity of the sample distribu-

tion isotherm (linear chromatography). Mass overload (or better, concentration overload) is a thermodynamic effect related to the nonlinearity of the distribution isotherm (nonlinear chromatography). Under these conditions, retention times and peak shapes depend on the quantity of sample injected.

Volume overload is well understood and has been precisely quantified. It is possible to optimize the column design (and/or the injection volume) in order to achieve a given throughput, purity, and recovery (*1–5*). Concentration overload is a more complex situation, and no accurate mathematical solution is yet available to describe the peak profiles (except at moderate overload). This is because this necessitates the knowledge of the distribution isotherm (in fact, the composite isotherm due to mutual interactions of solutes at high concentration). In addition, there is no analytical solution to the system of differential equations describing the combined effects of column dispersion and isotherm nonlinearity broadening (*9–13*). Some theoretical work is in progress in this direction (*6*, *14*), and it must be mentioned that a very interesting semi-empirical approach has been published (*5*). Mass overload is a very important problem in PLC because it is usually associated with much larger throughputs than volume overload.

Among the parameters characterizing the column design, the particle size is very critical. There are two philosophies concerning the size of the particles to choose for PLC (*15–18*). One is to use somewhat large particles (40–50 μm or more) packed in sufficiently long columns to generate the plate number required for the separation, and the other consists of using smaller particles (typically in the 10 to 20 μm range) packed in shorter and usually bulkier columns. The *apparent* advantages of the first approach are the low price of large particles compared to small ones, the ease of column preparation, and particularly the possibly to (mass) overload more of the column. This last point is very important and there seems to be some confusion in the literature concerning this issue (*17–19*).

Besides the particle size, there is another very critical and often neglected parameter concerning the column design in PLC: the performance of the pumping system in terms of flow rate and pressure rating (*9*, *20*). This is an important point because the throughput is directly proportional to the solvent flow rate. Accordingly, the column design must be such that the pumping system is used as efficiently as possible.

The purpose of this study is to present some data on the role of the particle size in PLC. It is not our purpose to propose an optimization scheme for the design of preparative columns, but rather to discuss some

simple considerations that should be useful as a first approach to the complex problem of optimization. This work only addresses concentration overload. The injected volumes were kept small enough not to produce additional broadening.

EXPERIMENTAL

The silicas used were Lichroprep Si-60 25–45 μm (Merck, Darmstadt, FGR) and Lichrosorb Si-60, 5 and 10 μm. The columns were made from 1/4″ o.d. tubing 4.6 mm i.d. The columns packed with the 10 and 25–45 μm particles were 30 cm long and that packed with the 5-μm particles was 15 cm long. The solutes investigated were nitrobenzene and benzophenone. The mobile phases were mixtures of *n*-heptane and chloroform (HPLC grade, Merck). The composition is given in volume fraction. In order to standardize as much as possible the surface properties of the different silicas tested (to assure similar retention characteristics), the silica samples were activated under identical conditions (250°C under nitrogen for 12 h). In addition, after being packed the columns were equilibrated with the same mobile phases until stable retention values were obtained. With this procedure, the 3 columns exhibited almost identical retention properties at low loadings (less than 5% variation in k' values).

The pumping system was a Constametric III (LDC Milton Roy, Riviera Beach, Florida). Solutes were detected at 254 nm with a Spectromonitor III UV/Vis detector (LDC Milton Roy). Retention times and plate numbers were determined from the first centered moments. These were measured using a data acquisition system based on a Commodore 128. The sampling frequency was adjusted in order to take at least 50 data points per peak.

RESULTS AND DISCUSSION

1. Particle Size and Loadability

The usual way to characterize a stationary phase for preparative applications is to give its specific mass loadability, q_0 (quantity of sample per unit mass of stationary phase). This specific load corresponds to a certain change in retention (for instance, 10% increase or decrease in capacity ratio) or efficiency (for instance, 10% increase in plate height).

The decrease in column efficiency with increasing injected quantity is

an important matter in PLC since a certain number of plates is required to obtain a given purity (*9*). Under severe mass (in fact concentration) overload conditions, the shape of the elution peak is only determined by the isotherm nonlinearity at the concentration at the column outlet (*11*). The adverse effect of overload on column efficiency appears when the broadening due to isotherm nonlinearity exceeds a certain fraction of the specific broadening due to the column (at zero injection). Consequently, an overload effect appears more rapidly on a system with little dispersion (high efficiency). This means that when comparing 2 columns of identical length and diameter but packed with particles of different sizes, the column packed with small particles will be overloaded more rapidly. However, this is not because the particles are smaller but rather because the column is more efficient. The specific loadability values that could be calculated from the efficiency versus specific load for these columns would be of little help in characterizing the packing materials because of the difference in plate numbers.

In loadability studies, the critical parameter is the plate number at zero injection, N_0 (*7*, *11*, *12*). Using a simple model, it is possible to define the apparent plate number N_A (calculated from the elution peak) according to:

$$1/N_A = (1/N_0) + (1/N_{OL}) \quad (1)$$

where N_{OL} is a "plate number" characterizing the overload effect. $1/N_{OL}$ is related to the specific load and characterizes the distribution isotherm. It is a thermodynamic parameter that does not depend on the column design (particularly the particle size). Equation (1) indicates that for a given specific load, columns with identical N_0 values but not necessarily the same design must experience the same change in efficiency with specific load. Equation (1) also shows that the effect of increasing the specific load must be less critical with columns of low plate numbers. Moreover, at a given specific load, columns for which $1/N_0$ is negligible compared to $1/N_{OL}$ should have the same plate number.

Some experimental results are shown in Figs. 1 to 3. They are very similar to those reported by Poppe et al. (*11*) and Knox et al. (*12*). Figure 1 shows plots of log (N_A) versus log (q_0) for the 3 different columns. At low loads the plots are straight lines parallel to the X-axis and at high loads they are superimposed on a common straight line. As explained above, this is because at high loads the variance of the injection is much larger than that of the column, and peak broadening is only controlled by the extent of overload, independent of the column efficiency (*12*). The horizontal part of the plots becomes shorter with increasing column

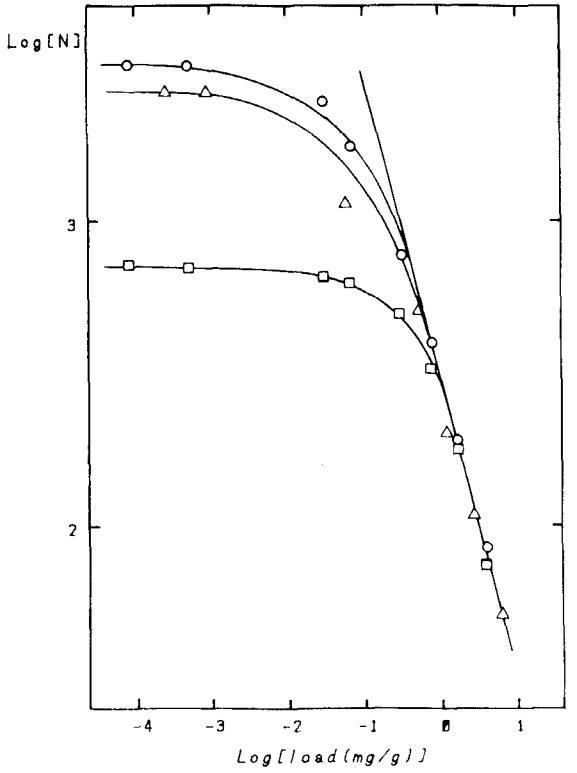

FIG. 1. Change in column efficiency with load. Sample: nitrobenzene. Solvent: *n*-heptane/chloroform 5:1 v/v. Flow rate = 2.5 mL/min. (○) 10 μm; (△) 5 μm; (□) 20–40 μm.

efficiency (N_0) because the *relative* importance of overload becomes larger. However, there is no specific effect of the particle size as demonstrated by the fact that for 2 columns designed to have the same efficiency but packed with particles of different sizes (5 and 10 μm), the curves are very similar. It is clear that if the loadability curves would have been made in terms of reduced plate height, the 5-μm support would have shown the same rate of increase with load as the 10-μm one. Similar conclusions can be drawn from Fig. 2 where the results obtained with a given column operated at different flow rates of solvent are shown. These results indicate that it is irrelevant to discuss the overload behavior of a column if its efficiency is not specified (*7*, *11*).

Reported in Figs. 3A, 3B, and 3C are the variations of $1/N_{OL}$ with q_0 for nitrobenzene measured with the 3 columns in different solvent conditions. The capacity ratio increases from about 0 in pure chloroform to 10

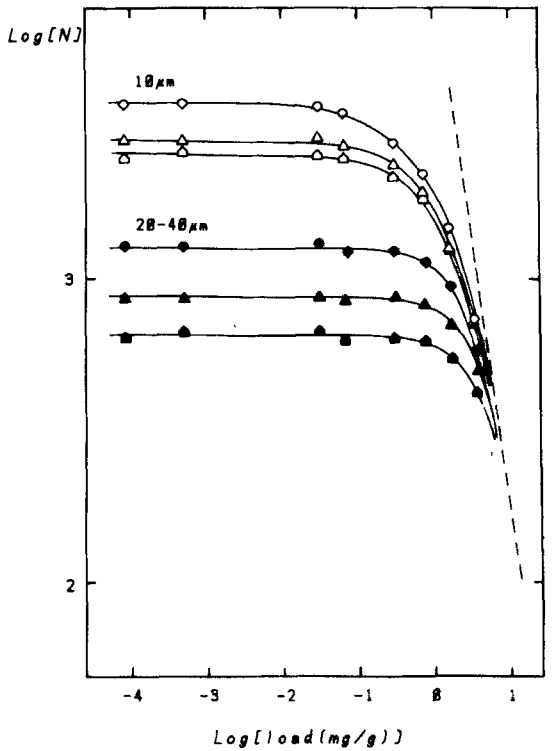

FIG. 2. Same as Fig. 1 except that (○) 1 mL/min; (△) 2.5 mL/min; (□) 4 mL/min.

in the mixture 9/1 *n*-heptane:chloroform. In each case the behaviors of the 5 and 10 μm particles are very similar. The agreement with the larger particles is not as good, particularly at low loadings. This is because the plate numbers are much smaller on that column and a small error in their determinations may induce very large differences in $1/N_{OL}$ values. For example, assuming N_0 is 500, it can be calculated that log $(1/N_{OL})$ decreases from -3.477 to -3.654 when N_A increases from 425 to 475. This means that information on the effect of low overloads can only be obtained with large plate numbers, but not necessarily with small particles.

The parameters of linear regressions made on the data in Figs. 3 are reported in Table 1. The results indicate that in each case the value of the slope S is close to unity (0.8–0.9). Very similar values were obtained with benzophenone. Observation of the data available in the literature reveals slope values different from unity (*11*, *19*). The value of the intercept I

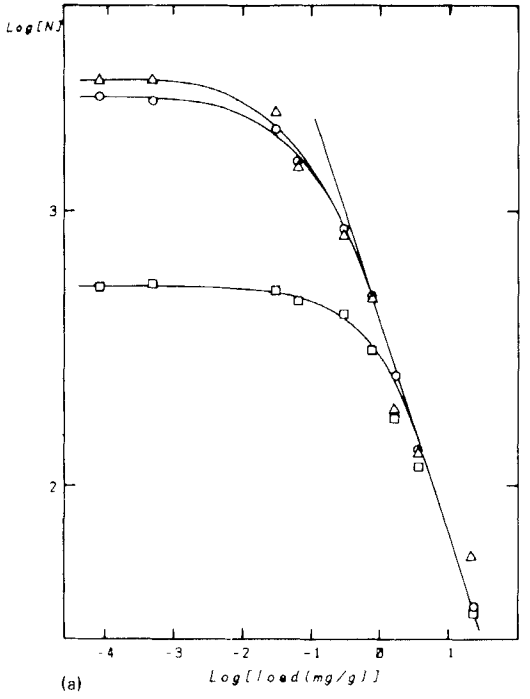

FIG. 3A. Same as Fig. 1 except for the solvent: chloroform.

depends on the extent of solute retention. The relationship between I and k' is shown in Fig. 4. It appears that I decreases quite rapidly with increasing k' and reaches a plateau at about $k' = 2$. The initial decrease is in agreement with the fact often reported that columns are more rapidly overloaded with increasing retention. The existence of the plateau can possibly be due to a compensation effect: with increasing k', the curvature of the distribution isotherm at the origin is more pronounced but the concentration in the mobile phase decreases because of increasing retention.

As mentioned by Poppe et al. (*11*), the relationship between $1/N_{OL}$ and q_0 is a useful aid to selecting optimum conditions in PLC. It seems that this relationship is probably a good way to characterize a system for preparative purposes. It would be interesting to know what the parameters that control $1/N_{OL}$ are, and particularly the effect of the solvent composition and the properties of the stationary phase (chain length, coverage, etc.).

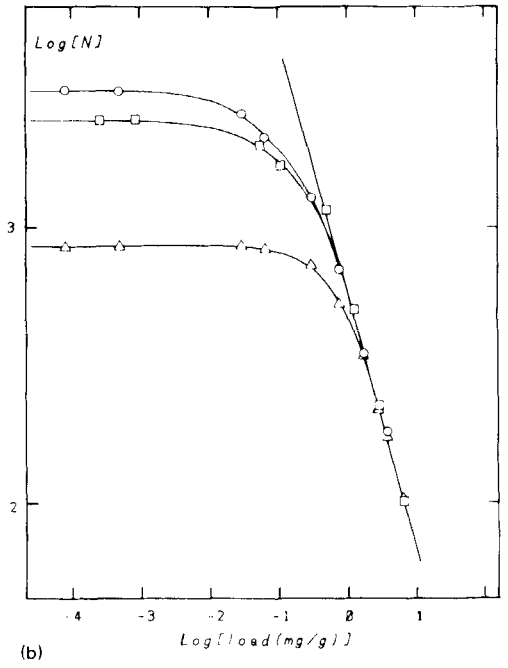

FIG. 3B. Same as Fig. 1 except for the solvent: *n*-heptane/chloroform, 1:1.

2. Considerations on Column Design

The previous results indicate that, independent of the particle size, there is a specific column load q_0 associated with a given plate number, N_C. If a column is designed to have a plate number N_0 larger than N_C, its efficiency will drop to N_C upon injection of q_0. The larger N_0, the more overloaded the column. The individual values of the length of the column and the diameter of the particles are not important, the only critical parameter is N_0. In other words, this suggests that, if a certain plate number N_0 is required for a separation, it will not be possible to achieve higher specific loads on a column giving more plates than required compared to a column giving N_0 plates (assuming the injection volume is small enough not to generate a significant decrease in plate number). Accordingly, when comparing columns of identical plate number and volume (in order to inject the same load), the column packed with small particles will be shorter and bulkier than that packed with large particles.

A critical aspect of the column design is the role of the solvent flow rate

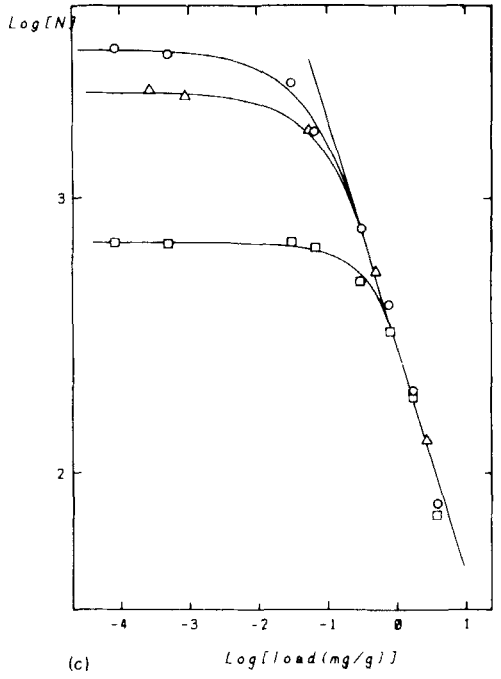

FIG. 3C. Same as Fig. 1 except for the solvent: *n*-heptane/chloroform, 9:1.

TABLE 1
Parameters of the Linear Regressions log (N_{OL}) vs log (q_0)

Regression coefficient	Intercept	Slope	Particle[a]	Solvent[b]
0.993	−3.450	1.043	10	0:1
0.836	−3.361	1.050	5	0:1
0.998	−2.808	0.892	10	1:1
0.997	−2.839	0.918	5	1:1
0.998	−3.018	1.254	20–40	1:1
0.996	−2.524	0.826	10	1:5
0.998	−2.498	0.809	5	1:5
0.985	−2.616	0.771	20–40	1:5
1.000	−2.529	0.918	10	9:1
0.995	−2.574	0.827	5	9:1
0.998	−2.724	1.105	20–40	9:1

[a]Particle size in μm.
[b]*n*-Heptane/chloroform mixture (v/v).

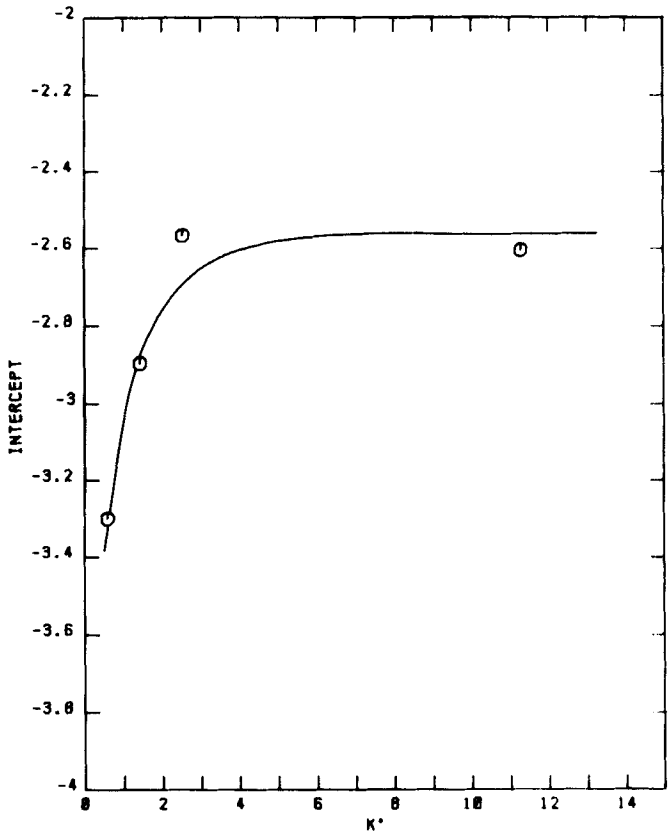

FIG. 4. Variation of the intercept (see text and Table 1) with the capacity ratio.

on the throughput, TP (ratio of the injected quantity to the analysis time). For a given recovery and purity, the higher the TP value, the better the system. The injected quantity, Q_{INJ}, is related to q_0 and the column volume V_C according to

$$Q_{INJ} = q_0 \rho V_C \tag{2}$$

where ρ is the packing density. The time of analysis is related to the column length and capacity ratio according to

$$t_R = (1 + k')V_C \varepsilon_T / F \tag{3}$$

where ε_T is the column total porosity and F is the solvent flow rate. Combination of Eqs. (2) and (3) gives

$$TP = Fq_0\varepsilon_T\rho/(1 + k') \quad (4)$$

Equation (4) reveals that, for a given chromatographic system (given k', ρ, q_0, and ε_T), TP does not depend on the column design and is proportional to the flow rate. It is assumed in the derivation of Eq. (4) that the column gives at least the critical plate number and the retention times do not change too much with the extent of overload. This is usually verified, unless the column is heavily overloaded. This effect can thus be neglected to a first approximation. It is also assumed in the derivation of Eq. (4) that the packing density and column porosity do not depend on the column design. This is usually true, unless the ratio of the column diameter to the particle size becomes too small (i.e., less than 100).

A straightforward implication of Eq. (4) is that it is recommended to operate the preparative system at the maximum flow rate. Although it is common practice in analytical chromatography to operate the pumping system much below the maximum flow rate (most of the separations are made at 1 or 2 mL/min whereas most of the systems can deliver up to 10 mL/min), this is probably not a good strategy to use in PLC. One reason is that the throughput is directly related to the flow of mobile phase in the column. Moreover, considering the price of a good pumping system for PLC, it is preferable to use its full capabilities of pressure and/or flow rate (in the limit of safe operation). The flow rate in the column is limited either by the pumping system (maximum flow rate and pressure) or by the rest of the equipment (such as the column pressure rating). The following discussion is based on the assumption of a pumping system flow rate limitation. It would also be possible to consider pressure limitation (*9*, *12*). The situation depends on the pumping system and column pressure rating as well as the solvent viscosity. Our experience is that flow rate limitations occur more often than pressure limitations, particularly when using stainless steel columns. The situation may be different with glass columns, particularly those of large diameter. The following calculations will also suggest that pressure limitation is not frequent.

It is clear that, for practical reasons, there is a maximum and a minimum limit to the length of the column. Because of the necessity to have end connectors, the column length must not be too small. On the other hand, it is not practical to use extremely long columns which, among other things, would be difficult to pack. Although the values are quite subjective, it was assumed that reasonable limits are 10 and 200 cm.

Based on these limits, the change in column diameter with the particle size and the pumping system flow rate was calculated for two values of the plate number: 1000 and 3000. The first one corresponds to an easy separation and the last one to a more difficult one (at the preparative level). It must be mentioned that in many cases more than 3000 plates are required, particularly with complex mixtures. The flow rate values are 100, 500, and 1500 mL/min. The first one is typical of a bench-top preparative system. The second one corresponds to a system intermediate between laboratory equipment and production scale, and the last one is production/process oriented.

The results are shown in Figs. 5, 6, and 7. The calculations were limited to columns with a diameter less than 30 cm (12″) and particle size between 10 and 100 μm. The results show that for a given column length, increasing the particle size most often corresponds to a very rapid increase in the column diameter, particularly at high flow rates. The shapes of some curves are interesting because they show the existence of

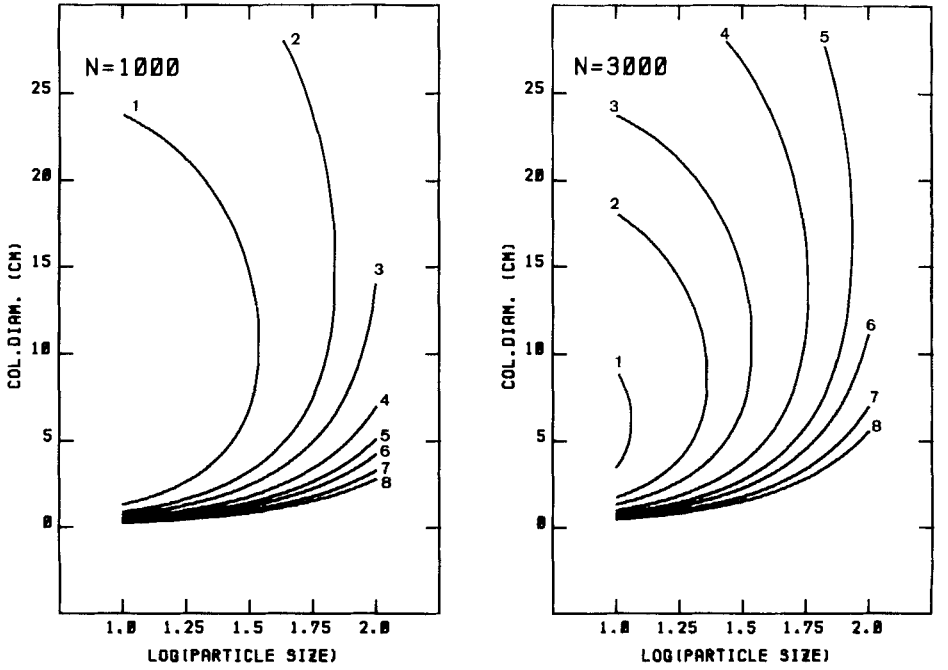

FIG. 5. Variation of the column diameter with the particle size (in μm) for columns of various length giving $N = 1000$ and $N = 3000$ for a flow rate of 100 mL/min. Column length (cm): 1, 10; 2, 20; 3, 30; 4, 50; 5, 75; 6, 100; 7, 150; and 8, 200.

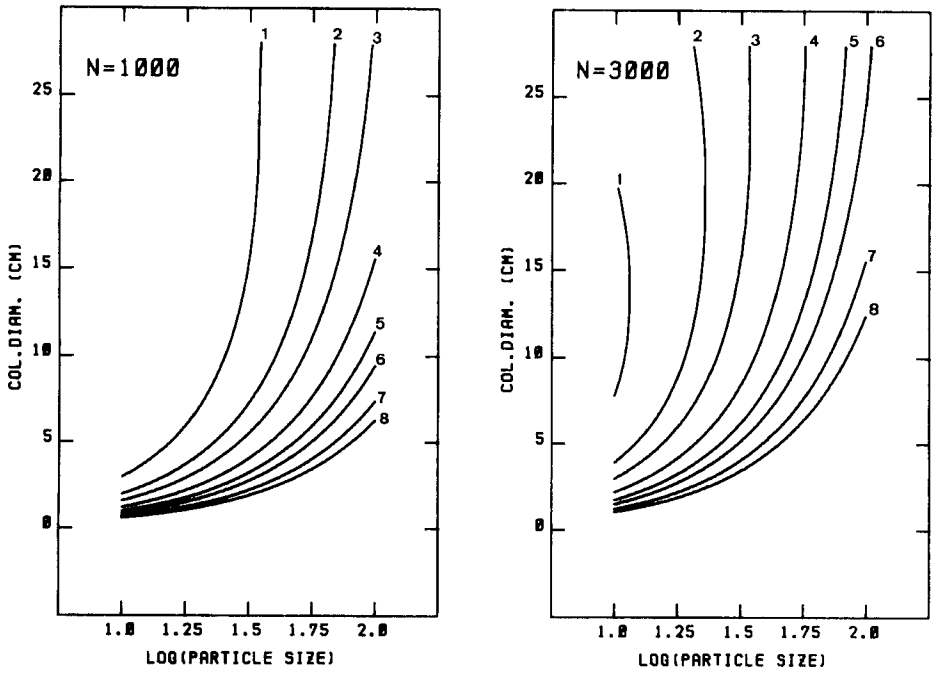

FIG. 6. Same as Fig. 5 except for a flow rate of 500 mL/min.

a maximum particle size. In fact, there exists a maximum particle size for each column length (corresponding to the minimum of the plate height curve), but it is only visible on the figures for certain combinations of the parameters. For a given particle size, there are 2 values of the column diameter on each curve. One is small and corresponds to a large solvent velocity and usually a high column pressure (particularly for small particles). The other one corresponds to a column of large diameter, operated at a solvent velocity smaller than the optimum. The column pressure is very low. The maximum particle size corresponds to the optimum solvent velocity; that is, the best use of the column. Examination of the curves reveals that increasing the pumping system flow capacity must be associated with a decrease in the size of the particles. In other words, small particles must be used for process chromatography (in the limits of cost constraints). It must be stressed that, although the cost of "reasonably" small particles (10–20 μm) is significantly larger than that of large ones, the gain in throughput and/or purity can largely compensate for the cost of the packing material.

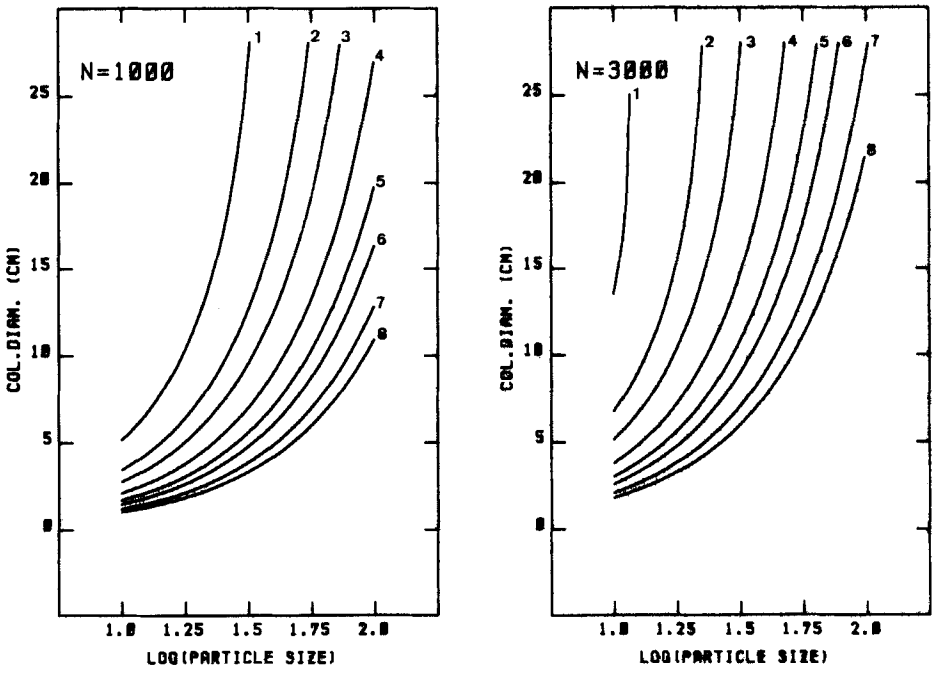

FIG. 7. Same as Fig. 5 except for a flow rate of 1500 mL/min.

The previous discussion does not take into account the column volume which is also an important parameter. The appropriate volume depends on the quantity of sample to purify and the specific loadability. In fact, the situation is more complex because of the possibility of multiple injections. There are two extreme strategies for the choice of the column volume. One is to select a column of sufficient volume to be able to purify all the sample in one injection and the other is to use a very small column (offering the required plate number) and make a very large number of small injections. In theory, if the small and the big columns are operated at the same flow rate, the throughput will be the same (see Eq. 4). The analysis time will be smaller on the short column, but the injected quantity will also be proportionally smaller too. In practice, the number of injections is limited by several parameters, however. One is the implausibility to operate a column at a very large solvent velocity because of the associated pressure drop and heat generation. In addition, it is sometimes necessary to regenerate the column after each cycle, such as in gradient elution, for instance. In that case, it takes more time to do

multiple injections on a small column than a single one on a bigger column. It is clear that the choice of the optimum column volume is not straightforward, particularly when economic factors have to be taken into account (*20*). It seems difficult to give general rules.

We show in Figs. 8, 9, and 10 several isochore (constant column volume) lines for typical column volumes, covering the range from the "small" laboratory purification column (V = 500 mL) to the medium size production column (V = 10,000 mL, a 20-cm long column 20 cm i.d.). On an isochore line, increasing the particle size results in a rapid decrease in column diameter and a corresponding increase in column length (since the plate number is constant). It also results in increasing column pressure, the rate of increase being extremely rapid above a certain particle size. In fact, there is an optimum value of the particle size for each isochore. However, the pressure change around the minimum is very small and the minimum can hardly be detected. The larger the required plate number and/or flow rate, the smaller the critical particle

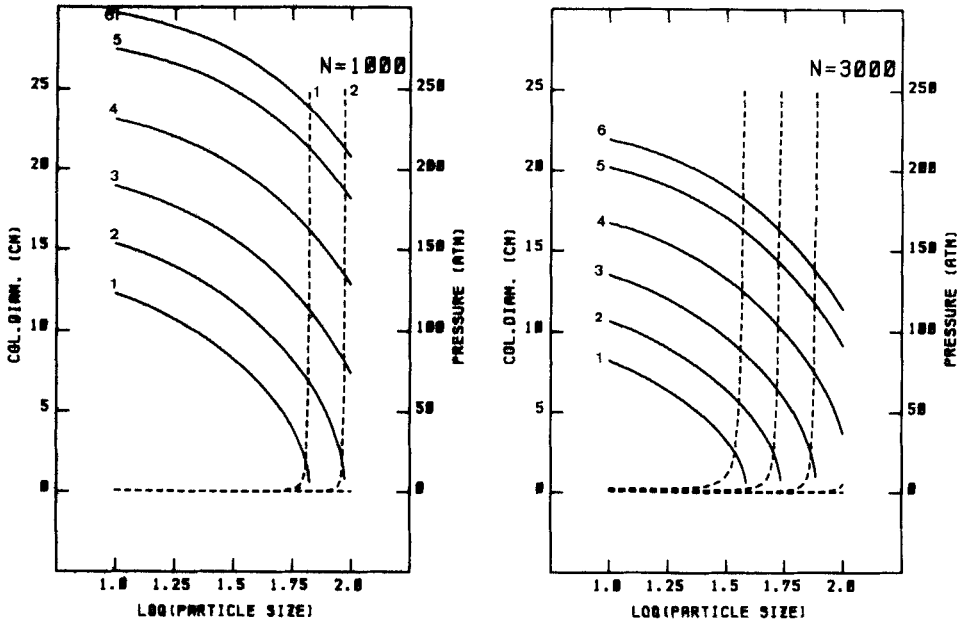

FIG. 8. Variation of the column diameter (solid lines) and the column pressure (dashed lines) for columns of various volumes giving N = 1000 and N = 3000 plates at a flow rate of 100 mL/min. Column volume: 1, 500 mL; 2, 1,000 mL; 3, 2,000 mL; 4, 4,000 mL; 5, 7,500 mL; and 6, 10,000 mL.

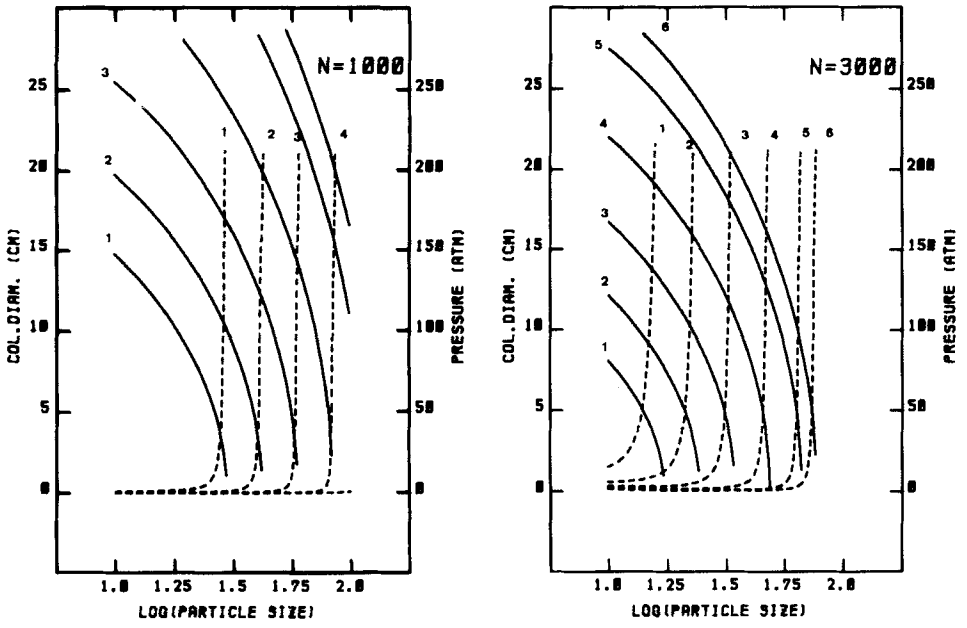

FIG. 9. Same as Fig. 8 except for a flow rate of 500 mL/min.

size. This observation is also in favor of using small particles for process chromatography.

CONCLUSION

Examination of Figs. 8 to 10 suggests that although it is not possible to define an "optimum" particle size for preparative chromatography, the range 10–20 μm seems to be very suitable, both in terms of column physical dimensions and operating pressure. Using a column of large diameter is usually not very well accepted. It is often claimed that the performance of such columns (especially in terms of sample distribution at the top and associated peak deformation) are not as good as those of smaller bore columns. This injection problem is very critical, and various technical solutions, more or less convenient to implement, have been proposed in the literature. In turns out that most of the problems experienced during injection on large columns originate from a poor column design. It has been shown (*21*) that large bore columns (up to 60 cm i.d.) can be as efficient as small bore ones, if not more efficient (*22*). A

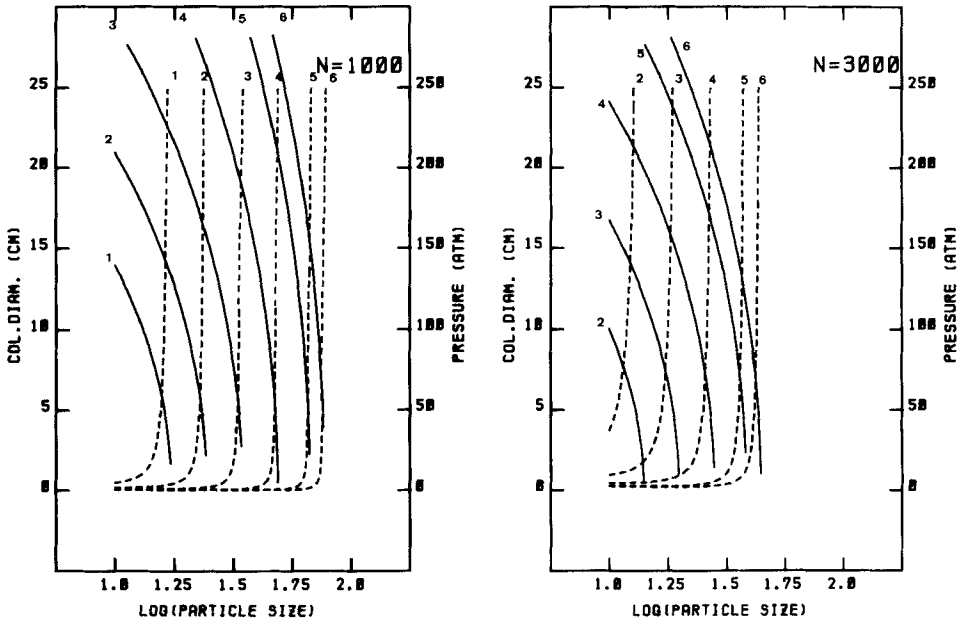

FIG. 10. Same as Fig. 8 except for a flow rate of 1500 mL/min.

dramatic advantage of short columns of large diameter packed with small particles compared to longer and skinnier ones packed with large particles is the possibility to use the axial compression technique (*23*). This technique makes column packing and unpacking very easy and fast (a very important point in terms of use for production purposes) and provides a very good column stability because the packing is continuously under pressure.

Finally, another advantage of small particles compared to larger ones appears at the thermodynamic level. It has been shown recently (*24*) that the sharpening effect of early eluting peaks resulting from solutes mutual exclusion on the stationary phase (composite isotherm effect) is more pronounced for small particles, resulting in an additional gain of efficiency.

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