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SELECTION OF OPTIMAL CONDITIONS IN PREPARATIVE LIQUID CHROMATOGRAPHY

I. THEORY

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SUMMARY

The amount of substance that can be separated per unit time (the production rate) and the purity of the isolated compounds are the determining quantities in preparative chromatography. Basing the design and operation of a preparative system on the optimal production rate means optimizing the latter with respect to time, solvent consumption and the smallest possible dilution of the sample in the effluent.

Equations describing the influence of the process variables are derived showing as the most important and striking result that the production rate is not a linear function of the column length. They predict that working under conditions where the contribution to mass dispersion of the injected volume and the column itself are about equal is to be preferred. The influence of the pressure limitation and pumping capacity of a chromatographic system on the production rate is discussed.

INTRODUCTION

The increasing use of liquid chromatography as a preparative tool and the general recognition of its practical importance for the recovery of pure substances has initiated a number of experimental and theoretical investigations in order to find rules for the selection of the optimal working conditions. Many published results relate with partial aspects of this subject, but a more general solution of the problem is still missing. They, showed, however that the amount of sample that can be isolated per unit time and its purity are determined by a number of parameters, of which the most important are column length and diameter, linear flow velocity, particle size, sample size and concentration, number of theoretical plates required and separation efficiency of the column in terms of plate height and selectivity.

The interdependence of these parameters seems to be very complex. In this paper a relatively simple relationship is derived that allows the determination of the appropriate values of the various parameters. In simplifying the mathematical model a number of assumptions have been introduced. However, they do not limit the general conclusions that can be derived from the proposed equations, even for those cases with far from idealized conditions.

THEORETICAL

The goal of preparative chromatography is the isolation of compounds in suitable amounts with a certain degree of purity. The latter requires a certain resolution between the compounds under consideration and is determined by the well known equation

$$R_{ji} = \frac{1}{4} \cdot \frac{\alpha - 1}{\alpha} \cdot \frac{k'}{1 + k'} \cdot \sqrt{N} \quad (1)$$

where $\alpha = k'_j/k'_i$ is the selectivity coefficient of the phase system with respect to components j and i , k'_j and k'_i are the capacity ratios of components j and i , k' is the arithmetic mean and N is the average number of apparent plates generated by the system. This equation also applies if the resolution is smaller than that required but the effluent is fractionated so that the necessary purity is achieved in this way¹⁻³. This technique only influences the yield, which is the ratio of the amount of a certain substance collected to that injected.

According to eqn. 1, a chromatographic system should be chosen with as high a selectivity as possible. However, if the selectivity cannot be adjusted properly, as is the case with complex mixtures or closely related compounds, the only way of achieving a sufficient purity is to improve the resolution by means of a high average plate number.

The most essential criterion for a preparative separation is the amount of component i that can be separated per unit time with a required number of theoretical plates to achieve the necessary resolution. This so-called production rate (P_{iN}) is defined as

$$P_{iN} = G_i/t_{Ri} \quad (2)$$

where G_i and t_{Ri} are the amount of i separated and its retention time, respectively. The term "production rate" was introduced by de Jong *et al.*⁴ and the considerations based upon this function are not restricted to large amounts of substances. They apply equally well if only microgram amounts are to be separated. As will be shown later, optimizing the production rate not only optimizes the system with respect to time but also to solvent consumption and dilution of the sample in the effluent. This optimization is valid both for separations which are carried out in one run and those in which repetitive injections are applied.

In eqn. 2 it is assumed that i , the substance under consideration, is the last eluting component and that the amount of i producing G_i is injected with a cycle time t_{Ri} . If instead of i component j , with a retention time t_{Rj} , is the last eluting component then eqn. 2 should be multiplied by the factor t_{Ri}/t_{Rj} .

Modifying eqn. 2 by means of the equations

$$t_{Ri} = t_{R0}(1 + k'_i) \quad (3)$$

and

$$t_{R0} = L/u_0 \quad (4)$$

where t_{R0} is the retention time of an unretained component, u_0 its linear velocity and L the column length, and defining

$$G_i = V_i c_i \quad (5)$$

where V_i is the volume in which i is injected and c_i its concentration, we obtain

$$P_{iN} = \frac{V_i c_i u_0}{L(1 + k'_i)} \quad (6)$$

With $L = NH$ one obtains

$$P_{iN} = \frac{V_i c_i u_0}{NH(1 + k'_i)} \quad (7)$$

where N is the required plate number according to eqn. 1 and H the apparent plate height describing the mass dispersion due to the injection volume, injection procedure and the column itself.

As is known from various publications on preparative liquid chromatography⁴⁻¹³ H is strongly influenced by the injected sample size. The latter can be increased in two ways: (1) by using a small and constant injection volume and increasing the sample concentration c_i ; and (2) by maintaining a concentration that lies in the linear part of the distribution isotherm and increasing the sample volume V_i . De Stefano and Beachell¹⁴ showed, that the linear capacity of a column is higher for diluted than for concentrated sample solutions if the same amount of solute is injected. In this work, the sample concentration was kept below the level where mass overload would occur, which means that the distribution coefficient was virtually constant and therefore the retention time was not affected by sample size. However, if this prerequisite is not fulfilled under practical conditions, the conclusions that can be drawn from the final results will still retain their qualitative validity.

As the total volume variance of the elution function for a certain component, $\sigma_{v_{tot}}^2$, generated by a system may be written as the sum of the variances of the subsystems which build up the system¹⁵, it follows that

$$\sigma_{v_{tot}}^2 = \sigma_{v_{inj}}^2 + \sigma_{v_{col}}^2 \quad (8)$$

where $\sigma_{v_{inj}}^2$ is the volume variance due to the finite width of the injected volume and its dispersion while entering the column and $\sigma_{v_{col}}^2$ is the volume variance of the elution profile generated by the column itself.

Modification of eqn. 8 with the equations $\sigma_{v_{tot}}^2 = (V_R^2/L)H$, $\sigma_{v_{inj}}^2 = V_i^2/D^2$ and $\sigma_{v_{col}}^2 = (V_R^2/L)H_0$ results in

$$H = H_0 + \frac{L}{D^2} \cdot \frac{V_i^2}{V_R^2} \quad (9)$$

where H is the apparent plate height of the total system, H_0 the theoretical plate height of the column for $V_i \rightarrow 0$, D the ratio of the injection volume to its volume

standard deviation at the column inlet and V_R the retention volume of the eluted component.

Solving eqn. 9 for V_i and substituting with $V_R = A\varepsilon_t(1 + k')L$ and $H = L/N$ we obtain

$$V_i = A\varepsilon_t(1 + k')LD[(1/N) - (H_0/L)]^{1/2} \quad (10)$$

where A is the column cross-section and ε_t the total porosity of the enclosed packing material.

Substituting eqn. 10 in eqn. 6 leads to the final expression for the production rate:

$$P_{iN} = A\varepsilon_t u_0 c_i D [(1/N) - (H_0/L)]^{1/2} \quad (11)$$

Eqn. 11 contains all the important process parameters and is well suited to a discussion of their influence on the production rate.

(i) *The capacity ratio, k' .* The capacity ratio does not appear in eqn. 11 and therefore does not affect the production rate. The reason for this is that longer retention times due to higher k' values are compensated for by a smaller variance of the injected volume. Therefore, the column can be shortened or the injection volume increased, which is in accordance with eqn. 6 and with the results obtained by Scott and Kucera⁶. If the column is concentration overloaded, however, the capacity ratios change with increasing concentration and a different situation arises, which was demonstrated by Coq *et al.*¹².

(ii) *The injection volume, V_i .* The injection volume is eliminated by the substitution of eqn. 10 into eqn. 6, which mathematically means that it is chosen such that the system always generates the required plate number. Injecting smaller volumes means that the column is not used properly, while larger volumes would result in a plate number smaller than that required, which violates our basic assumption.

(iii) *The square root term, $[(1/N) - (H_0/L)]^{1/2}$.* This term is the most interesting in eqn. 11. It suggests that the production rate increases with decreasing required plate number, N (easier separation), increasing column length (more stationary phase available) and decreasing column plate height H_0 (better separation efficiency). However, it is the relative magnitude of the terms under the square root which determines the production rate and the values to be selected. The most striking result of eqn. 11 is that the production rate is a monotonous but not a linear function of the column length. The following limiting cases can be distinguished:

$$(1) L < H_0 N.$$

Here a negative value is generated under the square root and a physically meaningless production rate is the result as the column itself has a plate number smaller than that required.

$$(2) L = H_0 N.$$

The column has a plate number equal to that required for the separation, but as for this case $V_i = 0$ then $P_{iN} = 0$. In the following this length is designated as the initial column length, L_0 .

$$(3) L > H_0 N.$$

The column has a plate number higher than that required, hence the production rate increases with increasing column length or decreasing column plate height. The

increase of the production rate with column length is steep for lengths close to the initial length ($L_0 = H_0N$) but slows down very quickly for longer lengths.

$$(4) L = \infty (L \gg H_0N).$$

The dispersion contribution of the column is small compared with the dispersion generated by the sample. The production rate reaches its maximum value:

$$P_{iN}^{\max} = A\varepsilon_i u_0 c_i D(1/N)^{1/2} \quad (12)$$

This result is in accordance with theoretical results of Wehrli⁵ and Scott and Kucera⁶. Wehrli *et al.*⁸ also discussed the limiting cases mentioned under (2) and (4). De Jong *et al.*⁴ investigated the limiting case mentioned under (2), omitting the influence of the injection volume.

The optimization of preparative chromatography, however, must not be discussed under limiting conditions only. As will be shown later, the preferable working point lies far from those points where either of the dispersion contributions may be neglected.

Fig. 1 shows the dependence of the production rate, P_{iN} , according to eqn. 11, on column length, L , for different particle sizes. As the limiting cases [(2) $P_{iN} = 0$ and (4) $L = \infty$] are impractical, the preferred working point must lie somewhere in between. The curves show a steep rise in production rate at relatively short column lengths. After a certain column length the slope of these curves decreases drastically and a further increase in length results in only a small increase in the production rate. Two points (A and B in Fig. 1) are considered to be the preferred working points for two columns packed with stationary phases having mean particle diam-

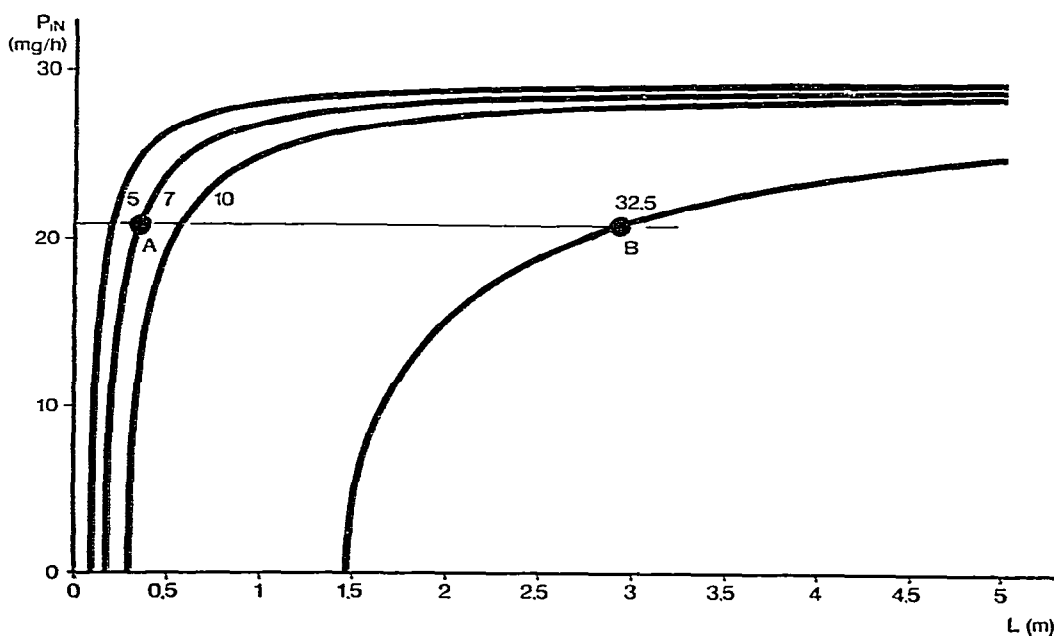


Fig. 1. Dependence of production rate on column length for different particle sizes (conditions as in Table I).

eters of 7 and 32.5 μm , respectively. These points have a special significance in that the respective column length is exactly twice the initial column length, L_0 , and the dispersion contribution of the column is equal to that of the volume injected in terms of their respective volume variances. At these points the system can be loaded with a sample size such that the apparent plate number equals the required plate number and is half the column plate number at $V_i \rightarrow 0$.

According to eqn. 10 the injection volume is

$$V_i = A\varepsilon_i(1 + k'_i)LD \left(\frac{1}{2N}\right)^{1/2} \quad (13)$$

and the resulting production rate is $P_{iN} = 0.71 P_{iN}^{\text{max}}$.

At this point it seems appropriate to discuss the term "loadability", which in general is defined as the amount of sample (in volume or mass) which preferably should be injected in order to work under optimal conditions.

Scott and Kucera⁶, taking Klinkenberg's work¹⁶ as a basis, defined the maximal sample load as the amount of sample that increases the final bandwidth (at $V_i \rightarrow 0$) by 5%. Snyder¹⁷ suggested a linear column capacity for which the limit is defined as the sample weight at which the sample equivalent retention volume decreases by 10%. Halász and co-workers^{18,19} proposed a graphical method for determining the loadability limit. Roumeliotis and Unger¹³ suggested working at that point where the sample size decreases the original column plate number by 20%. The definition of loadability factors is certainly useful for comparing different phase systems. They are not suitable, however, for estimating the optimal working point for a certain separation.

Fig. 1 also shows (and so does eqn. 11) that columns filled with packing materials of different sizes can generate the same production rate if the column length is adjusted appropriately. Even a column packed with 100- μm particles having a plate height of 1 mm and a length of 1 m fits Fig. 1 when the proper amount (about 56 cm^3) is injected and the sample is recycled 10 times. The production rate for this column then is as large as that for the highly efficient column packed with 5- μm material. It can therefore be concluded that if all other parameters are constant the ratio of column plate height to column length must be kept constant in order to obtain the same production rate. The decision as to whether to take column A or B will depend on practical aspects. Both columns are compared in Table I, which clearly shows that column B has distinct disadvantages over column A: (a) columns with this length are unwieldy and it is doubtful that if when dry packed they will show a reasonable performance; (b) the long cycle time and considerable dilution of small samples will make it very difficult to transfer and optimize the analytical separation to this scale; (c) although the price per gram of stationary phase is 3.3 times better the latter is over-compensated by an 8.7-fold increase in the requisite amount for this column.

An advantage of column B is reflected by its smaller pressure drop (93 bar). Separation problems that require a high number of apparent plates should be tackled with short, highly efficient columns (packed with small particles) unless the pressure becomes the limiting factor. If, however, Fig. 1 is constructed for a required plate number of 50, all curves would shift to the left-hand side, in which case the resulting preferred lengths ($L = 2L_0$) for small particles ($\leq 10 \mu\text{m}$) appear to be impractical

TABLE I

COMPARISON OF COLUMNS WITH PACKINGS OF DIFFERENT PARTICLE SIZES

Conditions: $R = 1$; $k' = 3$; $\alpha = 1.08$; $N = 5000$; column I.D. = 8 mm; $u_0 = 4.17$ mm/sec ($F = 10.06$ cm³/min); $c_t = 1$ mg/cm³; $D = \sqrt{12}$.

Parameter	Column	
	A	B
Particle diameter (μm)	7*	25-40**
Plate height, H_0 (μm)	34	296
Length (cm)	34	296
Injection volume (cm ³)	1.90	16.5
Production rate (mg/h)	21	21
Pressure (bar)	232	93
Volume (cm)	17	149
Cycle time, t_{Rt} (min)	5.45	47.42
Dilution factor	1.63	1.63
Weight of stationary phase (g)	10.3	89.3
Price of stationary phase (DM)	117	308

* Merck LiChrosorb RP-8; price 11.36 DM/g.

** Merck LiChroprep RP-8; price 3.45 DM/g.

(≤ 2.74 mm). In this case coarser particles should be chosen, which then allow one to make full use of their low price and low back-pressure. The influence of the required plate number on the production rate at constant particle size (7 μm) and linear velocity is shown in Fig. 2.

The meaning of the term $D[(1/N) - (H_0/L)]^{1/2}$ can be discussed by transforming eqn. 9 into

$$D[(1/N) - (H_0/L)]^{1/2} = V_i/V_R \quad (14)$$

From this equation it is obvious that the term under consideration is equal to the ratio of the volume V_i which can be injected into a column to the retention volume V_R . The column has a plate height H_0 and a length L , while the required plate number is N and the injection is performed with a certain value for D .

Substituting eqn. 14 into eqn. 11 and taking into account that $A\varepsilon_t u_0 = F$, the volume flow-rate, we obtain

$$P_{IN} = Fc_t V_i/V_R \quad (15)$$

with $0 \leq V_i/V_R \leq 1$. The limiting cases are:

$$\begin{aligned} V_i/V_R = 0 & \quad \text{for } V_i = 0 \text{ and therefore } P_{IN} = 0 \text{ (no injection)} \\ V_i/V_R = 1 & \quad \text{(continuous injection of sample, no separation)} \end{aligned}$$

Practical values of V_i/V_R lie between 0.001 and 0.1 depending on the conditions set by eqn. 10.

According to eqn. 15 the production rate can be interpreted as the product of a continuous mass flow, Fc_t , and a volume fraction determined by the parameters described in eqn. 14. The quantitative factor, Fc_t , in eqn. 15 has an upper limit

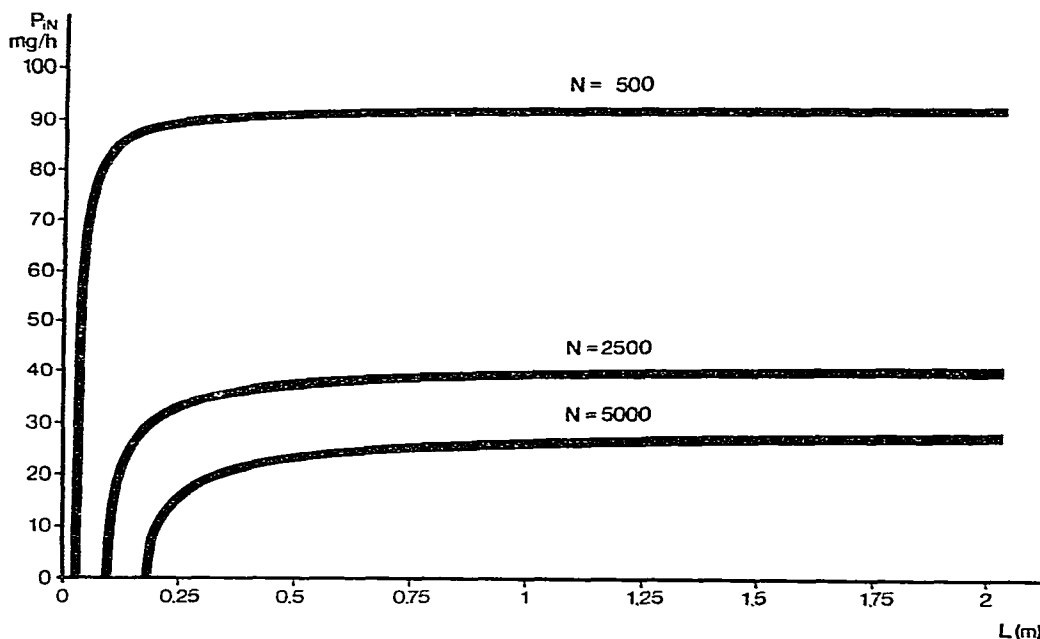


Fig. 2. Dependence of production rate on column length for different required plate numbers at constant particle size and linear velocity (conditions and assumptions as in Fig. 1).

which is set by the limiting pressure or the pumping capacity of the system. The qualitative factor, V_i/V_R , depends on the required plate number, N , the efficiency and length of the column, H_0 and L , and the quality of injection, D .

(iv) *The injection parameter, D .* The ratio of the injection volume to its volume variance while entering the column should be as high as possible. Its theoretical limit, however, is achieved for $D = \sqrt{12}$, which means that a volume is injected as a rectangular plug without being diluted on its way to the column. If the injection profile has a Gaussian shape then $D = \sqrt{2\pi}$. As will be shown in a subsequent paper²⁰, practical D values lie between 0.5 and 2.5, reflecting the fact that for various systems and techniques used very different results can be obtained and that therefore in this area ample space for improvements still exist.

(v) *The column cross-section, A .* According to eqn. 11 the production rate is directly proportional to A . The latter may therefore be selected according to the required amount of substance to be isolated and the flow-rate limitations of the available instrument. In contrast to gas chromatography, liquid chromatographic columns can be scaled up without a decrease in efficiency^{5,13,14,18,21-28}. However, the production rate not only increases with A but also with column length, L , as shown before. At a constant linear velocity an increase in column length will increase the column pressure drop while an increase in column cross-section will increase the volume flow-rate. A more detailed investigation with respect to these instrumental parameters will be discussed in a subsequent paper²⁰.

(vi) *The linear velocity, u_0 .* At first sight, eqn. 11 suggests that the production rate is directly proportional to u_0 . The latter, however, influences the magnitude of the

expression under the square root. This influence can be visualized by introducing a simplified expression for H_0 :

$$H_0 = H_0^{\min} + m(u_0 - u_0^{\min}) \quad (16)$$

where H_0^{\min} corresponds to the minimum in the H_0 versus u_0 curve at the linear velocity u_0^{\min} , m being its slope.

Two limiting cases can be distinguished:

- (1) for zero u_0 (no flow), $P_{iN} = 0$;
- (2) if u_0 increases, H_0 increases up to a value where the expression under the square root becomes zero and therefore $P_{iN} = 0$.

Between these two extremes the production rate must reach a maximum value. An example of this phenomenon is shown in Fig. 3, where P_{iN} is plotted versus u_0 for columns with different particle sizes and preferable lengths ($L = 2L_0$), taken from Fig. 1, with a required plate number of $N = 5000$. Fig. 3 shows that in this case (where the preferable length is an inherent parameter for each particle size) small particle sizes achieve the highest maximal production rate at the highest maximum linear velocity if pressure limitation is not taken into account. If the particle size is held constant (e.g., $7 \mu\text{m}$) but the column length and linear velocity varied simultaneously a plot as shown in Fig. 4 is obtained.

Two conclusions can be drawn from Fig. 4: (1) at constant particle size longer columns allow higher production rates at increasing velocity; (2) the locus of the points for which $dP_{iN}/du_0 = 0$ has a constant slope, which means that in those cases

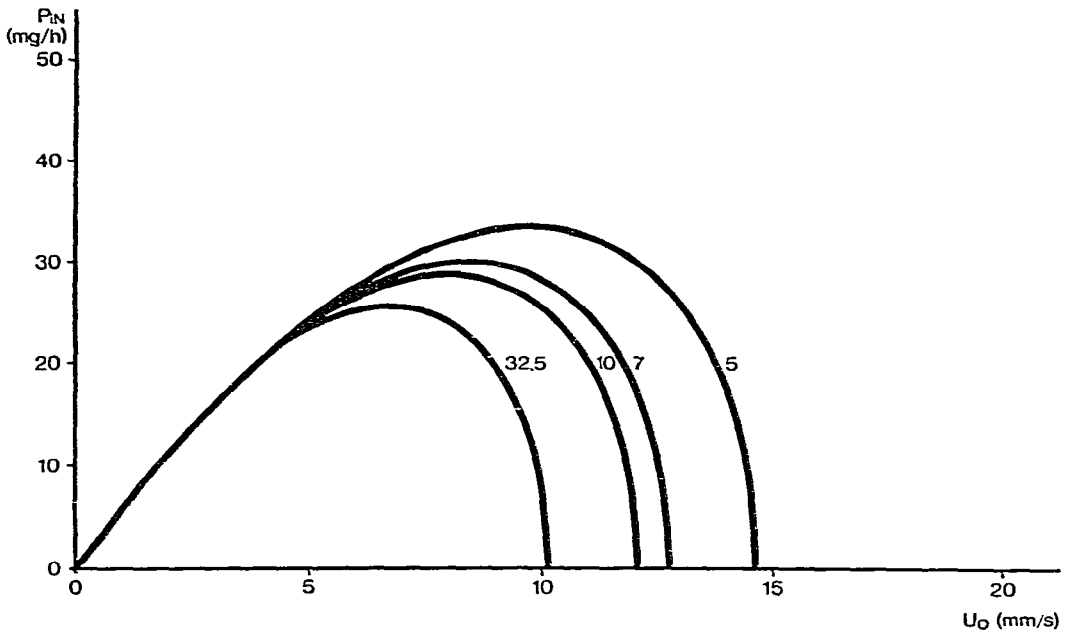


Fig. 3. Dependence of production rate on linear velocity for different particle sizes and inherent preferable column lengths.

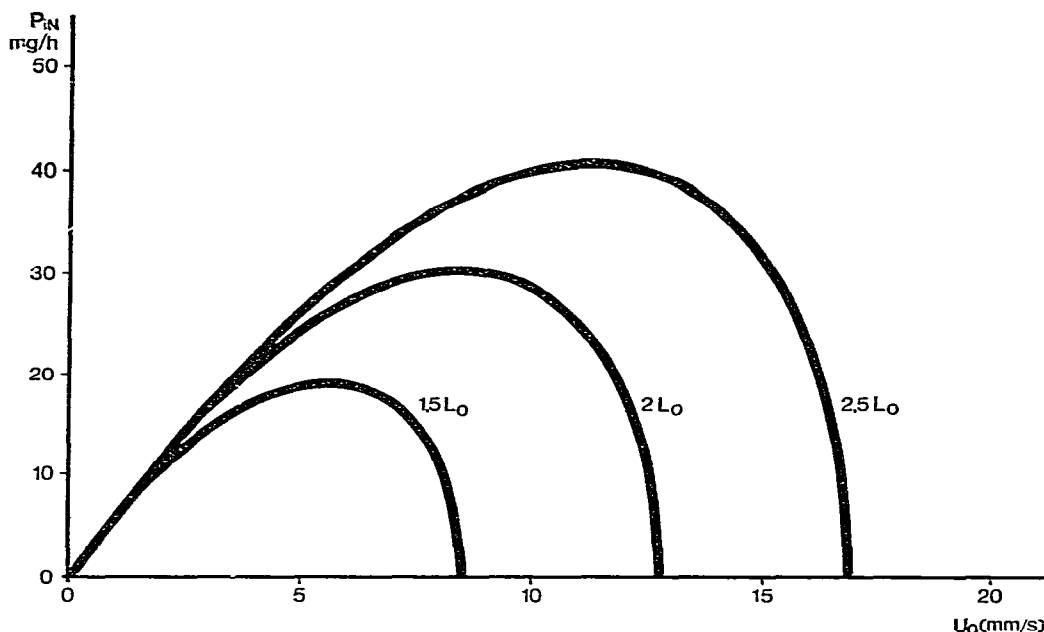


Fig. 4. Dependence of production rate on linear velocity and column length at constant particle size.

the injected volume per unit length is constant. It is also clear that in this case the pressure limitation becomes the dominant limiting factor.

The qualitative approach which shows that P_{IN} versus u_0 reaches a maximum value, P_{IN}^{max} , at a certain maximum velocity, u_0^{max} , and which is demonstrated in Figs. 3 and 4 can also be treated mathematically. Introducing eqn. 16 into eqn. 11 and differentiating with respect to u_0 results in a relationship for which P_{IN} reaches a maximum:

$$u_0^{max} = \frac{2L}{3mN} - \frac{2H_0^{min}}{3m} + \frac{2u_0^{min}}{3} \quad (17)$$

For a certain required plate number, N , eqn. 17 is a function of only two parameters, the column length, L , and the particle diameter, d_p , whereas H_0^{min} , m and u_0^{min} are only functions of d_p . This is also shown in the familiar relationship

$$u_0 = \frac{\Delta p d_p^2}{\varphi \eta L} \quad (18)$$

where φ is the dimensionless column resistance factor, η the dynamic viscosity of the solvent and Δp the pressure drop which u_0 generates over a column of length L .

It is obvious from eqn. 17 that pressure limitations of chromatographic systems will limit the column length and particle size and therefore u_0^{max} , which itself will influence the production rate. These interesting practical aspects will be considered in a forthcoming paper²⁰.

CONCLUSION

Within the limits of the available instrumentation, with respect to pressure and flow-rate, and within those limits discussed by Endelev *et al.*¹⁹, the parameters for a preparative separation should be selected as follows:

(i) A phase system should be chosen with as high a selectivity as possible for the components of interest.

(ii) A column length should be chosen which is about twice as long as is needed to perform the separation at an infinitely small injection volume; then the injection volume should be increased such that the apparent plate number drops to the required value, thereby making the dispersion contribution of the column and the injected volume of about equal sizes.

(iii) This means that preparative columns are always longer than those used for analytical purposes.

(iv) If, however, a certain column length is reached in order to increase further the production rate, it is of advantage to increase the column cross-section rather than its length.

(v) If the required plate number is high (>1000) then highly efficient columns, packed with small particles, should be used. For problems requiring low plate numbers (<100) columns packed with coarse particles may be used, giving the advantages of low price and low pressure.

(vi) A flow-rate should be chosen that gives an optimal value for the production rate, provided that is within the pressure and flow limitations of the system.

(vii) An injection system and a column interface should be used that do not generate excessive band broadening but instead give high D values.

(viii) Optimal production rates not only give high sample amounts per unit time, but also assure the smallest possible solvent consumption together with the smallest dilution of the sample in the eluent.

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