

# Optimization of the experimental conditions and the column design parameters in overloaded elution chromatography

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## ABSTRACT

A theoretical study of the optimization of the separation of a binary mixture in overloaded elution chromatography was performed. The elution band profiles were calculated using the semi-ideal model of chromatography and assuming competitive Langmuir isotherms. In a first step, the experimental (operating) conditions (*i.e.*, the reduced velocity of the mobile phase and the loading factor) were optimized using a simplex algorithm. In a second step, the column design parameters (*i.e.*, the column length and the average particle diameter) and the operating conditions were optimized for the maximum production rate of either the more or the less retained component. The optimum value of the capacity factor of the first component, was also determined. Binary mixtures having relative retentions between 1.1 and 1.8 and relative concentrations of 1:3 and 3:1 were studied. The maximum production rates were obtained for very low values of the capacity factor of the first component, of the order of 0.3–0.5, depending slightly on the relative retention.

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## INTRODUCTION

There is currently considerable interest in the preparative applications of liquid chromatography, especially in the pharmaceutical and the fine chemicals industries. However, there are great uncertainties regarding the procedures to be followed for optimizing the column design and operating conditions for a new separation. Although it has been shown that an overlapping band separation can provide a 5–10-fold increase in production rate, some persist in aiming for a touching band separation. Even the definition of the goal of an optimization procedure remains controversial. For an industrial-scale separation, the optimum conditions should correspond to the lowest cost possible. For

laboratory separations, time spent in preparing the products needed tends to dominate the costs and the maximum production rate becomes more desirable. However, finding rapidly sub-optimum conditions may permit considerable savings in time, and hence in cost. In an academic environment, it is difficult to price properly the various components of the cost of a separation. Hence, the optimization of the experimental conditions for the maximum possible production rate, with some constraints of product purity, recovery yield and inlet pressure, becomes the simplest goal. We adopted it in this work.

The achievement of a high production rate in preparative liquid chromatography means that the column has to be operated with a high feed load, and hence under non-linear conditions. The individual elution bands are asymmetric and overlap to some extent. Therefore, the composition and the size of the feed sample injected have a strong influence on the chromatograms obtained. Although the theory

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of non-linear chromatography has developed considerably in recent years [1,2], the system of mass balance equations for a binary mixture has no closed-form solution for the semi-ideal model, the best suited to account for the behavior of modern high-performance liquid chromatographic (HPLC) columns [1]. The optimum conditions must be determined using the numerical solutions of this model. There are two approaches to this problem, one theoretical and the other numerical.

Golshan-Shirazi and Guiochon [3-6] used the analytical solution of the ideal model of chromatography which can be derived for a binary mixture [7]. From this solution, they calculated analytical expressions for the optimum experimental conditions (optimum sample size, column length and particle size, cutting times) for the recovery yield and the production rate [3]. The ideal model assumes infinite column efficiency. Actual columns have a finite efficiency, a function of the mobile phase velocity. By using an approximate solution of the semi-ideal model of chromatography, expressions were also derived for the optimum conditions taking into account the finite column efficiency [4]. These results were later elaborated and applied to the study of several practical problems of optimization [8,9].

The other approach is purely computational. It involves combining the numerical solution of the system of mass balance equations with a maximum search method. Because it is entirely based on numerical solutions, it makes it very difficult to give general rules for the optimum experimental conditions in non-linear chromatography. The non-linear simplex method, a simple but, in most instances, efficient algorithm was used by Ghodbane and Guiochon [10] and by Katti *et al.* [11]. The simulations showed that the maximum production rate is achieved at a much higher value of the mobile phase reduced velocity than its efficiency optimum value, and the necessary resolution between components is usually lower than unity. The optimum values of the experimental parameters are very different, depending on whether one wishes to purify the more or the less retained component. If the relative amount of the second-eluting component is large enough, self-displacement may occur, which increases the production of the first component [12,13].

In previous work, the simultaneous optimization

of more than two parameters was not performed. Accordingly, these studies were focused on the optimization of the operating conditions using a given column. Much was learned regarding the influence of the mobile phase velocity, the sample size, the column length and the relative retention. The influence of other parameters, especially the retention factor of one of the components of the mixture, was neglected. This paper reports a more general approach, using a simplex procedure permitting the simultaneous optimization of the column length, particle size, mobile phase flow velocity and feed amount.

## THEORY

The individual elution profiles of the components of binary mixtures were calculated using an equilibrium-diffusive model of chromatography [2]. The calculations of these profiles are carried out by numerical integration of the system of mass balance equations of chromatography for the two components, using a finite difference method [2,14]. Constant equilibrium between the mobile and stationary phases is assumed, as in the ideal (equilibrium) model, and the competitive Langmuir isotherm model is used. The smoothing effect of the axial dispersion and the mass transfer resistance on the band profiles is accounted for by adjusting properly the numerical dispersion [14,15]. Then, given a purity requirement for both components, a simple integration routine permits the determination of the cutting times for the two fractions, the amounts produced, the recovery yields and the production rate [11].

### *Column characteristics*

For all the calculations of optimum conditions, we assumed the characteristics of the chromatographic system to be as follows: the column porosity was 0.80, the viscosity of the mobile phase was 1 cP, and the diffusion coefficient of each compound in the mobile phase was  $D_m = 1 \cdot 10^{-5}$  cm<sup>2</sup>/s, the column inside diameter was  $d = 4.6$  mm, the maximum inlet pressure allowed was assumed to be 125 atm, which was chosen as a compromise between the current performance of laboratory and industrial equipment used in preparative chromatography, and the sample was injected as a rectangular plug.

The column efficiency was calculated using the Knox equation [16]:

$$h = 2/v + v^{1/3} + v/10 \quad (1)$$

where  $h = H/d_p$  is the reduced plate height,  $H$  is the actual height equivalent to a theoretical plate,  $v = ud_p/D_m$  is the reduced mobile phase velocity and  $u$  is the actual mobile phase velocity.

In the first part of the work, we optimized the operating parameters of a given column for the chosen separation. The column dimensions were chosen arbitrarily as follows: column length  $L = 25$  cm and average particle diameter  $d_p = 20 \mu\text{m}$ . The experimental conditions (the loading factor and the reduced velocity of the mobile phase) were optimized. In the second part, the column design parameters (the column length and the average particle diameter) were also optimized.

#### Equilibrium isotherms

We assumed that the isotherms of the two compounds are given by the competitive Langmuir isotherm model [17]:

$$q_i = a_i C_i / (1 + b_1 C_1 + b_2 C_2) \quad i = 1, 2 \quad (2)$$

where  $q_i$  and  $C_i$  are the concentrations of the component  $i$  at equilibrium in the stationary and the mobile phase, respectively. All concentrations are in mg/ml.  $a_i$  and  $b_i$  are numerical coefficients. The choice of the Langmuir model is a simplification. This model is valid only if the column saturation capacities for the two components, *i.e.*, the ratios of their coefficients  $a_i/b_i$ , are the same [18], otherwise major deviations from this model may be observed. When the column saturation capacities of the components of a binary mixture are different, the Levan-Vermeulen model [18] is much more satisfactory [19]. In keeping with the theoretical rigor of the model, we selected values of the isotherm coefficient giving the same value of the column saturation capacity for the two components.

The numerical values chosen for the isotherm coefficients were  $a_1 = k'_1/0.25$ ,  $a_2 = \alpha k'_1/0.25$ ,  $b_1 = k'_1/325$  ml/mg and  $b_2 = \alpha k'_1/325$  ml/mg. With this choice of coefficients,  $q_1 = q_2 = 1300$  mg/ml.  $k'_1$  is the retention factor, or column capacity factor, of the first component at infinite dilution and  $\alpha$  is their separation factor.

#### Definitions

The production rate was defined as the amount of the purified component produced per unit cross-sectional area of the column per unit time ( $\text{mg}/\text{cm}^2 \cdot \text{s}$ ). The cycle time, *i.e.*, the time between two consecutive injections, was defined as  $\Delta t_c = t_{c,2} - t_{c,1}$ , where  $t_{c,1}$  is the time when the concentration of the first component reaches  $1 \cdot 10^{-6}$  mg/ml and  $t_{c,2}$  is the time when the concentration of the second component drops below  $1 \cdot 10^{-6}$  mg/ml. Although arbitrary, especially as far as the concentration thresholds are chosen, this definition has some merit over the alternative,  $\Delta t = t_{R,20} - t_0 = k'_2 t_0$ , where  $t_{R,20}$  is the limiting retention time of the second component at infinite dilution and  $t_0$  is the column hold-up time [3–6,8,9].

#### Calculation procedures

The modified simplex algorithm described by Dose [20] was used. The calculations of the optimum values of the parameters assumed a desired purity of both components of 99%. Two constraints were applied, a maximum inlet pressure, which determines the maximum allowed reduced velocity, and a minimum required recovery yield. The standard deviations of the coordinates of the simplex vertices were used as convergence criteria. The convergence was reached when the standard deviation for all parameters to be optimized dropped below 0.1%.

The optimum experimental conditions of the separation were calculated for both the less and the more retained components. As we see, they are often very different. Two mixture compositions (relative concentrations 1:3 and 3:1) were chosen and four values of the separation factor ( $\alpha = 1.1, 1.2, 1.5$  and  $1.8$ ) were studied.

#### RESULTS AND DISCUSSION

In Fig. 1 we compare typical results obtained in the calculation of an optimum loading factor. As can be seen, if the cycle time is defined as the corrected retention time of the second component [4], the production rate of the second component (dotted line) rises very fast at first, then increases slowly, but indefinitely with increasing loading factor. The production rate has no well defined maximum, and this result is not practical for the purpose of optimi-

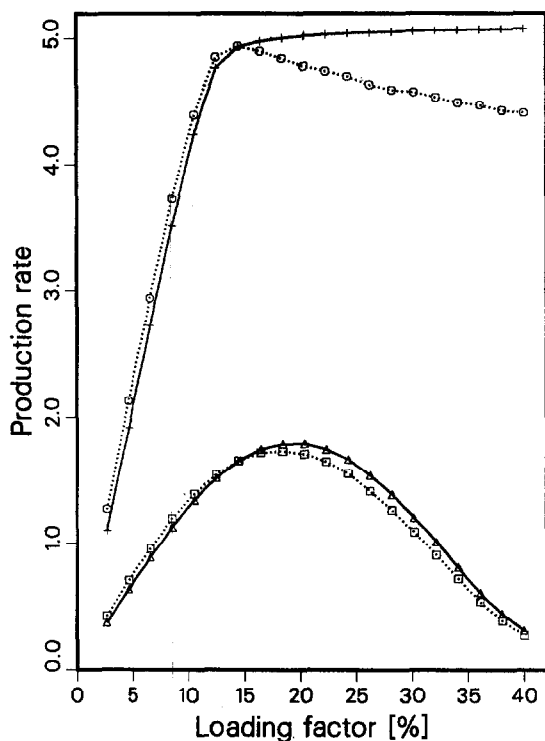


Fig. 1. Effect of the definition of the cycle time on the production rate of the two components of a binary mixture. Solid lines: production rate of ( $\Delta$ ) the first and (+) the second component if the cycle time is  $\Delta t_c = t_{R2,0} - t_0$ . Dotted lines: production rate of ( $\square$ ) the first and ( $\circ$ ) the second component if the cycle time is  $\Delta t_c = t_{c,2} - t_{c,1}$ . In both instances, the mixture composition is 1:3,  $\alpha = 1.5$ ,  $k'_1 = 6$ ,  $v = 300$ . The reduced velocity of the mobile phase and the loading factor have been optimized.

zation by numerical calculations. More importantly, it leads to misleading conclusions for the practical applications of the results. There would be no point in injecting large sample amounts if most of the throughput has to be cut off and recycled or, still worse, wasted.

On the contrary, with the definition of the cycle time used in this work, the higher the loading factor the longer is the cycle time. Hence the production rate of the second component (Fig. 1, dotted line) decreases after reaching a maximum. Below this maximum, the two curves are almost identical. For the first component, both the location of the optimum and the maximum production rate differ slightly, but the two plots of the loading factor *versus* the production rate are essentially identical.

#### Optimization of experimental conditions for a given column

In this first part of the work a two-parameter optimization was performed to determine the operating conditions giving the maximum production rate for 99% purity. The loading factor and the reduced velocity of the mobile phase were optimized simultaneously, the other parameters remaining unchanged.

*Influence of retention factor.* The optimization procedure for  $L_r$  and  $v$  was repeated for several values of  $k'_1$  between 0.5 and 6. The values of the retention factor giving the maximum production rate were determined for the two components for several values of the retention factor and of the mixture composition. The numerical results, including the optimum values of the parameters and the maximum production rate, are listed in Table I.

The maximum production rate is plotted *versus* the retention factor,  $k'_1$ , in Figs. 2 and 3 for mixtures with relative concentrations of 1:3 and 3:1 and a relative retention  $\alpha = 1.2$ . The production rate increases very rapidly at first with increasing retention factor. An optimum is reached for  $k'_1$  in the range 1.5–1.9, depending on the relative concentration and on whether the production rate of the first or of the second component is being maximized. The optimum capacity factor is slightly smaller if the component at the higher concentration is being purified.

This result is in agreement with previous results derived on a purely theoretical basis [5,6]. Optimum values of the order of unity for the retention factor had been expected.

*Influence of recovery yield constraint.* The maximum production rate was determined without yield constraint and with 60% and 90% yield constraints. The recovery yield is poor at the optimum value of the retention factor; it is only 27–48% for a separation factor  $\alpha = 1.2$ . When setting a 60% constraint on the recovery yield, the loss of production rate is only *ca.* 3–17%, whereas the yield actually doubles. The decrease in production rate is much more significant (38–62%) when a constraint of 90% is set on the recovery yield. To increase the recovery yield as required, both the reduced velocity and the loading factor must be lowered significantly. As a consequence, the optimum value of the retention factor,  $k'_1$ , is shifted towards higher values

TABLE I

OPTIMUM VALUES OF THE EXPERIMENTAL OPERATING CONDITIONS FOR A GIVEN COLUMN

 $L = 25 \text{ cm}; d_p = 20 \mu\text{m}$ 

$\alpha$	$C_1/C_2$	Component No.	$k'_1$	Production rate (mg/cm <sup>2</sup> · s)	Recovery yield (%)	$v$	$L_r$ (%)
1.2	3:1	1	1.5	2.371	37.0	124	6.84
1.2	3:1	1	1.6	1.970	60.0	102	4.14
1.2	3:1	1	2.3	0.909	90.0	62	2.21
1.2	3:1	2	1.7	0.278	27.5	89	4.08
1.2	3:1	2	2.0	0.241	60.0	69	2.17
1.2	3:1	2	2.7	0.137	90.0	45	1.35
1.2	1:3	1	1.8	0.413	48.7	55	6.11
1.2	1:3	1	1.9	0.401	60.0	52	4.79
1.2	1:3	1	2.4	0.252	90.0	39	2.90
1.2	1:3	2	1.5	1.725	26.3	171	5.53
1.2	1:3	2	1.7	1.547	60.0	121	2.79
1.2	1:3	2	1.9	1.064	90.0	64	2.15
1.5	3:1	1	0.9	18.354	59.0	400	12.48
1.5	3:1	1	0.9	18.338	60.0	368	12.45
1.5	3:1	1	1.3	12.233	90.0	179	10.53
1.5	3:1	2	1.2	3.534	49.5	294	12.76
1.5	3:1	2	1.3	3.392	60.0	235	12.40
1.5	3:1	2	1.4	2.329	90.0	163	6.60
1.5	1:3	1	1.2	3.513	62.7	195	13.76
1.5	1:3	1	1.7	2.608	90.0	152	11.28
1.5	1:3	2	1.0	15.536	53.8	400	13.65
1.5	1:3	2	1.0	14.991	60.0	400	10.55
1.5	1:3	2	1.3	11.034	90.0	247	8.56
1.8	3:1	1	0.7	42.596	61.7	400	36.56
1.8	3:1	2	1.0	8.736	62.4	400	27.75
1.8	1:3	1	1.0	8.295	66.2	400	20.34
1.8	1:3	2	0.7	30.132	61.6	400	23.73

(up to 2.65 if we want to recover the more retained component at 99% purity from a 3:1 mixture, with a 90% recovery yield at  $\alpha = 1.2$ ).

The production rate of the first component is always higher than that of the second if account is taken of the difference in relative concentration. This was found to be true not only for the 1:3 mixture but in all instances when the concentration of the more retained component is higher than that of the less retained component. This can be explained by the effect of self-displacement [13], which is significant only if the loading factor of the second component is higher than that of the less retained component. The optimum loading factor for maxi-

imum production rate is always higher for the purification of the first component. On the other hand, the optimum reduced velocity is higher if one optimizes the purification of the component of higher relative concentration, whether it is the more or the less retained component.

*Influence of relative retention or separation factor.* The same calculations were repeated for binary mixtures at a relative retention  $\alpha = 1.5$ . The dependence of the maximum production rate on the retention factor in this instance is shown in Fig. 4 (3:1 mixture) and 5 (1:3 mixture). We observe that the optimum value of the retention factor is markedly shifted towards smaller values (0.9–1.2, instead

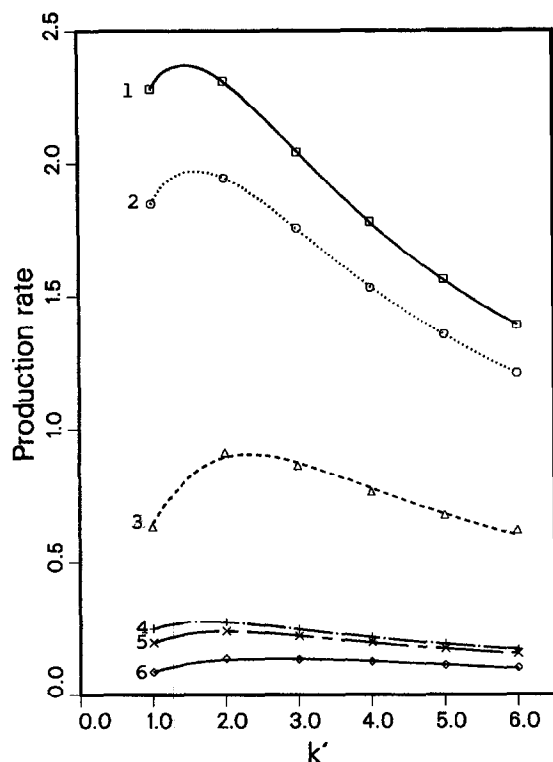


Fig. 2. Plots of the maximum production rates of either component *versus* the retention factor of the less retained component and influence of a yield constraint. Feed composition, 3:1; separation factor,  $\alpha = 1.2$ . 1, First component without yield constraint; 2, first component, 60% recovery yield; 3, first component, 90% recovery yield; 4, second component without yield constraint; 5, second component, 60% recovery yield; 6, second component, 90% recovery yield.

of 1.5–1.9) and that the recovery yield is much better at the optimum retention factor than for  $\alpha = 1.2$ . The yield exceeds 60% at the maximum production rate of the first component from a 1:3 mixture. When the retention factor is higher than 2, the recovery yield under the optimum conditions is always better than 60%. The larger separation factor allows the use of a 3–4 times higher reduced velocity and of a 2–4 times higher loading factor. The combination of these changes results in an 8–12 times higher production rate than with  $\alpha = 1.2$ . This increase is intermediate between a dependence on  $(1 - \alpha)^2$  and on  $(1 - \alpha)^3$ , as was predicted by theory [3–7]. In some instances, the optimum reduced velocity is below the maximum ( $v = 400$ ) allowed

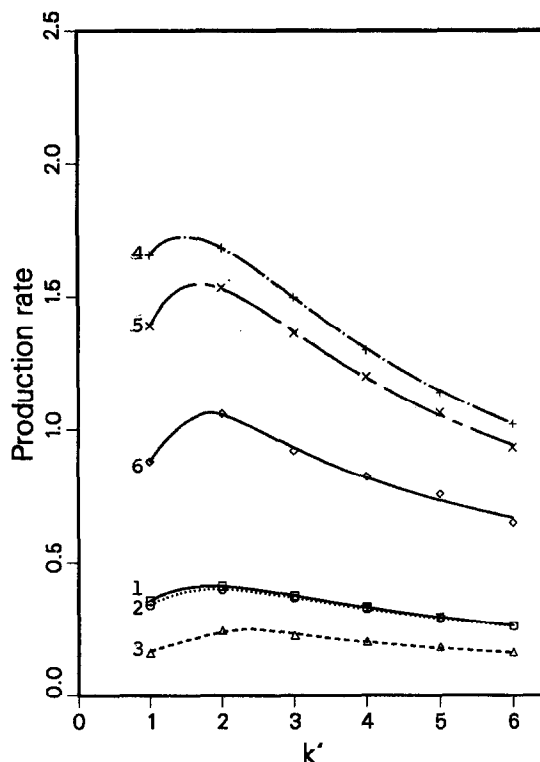


Fig. 3. Same as Fig. 2, except feed composition 1:3.

by the maximum inlet pressure constraint ( $p = 125$  bar).

Calculations were also performed with a very high relative retention,  $\alpha = 1.8$ . In this instance, the optimum capacity factor is shifted towards still lower values, 0.7–1.0 (Fig. 6). The recovery yield under optimum experimental conditions always exceeds 60%. However, the gain in production rate is only about 2.3-fold, compared with the production rate obtained for  $\alpha = 1.5$ . This gain is not as significant as when the separation factor was raised from 1.2 to 1.5. It corresponds barely to a dependence on  $(1 - \alpha)^2$ . One of the reasons for this is the pressure constraint. The optimum reduced velocity increases with increasing retention factor. When the retention factor increases from 1.2 to 1.5, the optimum reduced velocity increases to near the upper limit corresponding to the pressure constraint of 125 atm ( $v = 400$ ). When the separation factor is increased to 1.8, the reduced velocity cannot be raised. The loading factor only can be increased, by about 20–

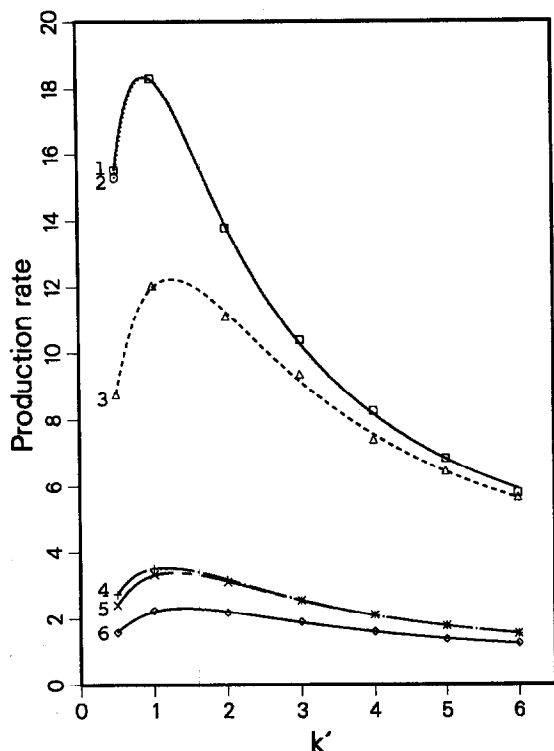


Fig. 4. Same as Fig. 2, except  $\alpha = 1.5$ .

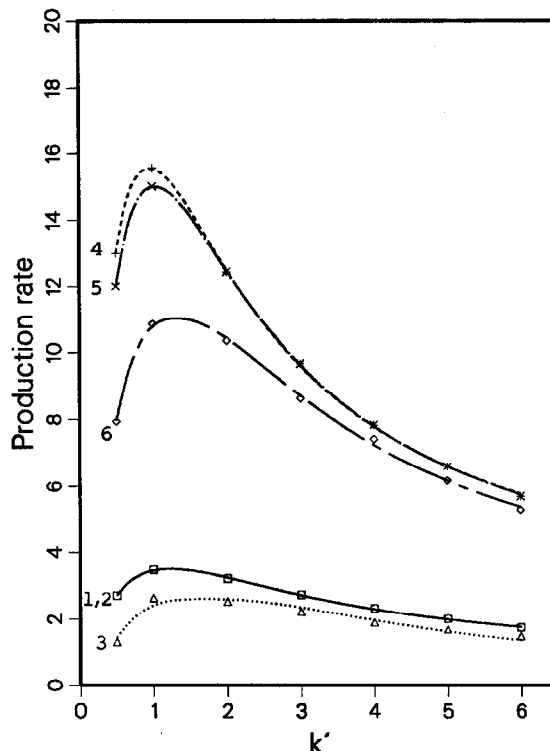


Fig. 5. Same as Fig. 4, except feed composition 1:3.

36%, to raise the production rate, as the cycle time cannot decrease.

The use of a higher inlet pressure would permit significant improvements in the production rate of components that are easy to separate, as we do not need a high efficiency and we are using a given column, of constant length.

#### *Simultaneous optimization of column design parameters and operating conditions*

In the second part of the work, the column characteristics (column length and average particle diameter) and the operating conditions (reduced velocity of the mobile phase and column loading factor) were optimized simultaneously. The same values were used for the relative composition of the mixture (1:3 and 3:1) and for the separation factor ( $\alpha = 1.1, 1.2, 1.5$  and  $1.8$ ). The optimum conditions were determined without any yield constraint, however.

*Optimum value of the ratio  $d_p^2/L^a$ .* A detailed investigation of the solution of the ideal model of chromatography [7] shows that, for infinitely efficient columns, there is no separate optimum value of either the column length or the particle diameter, but that there is an optimum for the ratio  $d_p^2/L$  [3]. The reason for this phenomenon is that short columns packed with small particles have a short cycle time but accept small sample sizes, whereas longer columns packed with coarser particles may accommodate larger amounts of feed but have a longer cycle time. It was shown that, with certain assumptions, this result can be reasonably extended to col-

<sup>a</sup> The values of  $d_p^2/L$  should be given in length units. As  $d_p$  is conveniently expressed in  $\mu\text{m}$  and  $L$  in  $\text{cm}$ , the natural unit (or rather the most convenient) is  $1 \mu\text{m}^2/\text{cm} = 1 \text{ \AA}$ . This is the unit used for the ratio  $d_p^2/L$  throughout this paper. Such a small unit should not impress;  $d_p^2/L$  has no physical reality, it is merely a convenient parameter.

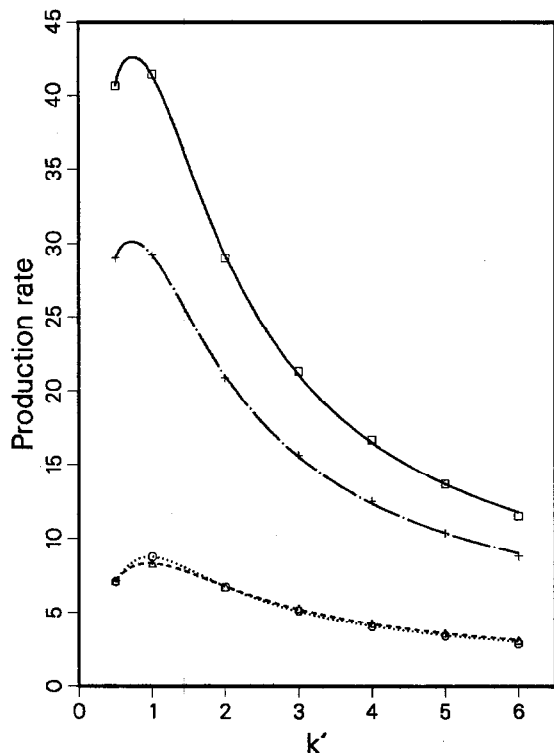


Fig. 6. Plot of the maximum production rate of the two components of a mixture *versus* the retention factor of the first component. ( $\square$ ) First and ( $\circ$ ) second components of a 3:1 mixture; ( $\Delta$ ) first and ( $+$ ) second components of a 1:3 mixture. Relative retention,  $\alpha = 1.8$ .

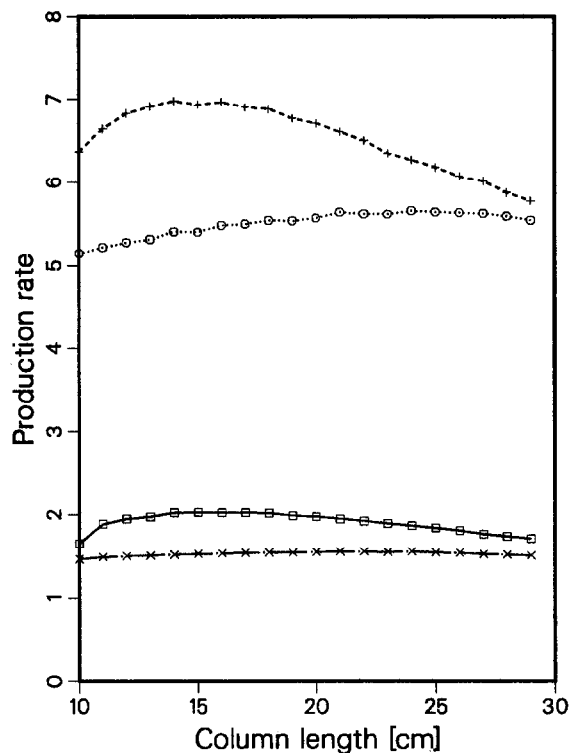


Fig. 7. Plot of the maximum production rate *versus* the column length. The particle diameter is optimized for the maximum production rate for each column length. ( $+$ ) First and ( $\times$ ) second components in a 3:1 mixture; ( $\square$ ) first and ( $\circ$ ) second components in a 1:3 mixture.  $\alpha = 1.5$ .

umns of finite efficiency (semi-ideal model) [4]. To investigate the range of validity of this rule, numerical optimization was carried out to investigate the degree and range of validity of this conclusion by calculating the exact optimum conditions for various combinations of these two parameters.

Calculations were performed for 3:1 and 1:3 binary mixtures with  $\alpha = 1.5$  and  $k'_1 = 6$ . The optimum particle size was searched for with values of the column length set successively between 10 and 30 cm. In Fig. 7, we see that the maximum production rate varies only very slightly. It is almost constant for the production of the second component from 3:1 mixture and has a very weak optimum around  $L = 15$  cm for the production of the first component. In Figs. 8 and 9, we show contour plots of the production rate as a function of the column length (ordinate) and the particle size (abscissa) for

the first (Fig. 8) and the second (Fig. 9) component of a 3:1 mixture.

By comparing these two figures and Fig. 10, which shows the lines along which  $d_p^2/L$  is constant in an  $L$  *versus*  $d_p$  graph, we observe that the contour plots of the production rate of the second component (Fig. 9) and the lines along which  $d_p^2/L$  is constant (Fig. 10) are parallel. Hence the production rate of the second component is nearly constant along these curves. Only a very slight maximum is found at  $L = 21.2$  cm and  $d_p = 17.4$   $\mu\text{m}$  ( $d_p^2/L = 14.3$ ). For the first component, the contours of constant production rate and constant  $d_p^2/L$  are not exactly parallel. They intersect each other, but with a very shallow angle. There are separate optimum values of the column length and the particle size. However, at a constant value of  $d_p^2/L$ , the column length optimum is very flat, at  $L = 15$  cm and  $d_p = 16.2$   $\mu\text{m}$  ( $d_p^2/L = 17.5$ ).



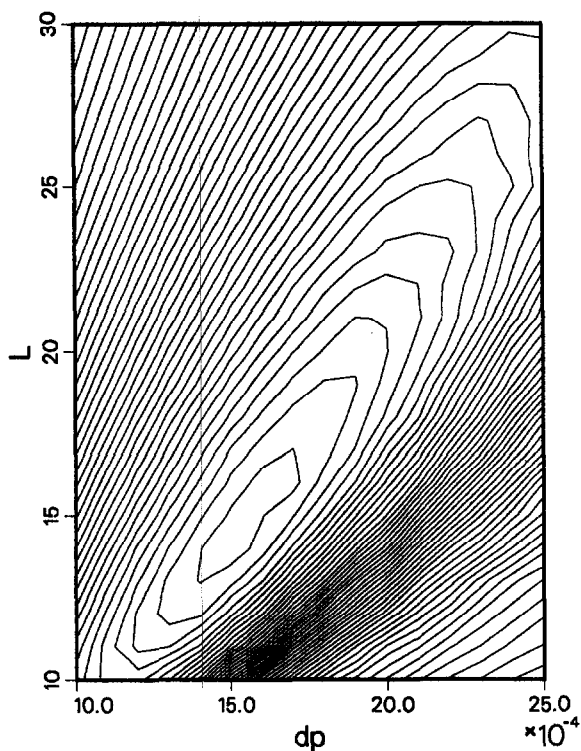


Fig. 8. Contour plots of the production rate of the first component as a function of the column length ( $L$ ) and the average particle diameter ( $d_p$ ). Mixture composition, 3:1;  $\alpha = 1.5$ ;  $k'_1 = 6$ .

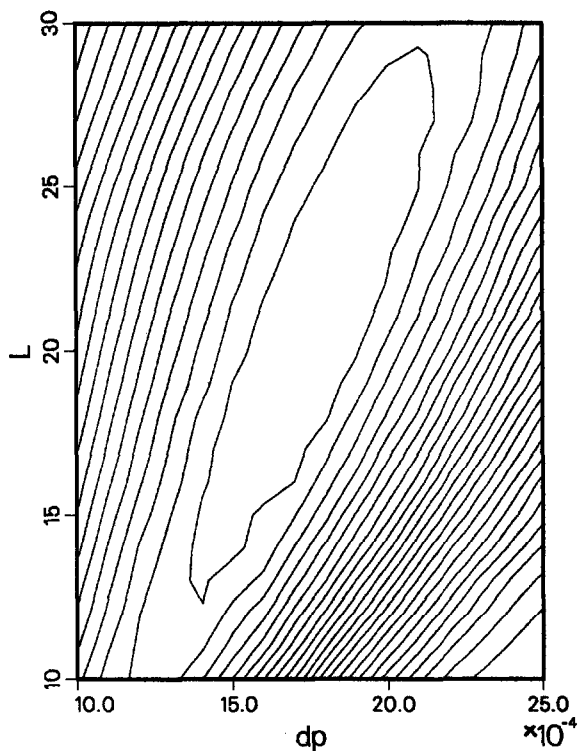


Fig. 9. Same as Fig. 8, but for the second component.

As the expressions derived for the optimum parameters using the approximate solution of the semi-ideal model [4] are correct only for high values of the reduced velocity, it can be expected that for smaller values of the separation factor and the retention factor, where the optimum reduced velocity is also smaller, the maximum is better defined. In consequence, when one tries to optimize the column design parameters, both the column length and the particle diameter should be optimized separately.

**Optimization of retention factor.** Compared with the value obtained for a given column, the optimum retention factor is much smaller when the column design parameters are also optimized (see Figs. 11–14 and Table II). The optimum value of  $k'_1$  was found to be between 0.3 and 0.5 in all the cases investigated. Only a very slight dependence on the separation factor can be observed. The optimum value of the retention factor is found in the range

$k'_1 = 0.4$ – $0.5$  for  $\alpha = 1.1$  (Fig. 11) and in the range  $k'_1 = 0.3$ – $0.4$  for  $\alpha = 1.8$  (Fig. 14).

Figs. 15 and 16 show chromatograms corresponding to the maximum possible production rate for values of the retention factor around the optimum value. The production rates of the less and the more retained component were optimized in Figs. 15 and 16, respectively, for a binary mixture having a relative concentration of 1:3; the separation factor is  $\alpha = 1.5$ . These chromatograms show that the band width increases rapidly with increasing retention factor, as the optimum column efficiency decreases as the retention factor increases. Consequently, the concentration of the purified components collected decreases with increasing retention factor.

For a separation factor  $\alpha = 1.2$ , the maximum production rate under the optimum conditions (including the optimum retention factor) is 3.5–6 times higher than it was for the 25-cm column packed

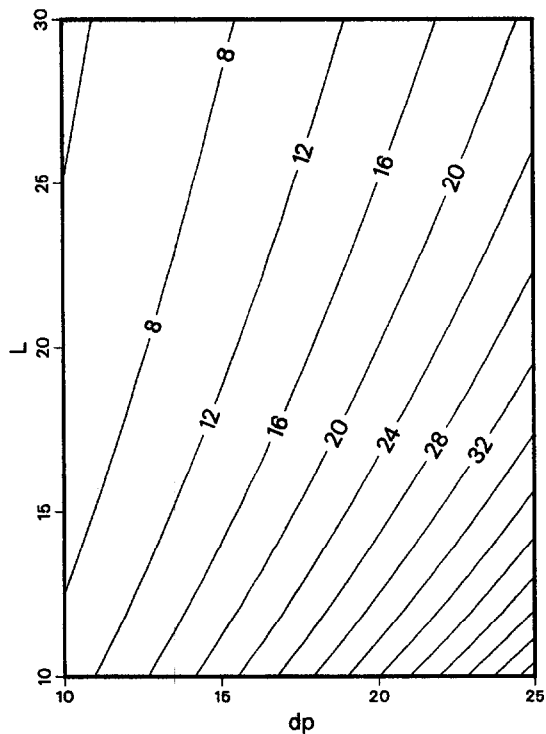


Fig. 10. Contour plots of constant  $d_p^2/L$  ( $\mu\text{m}^2/\text{cm}$ ) as a function of  $L$  and  $d_p$ .

with 20- $\mu\text{m}$  particles operated under its optimum conditions. The production rate gain is only 1.5–2.3 times at  $\alpha = 1.5$  and 1.3–1.6 times for  $\alpha = 1.8$  (compare Tables I and II).

**Dependence of maximum production rate on retention factor.** It is not always possible to optimize the retention factor to such low values as required for  $k'_1$ . Other considerations may require the separation to be performed at high values of  $k'_1$ . The gain in production rate that we can achieve by optimizing the column characteristics at a constant value of the retention factor is smaller when we want a high retention factor. For a constant value of the retention factor of 6, this gain is 50–150% for a  $\alpha = 1.2$ , 1–20% for  $\alpha = 1.5$  and 0–5% for  $\alpha = 1.8$ . This means that the 25-cm column packed with 20- $\mu\text{m}$  particles considered in the first part of this work is almost optimum for the separation of a mixture of relative retention  $\alpha = 1.8$  and a retention factor  $k'_1 = 6$ . We note that the value of the ratio  $d_p^2/L$  is 16 for the above column, whereas the optimum ra-

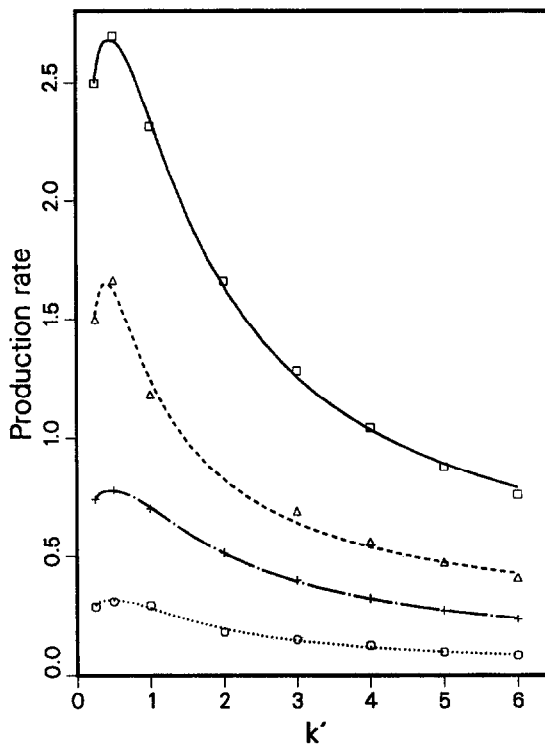
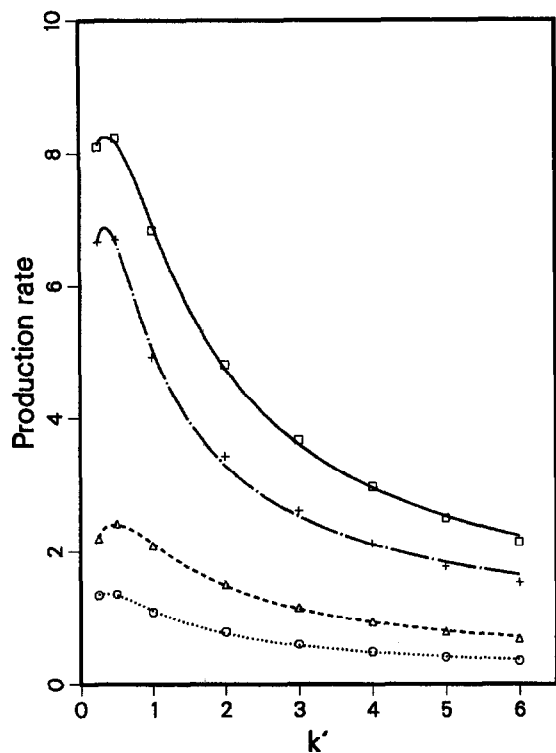
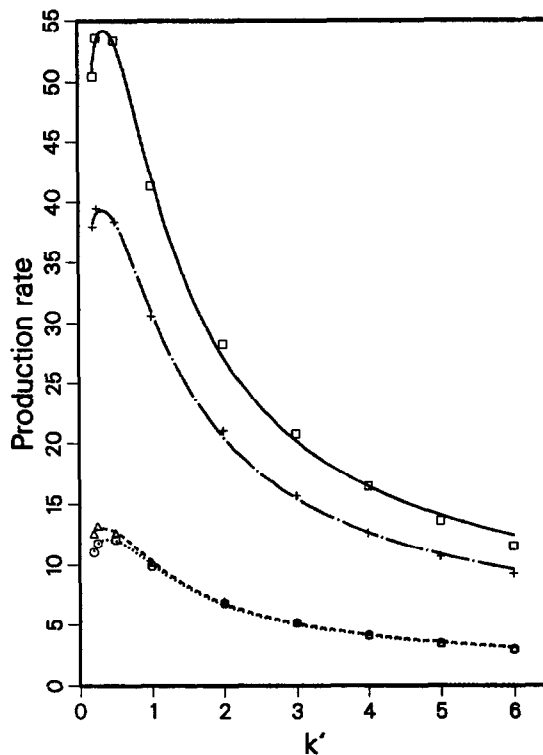
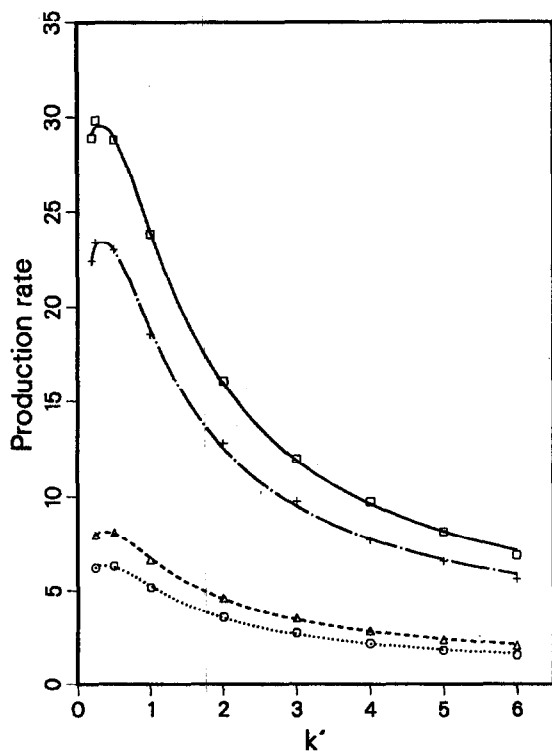


Fig. 11. Plots of the maximum production rates of either component versus the retention factor of the less retained component. ( $\square$ ) First and ( $\circ$ ) second components of a 3:1 mixture; ( $\triangle$ ) first and (+) second components of a 1:3 mixture. Separation factor,  $\alpha = 1.1$ . The mobile phase reduced velocity, the loading factor, the column length and the particle diameter have been optimized.

tio for such a mixture ( $k'_1 = 6$ ,  $\alpha = 1.8$ ) was found to be between 14 and 22, depending on the composition of the mixture.

The dependence of the optimum value of  $d_p^2/L$  on the first component retention factor in a 1:3 mixture is illustrated in Fig. 17 for the four values of the separation factor considered. The plots are almost linear, and the slope of the lines increases with increasing separation factor. The optimum value of the ratio also depends on the sample composition and on whether we want to purify the less or the more retained component of the mixture, but to a much smaller extent. The optimum ratio is usually smaller when we need to optimize the separation for the less concentrated component of the mixture than for the more concentrated component.

Fig. 12. Same as Fig. 11, except  $\alpha = 1.2$ .Fig. 14. Same as Fig. 11, except  $\alpha = 1.8$ .Fig. 13. Same as Fig. 11, except  $\alpha = 1.5$ .

*Optimum column design.* As we have shown earlier, there are an optimum column length and an optimum particle size (Table II), although significant variations around these optimum values do not cause a large decrease in production rate provided that  $d_p^2/L$  is kept constant. We see in Table II that the separation factor has a strong influence on the optimum column length. This length also depends on whether we need to purify the less or the more retained component. For the purification of the more retained component, the optimum column is much longer than that for the purification of the less retained component.

We see in Table II that the separation factor has a strong influence on the optimum column efficiency. This results from the influence of the separation factor on both the optimum column length and the optimum flow velocity, but also from the low value of the optimum retention factor. This latter relationship is illustrated in Fig. 18. If the column is operated at  $k'_1 = 0.25$ , we need a column that is nearly ten times more efficient than the optimum

TABLE II

## OPTIMUM VALUES OF THE PARAMETERS OF OPERATING CONDITIONS AND COLUMN DESIGN

$\alpha$	$C_1/C_2$	Component No.	$k'_1$	Production rate (mg/cm <sup>2</sup> · s)	Yield (%)	$\nu$	$L_f$ (%)	$L$ (cm)	$d_p$ ( $\mu$ m)	$d_p^2/L$ ( $\mu$ m <sup>2</sup> /cm)	$N$
1.1	3:1	1	0.5	2.675	42.3	28	3.72	93.4	12.9	1.78	12 453
1.1	3:1	2	0.5	0.318	67.6	27	0.67	189.3	14.8	1.17	18 700
1.1	1:3	1	0.5	0.775	57.6	17	2.88	120.0	12.0	1.19	22 755
1.1	1:3	2	0.4	1.653	49.5	32	0.86	227.0	18.1	1.44	19 791
1.2	3:1	1	0.4	8.248	53.4	36	7.84	50.5	11.5	2.62	6310
1.2	3:1	2	0.4	1.362	58.4	39	3.42	114.8	15.6	2.13	9998
1.2	1:3	1	0.5	2.399	64.5	25	6.75	51.5	10.7	2.24	8710
1.2	1:3	2	0.4	6.881	54.4	69	4.36	142.0	20.4	2.93	6310
1.5	3:1	1	0.3	29.506	59.4	60	21.67	17.4	9.6	5.32	1820
1.5	3:1	2	0.4	6.345	72.5	66	12.40	29.5	12.1	4.93	2291
1.5	1:3	1	0.4	8.133	74.6	40	17.27	19.9	8.8	3.92	3020
1.5	1:3	2	0.3	23.404	81.4	70	11.45	26.4	11.3	4.86	2082
1.8	3:1	1	0.4	54.154	70.3	88	34.34	11.9	9.6	7.75	933
1.8	3:1	2	0.4	12.112	77.7	85	21.92	15.6	10.2	6.73	1175
1.8	1:3	1	0.3	13.033	74.6	60	25.00	13.9	8.8	5.57	1589
1.8	1:3	2	0.3	39.312	80.3	76	19.61	17.4	10.8	6.72	1202

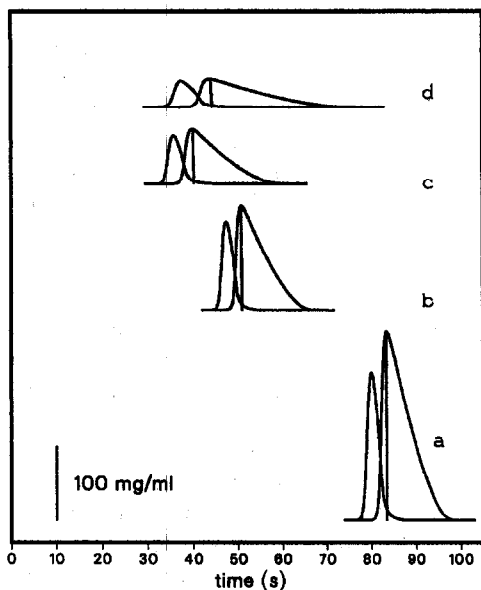


Fig. 15. Optimum chromatograms corresponding to the maximum production rate of the less retained component in a mixture of relative concentration 1:3; the separation factor is  $\alpha = 1.5$ . Retention factor,  $k'_1$ : (a) = 0.25; (b) = 0.5; (c) = 1.0; (d) = 2.0.

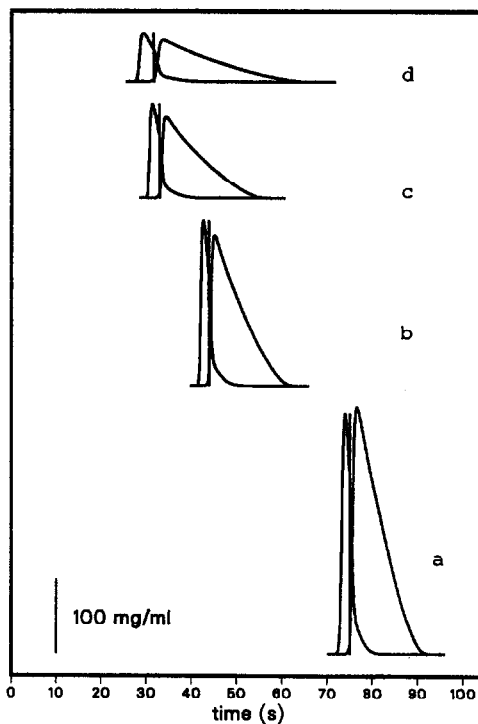


Fig. 16. Same as Fig. 15, except the production rate of the more retained component is optimized.

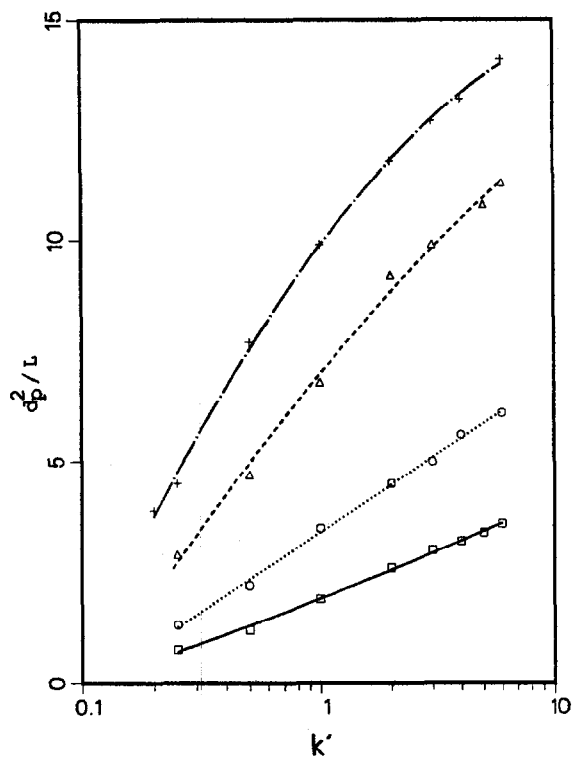


Fig. 17. Plot of the optimum value of the ratio  $d_p^2/L$  versus the retention factor of the less retained component of a 1:3 mixture. Separation factors:  $\square = 1.1$ ;  $\circ = 1.2$ ;  $\triangle = 1.5$ ;  $+ = 1.8$ .

column needed when the same separation is carried out at  $k'_1 = 6$ . For the separation of a mixture with  $\alpha = 1.1$  a column having more than 20 000 theoretical plates is needed, whereas a column with 1000 theoretical plates is sufficient at  $\alpha = 1.8$ . Generally, a more efficient column is needed for the preparation of the more retained component and for the purification of the component which has the smaller relative concentration.

**Mobile phase velocity.** We found that the optimum reduced velocity of the mobile phase is usually small at the optimum retention factor, although it is markedly higher than the reduced velocity corresponding to the maximum column efficiency. It is rarely limited by the maximum inlet pressure (never in Table II, and only for the easiest problems in Table I). For example, the reduced velocity is small (15–30) at the optimum capacity factor for  $\alpha = 1.1$  (i.e.,  $k'_1 = 0.5$ ), whereas the optimum column

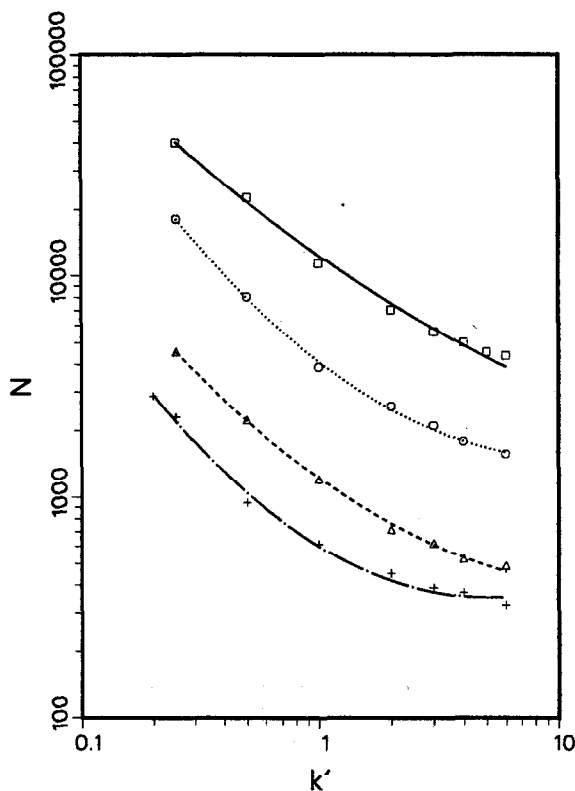


Fig. 18. Plot of the column efficiency required to perform the separation under the same experimental conditions as in Fig. 17.

length is large (100–200 cm) and the optimum particle size moderate (12–15  $\mu\text{m}$ ). Whereas the optimum column length depends only slightly on the composition of the mixture, it depends much more on whether we want to prepare the more or the less retained component. The column should be operated at a lower mobile phase reduced velocity if we want to prepare the component with the smaller relative concentration.

In contrast, if the column is operated at a non-optimum value of the retention factor, the optimum reduced velocity may be high (compare Tables I and II). The optimum reduced velocity increases almost linearly with increasing  $\log k'_1$ , as illustrated in Fig. 19 for the less retained component in a 1:3 mixture. This result explains the apparent disagreement between previous results. General theoretical considerations on the optimization problem lead to the prediction of a moderate value of the mobile phase

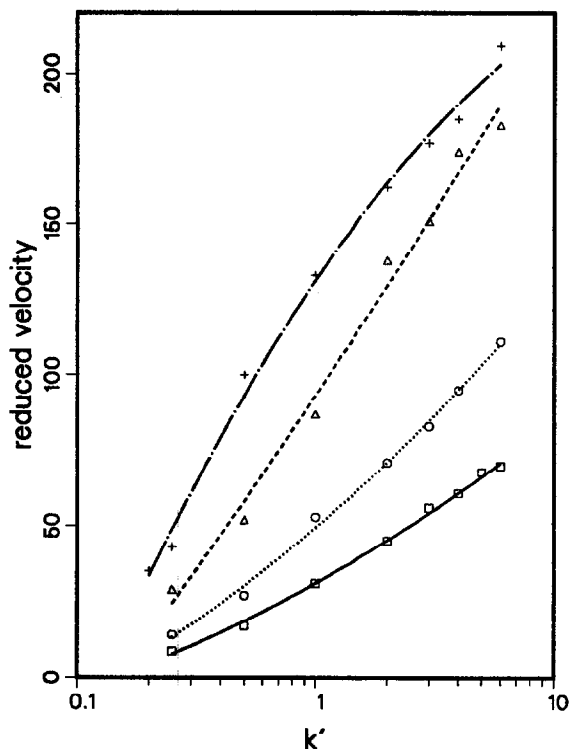


Fig. 19. Plot of the optimum reduced velocity of the mobile phase versus the retention factor of the first component. Same experimental conditions as in Fig. 17.

reduced velocity [4]. On the other hand, numerical optimization of the experimental conditions for a given column and chromatographic system resulted in large values of the optimum reduced velocity [11]. However, in these numerical calculations, we have assumed constant values of  $\alpha$  and  $k'_1$ , and the selected value of  $k'_1$  was large compared with the optimum values recommended in this work.

**Optimum sample size.** Based on the ideal model of liquid chromatography, an equation was derived for the optimum loading factor of the sample when the purification of the more retained component is optimized [3]. In Table III, we compare the optimum loading factors predicted by the ideal model for different values of the separation factor and of the feed composition with the results of the numerical optimization obtained in this work.

To check whether or not the difference is due only to the error caused by the simplex algorithm, we computed the standard deviation of the optimum

TABLE III

OPTIMUM LOADING FACTORS BASED ON THE IDEAL MODEL AND ON THE SEMI-IDEAL MODEL

The purification of the more retained component is optimized.

$\alpha$	3:1 mixture		1:3 mixture	
	$I^a$	$S^a$	$I^a$	$S^a$
1.1	0.89	0.67	1.12	0.86
1.2	3.17	3.42	3.87	4.36
1.5	13.57	12.40	14.30	11.45
1.8	19.14	21.92	18.11	19.61

<sup>a</sup>  $I$  = optimum loading factor predicted by the ideal model of chromatography;  $S$  = optimum loading factor calculated by the semi-ideal model.

parameters by repeating the simplex optimization 25 times, starting the simplex algorithm with a very different initial vertex. The relative standard deviations of the optimum values obtained for the column length, the average particle diameter and the loading factor are all between 4 and 5%. The relative standard deviation of the mobile phase reduced velocity is twice as high (8.5%), whereas that of the maximum production rate is very small (less than 1%).

The difference between the two sets of data in Table III is approximately within the  $\pm 2\sigma$  confidence interval, although in five cases out of eight it is slightly higher.

## CONCLUSIONS

One of the most important results of this work is the confirmation of an optimum value of the retention factor of the first component of a pair of components to be separated in preparative chromatography. Of equal importance is the fact that this optimum retention factor is very low, always below unity when all the parameters are optimized for a given separation (Table II), rarely above 1.5 when the operating conditions of a given column are optimized (Table I). The optimum retention factor decreases slightly with increasing separation factor when all parameters are optimized simultaneously. It decreases more rapidly when the operating parameters of a given column are optimized. Although typical of most columns currently used in preparative applications, the column considered here ( $L = 25$

cm,  $d_p = 20 \mu\text{m}$ ) is close to the optimum design only for the relatively simple separation of a binary mixture with a high separation factor,  $\alpha = 1.8$ , and at a high value of the retention factor, between 5 and 6.

In contrast, if all the parameters of the separation are optimized together, the optimum retention factor is very small, of the order of 0.3–0.5, depends only slightly on the separation factor and is almost independent of the composition of the mixture and of whether the more or the less retained component is prepared. The optimum is sharp, and small changes in the retention factor around the optimum may lead to significant losses of the production rate.

It could appear unacceptable to a chromatographer to operate a preparative column under conditions where the retention factor is a fraction of a unit. It is recognized that optimum analytical results are obtained when the retention factor is between 3 and 6. This difference between the two results is a further illustration of the dangers encountered in applying carelessly the principles of analytical chromatography to preparative chromatography. In addition to permitting the achievement of the maximum production rate possible, the choice of a low retention factor has the further advantage of giving a relatively low optimum mobile phase velocity.

Another important result is the strong dependence of the column design parameters on the retention factor of the first component of the sample if a non-optimum value of this retention factor has to be chosen. Also, there is a strong dependence of the column design parameters on whether we prefer to optimize the separation for the production of the more or the less retained component of the mixture. For the production of the more retained component we need a longer column.

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