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Comparing the optimum performance of the different modes of preparative liquid chromatography

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Abstract

A comparative study of the optimization of the different modes of the preparative separation of binary mixtures by liquid chromatography is presented. Band profiles were calculated by means of the equilibrium-dispersive model of chromatography in the cases of isocratic elution, gradient elution, and displacement chromatography. The objective function to be maximized was the product of the production rate and the recovery yield. The production rate was calculated using the same definition of the cycle time in all cases. This common definition accounts for column regeneration after each run in each mode of the separation. The calculations reveal that the number of experimental parameters to be adjusted to achieve optimum separations is relatively small. The major parameters are the loading factor and the number of theoretical plates, besides the displacer concentration in displacement chromatography, or the gradient steepness in gradient elution. The relative advantages of the different modes of preparative chromatography are discussed.

Keywords: Preparative chromatography; Optimisation; Band profiles; Production rate

1. Introduction

Several recent studies have focused on the determination of the optimum experimental conditions of binary separations in preparative liquid chromatography [1–14]. The nonlinear nature of preparative chromatography complicates the separation process so much that the derivation of general conclusions regarding these optimum conditions is a rather difficult task. Furthermore, the very choice of the objective function of preparative chromatography is not simple. In industrial applications, the production cost would be the major factor to consider. However, many components of the production cost (e.g., overhead cost) are beyond the scope of the separation process itself. Accordingly, most previous authors have elected to study the more straightforward approach of maximizing the production rate.

Separate studies have determined the optimum experimental conditions for the maximum production rate in overloaded isocratic elution using either the ideal model [1,2] or the equilibrium-dispersive model of nonlinear chromatography [3,4]. Other works have discussed the optimization of the experimental conditions in displacement chromatography for maximum production rate [3,5–8]. Production rate in gradient elution chromatography was optimized using the linear solvent strength theory [9,10]. Recently, Jandera et al. studied the optimization of gradient elution chromatography when deviations from the linear solvent strength theory take place [11].

The economic consequences of the conditions under which a separation is carried out have also

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been discussed. Optimum experimental conditions were determined in situations in which the cost of the solvent — a major cost factor in certain applications of preparative liquid chromatography — was also taken into account [12,13]. A hybrid objective function was recently introduced in order to weigh the importance of both the production rate (which should be as high as possible) and the solvent consumption (which should be as low as possible) [13]. Because all the modes of operation considered in this study are applied as batch processes, the recovery yield during each run is lower than unity. Some optimization for maximum production rate were undertaken with constraint of a minimum yield [3,4,8]. A more recent study introduced a very beneficial objective function, the product of the production rate and the recovery yield [14]. It was shown that, by means of that objective function, experimental conditions can be found which are such that the production rate is only slightly lower than when the production rate is the objective function while the recovery yield is significantly improved. This trade-off of a slight decrease in the production rate for a considerable yield improvement would be most economical. The optimization of gradient elution chromatography was studied with that objective function by Felinger and Guiochon [15].

The optimization of the different modes of preparative chromatography allowed the comparison of elution and displacement chromatography [3,16], revealing the relative advantages of either mode of separation. These studies suggested that elution can offer a larger production rate than displacement chromatography but delivers less concentrated fractions, which may significantly increase the cost of downstream processing.

The practical interest of these investigations of the optimization of the experimental conditions arises from the current availability of powerful personal computers. These machines allow the convenient calculation of the optimum conditions by using accurate models of the nonlinear separation process. The a priori knowledge of the competitive isotherms for the binary mixture of interest and of the parameters of the Knox efficiency correlation allow the rapid calculation of the band profiles of both components, their integration, the determination of the positions of the cut points, the production rate, and the recovery yield. Combining the algorithms which performs these tasks with an optimization algorithm permits the determination of the experimental conditions which maximize the relevant objective function. Then, the experimental conditions obtained by computation can be fine tuned experimentally. The introduction of the modeling step just described into the optimization process highly reduces its time and cost demand [17].

In this study, we introduce a uniform handling for the calculation of the production rate and the recovery yield achieved with the different modes of chromatography. This allows a direct comparison of their performance and an illustration of their relative advantages and drawbacks. Finally, guidelines are given to chose the parameters to be optimized.

2. Theory

The band profiles were calculated by means of the equilibrium-dispersive model of chromatography [17]. In this model instantaneous equilibrium is assumed between the stationary and the mobile phase. The concentrations in these two phases at equilibrium are simply related by the isotherm equation. An apparent dispersion term accounts for the contributions of all the sources of band broadening: axial diffusion, eddy dispersion, and the finite rate of the mass transfers. A mass balance equation is written for each component of the sample:

$$\frac{\partial C_i}{\partial t} + F\frac{\partial q_i}{\partial t} + u\frac{\partial C_i}{\partial z} = D_a\frac{\partial^2 C_i}{\partial z^2} \quad (1)$$

where C_i and q_i are the concentrations of component i in the mobile and the stationary phase, respectively; z is the column length, t the time, u the mobile phase linear velocity, and F the phase ratio, $F=(1-\varepsilon)/\varepsilon$, where ε is the total porosity of the column. D_a is the apparent axial dispersion coefficient. The model is completed by (1) a set of relationships, $q_i=f_i(C_1, C_2)$, between the concentrations in the two phases, stating the competitive isotherms of the feed components (see below); (2) a plate height equation allowing a correlation between the apparent axial dispersion coefficient and the experimental conditions; and (3) by a set of initial and boundary

conditions describing the actual experiment performed.

From a first glance at Eq. (1), it appears that the number of parameters that should be optimized in preparative chromatography is rather high. However, a more detailed analysis of the model allows an important reduction of that number and reveals the major factors to be considered in the optimization process.

Eq. (1) can be reformulated using as variables the reduced time, ($\tau = t/t_0$), and the reduced column length, ($x = z/L$), where t_0 is the hold-up time and L the actual column length (with $u = L/t_0$). Using these variables and substituting the apparent dispersion coefficient by $D_a = H\,u/2 = L^2/(2Nt_0)$, — where N is the number of theoretical plates assuming an analytical size injection, i.e., under linear conditions — the mass-balance equation can be rewritten as [17]

$$\frac{\partial C_i}{\partial \tau} + F\frac{\partial q_i}{\partial \tau} + \frac{\partial C_i}{\partial x} = \frac{1}{2N}\frac{\partial^2 C_i}{\partial x^2} \qquad (2)$$

The band profile obtained as a numerical solution of Eq. (2) gives the concentration distribution as a function of the reduced time at the column end, i.e. at location $x = 1$, regardless of the column length. It depends only on the column efficiency, the boundary conditions (see later), the phase ratio, and the sample size (which is part of the boundary conditions). Note that the mobile phase velocity has been eliminated from Eq. (2) and that the apparent axial dispersion coefficient has been replaced by the plate number.

As long as the boundary and initial conditions remain unchanged, the band profiles on the reduced time and length scale depend only on the column efficiency. The conventional boundary and initial conditions for all modes of chromatography state that (1) the column is equilibrated with the mobile phase prior to the beginning of the separation (2) the sample is then injected as a rectangular pulse and (3) the separation proceeds as required by the specific mode selected. The amount of sample injected is determined by the volume and the concentration of the feed injected. As long as we avoid serious volume overload, the actual values of these two parameters are immaterial. Only their product, i.e., the amount injected, will influence the band profile.

In conclusion, two major parameters only affect the band profiles in nonlinear chromatography: the column efficiency, and the amount injected or loading factor. The parameter F in Eq. (2) depends on the total porosity of the packing and cannot be changed in practice.

Previous studies have shown that, in order to maximize the production rate of preparative separations, the column should be operated at the highest possible flow-rate that corresponds to the maximum pressure drop allowed [4,8,13,14]. It has also been demonstrated that, under optimum conditions, the column length and the average particle diameter are not independent parameters; instead the term d_p^2/L has an optimum value [2,4]. Either the column length or the particle diameter could remain unchanged during the optimization, and the optimum plate number be reached by changing the other column design parameter. Therefore, the following strategy should be observed during the optimization of preparative separations:

1. The column should be operated at the maximum allowed pressure drop;
2. The optimum column efficiency (N) should be determined by changing only one of the column design parameters (L or d_p);
3. The loading factor and the column efficiency should be optimized simultaneously.

There are additional parameters to be optimized in modes of chromatography other than overloaded elution: the displacer concentration in displacement chromatography, the gradient steepness in overloaded gradient elution chromatography.

We need to introduce the following definitions of the parameters used in this study.

The loading factor is the ratio of the total amount of the components in the sample to the column saturation capacity. For a binary mixture it is

$$L_f = \frac{V_s(C_1^0 + C_2^0)}{(1-\varepsilon)S_A L q_s} \qquad (3)$$

where V_s is the sample volume, S_A is the column cross-section area, ε is the total porosity of the column, C^0 is the injected concentration, and q_s is the saturation capacity of the isotherm.

The cycle time is the time elapsed between two successive injections. In order to achieve uniform handling of the different modes of chromatographic

separation, the cycle time should be introduced in such a way that the definition is applicable to all modes. The column must be regenerated after each run in both displacement and gradient elution. The same operation must also be performed occasionally in isocratic elution. The cycle time should account for the time required by this column regeneration step. Unless we introduce a new parameter, which would complicate markedly the issue, an arbitrary decision must be made. The cycle time will be defined in this work as the sum of the retention time of the more retained component under analytical (i.e., linear) conditions and the time needed for the regeneration of the column with six column volumes of mobile phase, i.e.:

$$t_c = t_{R,2} + 6t_0 = t_0(7 + k'_2) \tag{4}$$

The consequences in isocratic elution will be discussed later. Note that, under the optimum conditions, the column must be operated under the maximum pressure allowed. Thus, the regeneration time cannot be reduced by pumping the solvent faster.

The production rate is the amount of a purified component produced per unit column cross-section area, per unit time, in mg cm^{-2} s^{-1}

$$\mathrm{Pr}_i = \frac{V_s C_i^0 Y_i}{\varepsilon S_A t_c} \tag{5}$$

The recovery yield of component i, Y_i, is defined as the ratio of the amount of component I recovered in the collected fraction to the amount of the same component injected in the sample.

The purity of a component is its concentration in the collected fraction. In all the calculations discussed in this work, we have assumed a purity requirement of 99%.

The objective function of the optimization study that has been maximized in each mode of preparative chromatography is the product of the production rate and the recovery yield, PrY.

In gradient elution chromatography, the rate of change of the mobile phase composition during gradient elution can be described by the gradient slope, $\Delta\varphi/t_G$, i.e. the ratio of the change in the modifier volume fraction and the gradient time [18].

The gradient steepness, G, is proportional to the gradient slope:

$$G = \frac{\Delta\varphi}{t_G} t_0 S \tag{6}$$

where φ is the volume fraction of the modifier, t_G is the gradient time, and S is the solvent strength parameter (the slope of the ln k' vs. φ plot).

2.1. Column characteristics

The column efficiency was calculated using the Knox plate height equation [19]

$$h = \frac{2}{\nu} + \nu^{1/3} + \frac{\nu}{10} \tag{7}$$

where $h = H/d_p$ is the reduced plate height, H the height equivalent to a theoretical plate, d_p the average particle diameter, and $\nu = ud_p/D_m$ the reduced mobile phase velocity (or particle Peclet number). The numerical constants of Eq. (5) correspond to a well packed, efficient column.

The pressure drop within the column was calculated by means of the following equation [18,20] derived from Darcy equation

$$\Delta P = \frac{u\eta L}{k_0 d_p^2} \tag{8}$$

where ΔP is the pressure drop and k_0 the specific column permeability (ca. $1 \cdot 10^{-3}$). A maximum pressure drop of 125 atm was allowed in all calculations (1 atm = 101 325 Pa).

The total column porosity is assumed to be $\varepsilon = 0.8$, the viscosity of the mobile phase is $\eta = 1$ cP, and the molecular diffusivity of each component in the mobile phase is $D_m = 1 \cdot 10^{-5}$ cm^2/s. This last value represents a good approximation for small molecules.

3. Isotherm model

We have assumed that the adsorption behavior of the two components of the sample (and that of the displacer in displacement chromatography) can be described by competitive Langmuir isotherms

$$q_i = \frac{a_i C_i}{1 + \sum b_j C_j} \tag{9}$$

The numerical coefficients were chosen so that the saturation capacity of each solute — and that of the displacer — be the same, $q_s = 260$ mg/ml.

In reversed-phase gradient elution chromatography, the linear solvent strength model connects the mobile phase composition with the retention factor through:

$$\ln k'_i = \ln k'_{0,i} - S_i \varphi \tag{10}$$

where φ is the volume fraction of the modifier in the mobile phase. In this case, the strong solvent was supposed to be nonadsorbed on the stationary phase, which is a reasonable assumption [21].

As the retention factor at infinite dilution (k'_i) and the first (a_i) parameter of the Langmuir isotherm are related through $k' = aF$, the isotherm parameters can be determined as a function of the mobile phase composition, utilizing the equation above, rewritten as:

$$a_i(\varphi) = a_{0,i} \exp(-S_i \varphi) \tag{11}$$

It has been shown by El Fallah and Guiochon that, in many cases and especially during the separation of small molecules, the saturation capacity, $q_{s,i} = a_i / b_i$, of the stationary phase remains practically unchanged when the composition of the mobile phase varies [21]. If this statement holds true, the dependences of the coefficients a_i and b_i on the mobile phase composition are the same or very similar.

3.1. Computations

The mass balance equation was integrated numerically using a finite difference method. The continuous (z,t) plane is replaced by the grid (Δz, Δt), and the differential equation is replaced by the appropriate difference equation. The band profiles of displacement chromatography were calculated with the conventional Rouchon algorithm [22]. That algorithm replaces the right hand side of the mass balance equation with zero, and the length and time increments of the numerical integration are chosen so that the numerical dispersion be identical to the dispersion effect caused by the apparent diffusion coefficient D_a.

The finite difference equation of the Rouchon algorithm is written as

$$\frac{C_{z,t} - C_{z,t-1}}{\Delta t} + u \frac{C_{z+1,t} - C_{z,t}}{\Delta z} + F \frac{q_{z,t} - q_{z,t-1}}{\Delta t} = 0 \tag{12}$$

which can be rearranged into

$$C_{z+1,t} = C_{z,t} - \frac{\Delta z}{u \Delta t} \left[C_{z,t} + F q_{z,t} - C_{z,t-1} - F q_{z,t-1} \right] \tag{13}$$

In the case of isocratic elution, the modified Rouchon algorithm was applied [23]. This algorithm ignores the empty sections of the column during the integration, which significantly increases the speed of computation. Gradient elution was also modeled with a finite difference integration of the mass balance equation. In that case, however, one has to consider that the mobile phase composition — and consequently the isotherm of each solute — changes along the column length (cf. Eq. (11)). Another finite difference algorithm should be used for modeling gradient elution chromatography:

$$\frac{C_{z-1,t} - C_{z-1,t-1}}{\Delta t} + u \frac{C_{z,t} - C_{z-1,t}}{\Delta z} + F \frac{q_{z,t} - q_{z,t-1}}{\Delta t} = 0 \tag{14}$$

which rearranges into

$$C_{z,t} + F q_{z,t} = C_{z,t-1} + F q_{z,t-1} - u \frac{\Delta t}{\Delta z} (C_{z,t-1} - C_{z-1,t-1}) \tag{15}$$

This algorithm becomes identical to that of the Craig machine when the length increment is $\Delta z = L / n_c$, where n_c is the number of cells in the Craig machine equivalent to the column ($n_c = N\, k'/(k'+1)$ [17]), and the time increment is $\Delta t = \Delta z / u$. This means that the mobile phase moves exactly by one cell for each time unit, Δt. This latter finite difference algorithm requires a longer computational time than the Rouchon algorithm because the numerical integration does not provide directly the mobile and stationary phase concentrations. Instead, the total concentration ($C_{z,t} + F q_{z,t}$) is obtained. The individ-

ual mobile and stationary phase concentration must be determined via time consuming iterations in the case of multicomponent separations [17].

Band profiles were calculated as described above. After integration of the peak areas, it is easy to determine the position of the cut points which allow the achievement of the required purity. The recovery yield and the production rate were derived from these results. The numerical solution of the mass balance equation was combined with a sequential simplex routine in order to maximize the objective function by changing the experimental parameters [4,8,13–16].

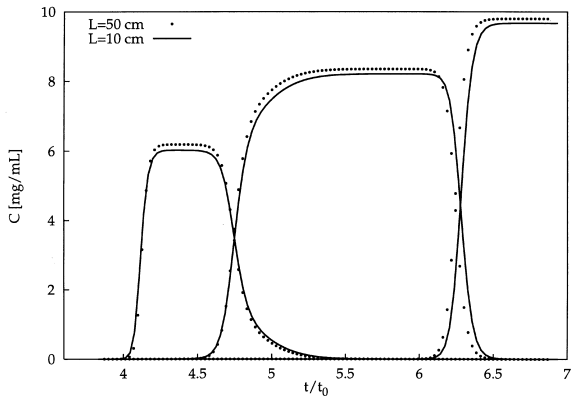

Fig. 1. Optimum displacement chromatograms. The loading factor, the plate number, and the displacer concentrations were optimized simultaneously at $k_1' = 10$ and $\alpha = 1.5$. The column lengths are 10 cm (solid line) and 50 cm (dotted line). The optimum loading factors (L_f) were 25.1% and 24.1%, respectively, the optimum plate number, 1096 and 1271 theoretical plates, respectively, and the displacer concentrations, (C_d), 9.6 and 9.4 mg/ml, respectively. The optimum conditions for the 10 cm column were: $\mathrm{Pr}Y_{max} = 0.164$. As a consequence, the other optimum experimental conditions are: $d_p = 8.2$ μm, $\nu = 69.9$, $u = 0.848$ cm/min, $Y = 78.5\%$ and $\mathrm{Pr} = 0.209$. For the 50-cm long column, they were $\mathrm{Pr}Y_{max} = 0.182$, $d_p = 18.5$ μm, $\nu = 159$, $u = 0.858$ cm/min, $Y = 84\%$ and $\mathrm{Pr} = 0.218$.

4. Results and discussion

As shown above, the parameters which have to be optimized in a preparative separation are the loading factor and the number of theoretical plates in all modes of chromatography, as well as the displacer concentration in displacement chromatography and the gradient steepness in gradient elution.

4.1. Column length

It is clear from Eq. (2) that band profiles are identical on a normalized time scale at constant values of the loading factor and plate number. However, it is not obvious that the optimum chromatograms obtained under experimental conditions leading to the same values of these parameters should also be identical. In order to check whether there is an influence of the column length, the optimization was carried out successively with two different column lengths: $L = 10$ cm and $L = 50$ cm, respectively. Fig. 1 shows the optimum chromatograms obtained in the case of displacement chromatography. In each of the two successive calculations, the loading factor, the plate number, and the displacer concentration were optimized simultaneously. All the other parameters were kept constant and identical for the two column lengths. Fig. 1 demonstrates that, except for the minor, unavoidable consequences of the uncertainty of numerical origin in finding the global optimum, the chromatograms are indeed identical when plotted on the reduced time scale.

Similar calculations were repeated in gradient elution chromatography. Again, column lengths of $L = 10$ cm and $L = 50$ cm, respectively, were considered and the loading factor, the plate number, as well as the gradient steepness were optimized simultaneously in both cases. As seen in Fig. 2, when the optimum band profiles are plotted on the reduced time scale they match perfectly.

Based on these results, we can state that when the column length is fixed and the plate number is varied, e.g., by adjusting the average particle diameter, the optimum chromatograms are always identical when plotted on the reduced time scale. These results confirm the assumption made earlier on a theoretical basis that the loading factor and the plate number are the essential experimental parameters to be optimized in all modes of preparative chromatography.

4.2. Cycle time

Our previous optimization studies [4,8,13–16]

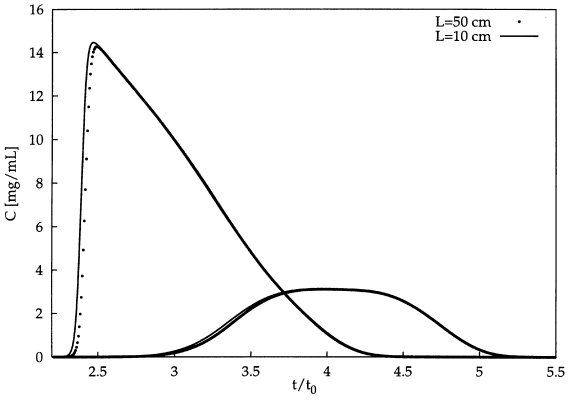

Fig. 2. Optimum gradient elution chromatograms. The loading factor, the plate number, and the gradient steepness were optimized simultaneously at $k'_1=10$. The column lengths are 10 cm (solid line) and 50 cm (dotted line). The optimum loading factors (L_f) were 26.5 and 25.8%, respectively, the optimum plate number, 162 and 173 theoretical plates, respectively, and the gradient steepness, (G), 0.50 and 0.50 mg/ml, respectively. The optimum conditions for the 10 cm column were: $PrY_{max}=1.34$. As a consequence, the other optimum experimental conditions are: $d_p=14.2$ μm, $\nu=361$, $u=2.53$ cm/min, $Y=69.5\%$ and $Pr=1.92$. For the 50-cm long column, they were $PrY_{max}=1.39$, $d_p=32$ μm, $\nu=812$, $u=2.54$ cm/min, $Y=72\%$, and $Pr=1.94$.

utilized a different definition of the cycle time and another objective function. Thus, some properties have to be re-evaluated here in the cases of isocratic elution and displacement chromatography to clarify the origin of slightly different conclusions. By contrast, the optimization of overloaded gradient elution chromatography was investigated using the new objective function and cycle time definition [14,15].

In isocratic elution, the presence of strongly retained impurities (not infrequent in binary separations and not always easy to eliminate in a purification step prior to preparative HPLC) may call for column regeneration. Depending on the specifics of the case, the impurity can be washed off the column more or less rapidly. We can consider two extreme case scenarios. In the first one, there are no impurities and the cycle time is the conventional analysis time under linear conditions [$t_c=(1+k'_2)t_0$]. In the second case, a regeneration step of complexity comparable to the one required in displacement or in gradient elution chromatography is needed and will take about the same time. However, because the displacer must be more strongly retained than all feed impurities, it is only in rare cases that it will take the same time to regenerate the column in elution and in displacement chromatography. Fig. 3 compares the performance achieved in isocratic elution and in displacement chromatography when the definition of the cycle time was altered in the elution mode. The solid line in this figure gives the product of the production rate and the recovery yield in displacement chromatography as a function of the retention factor of the less retained component of a binary mixture with a 3:1 relative concentration, at a separation factor of $\alpha=1.5$[1]. In this case, the new definition of the cycle time was used (i.e., the column regeneration requires six column volumes of solvent). The same product, PrY, calculated for isocratic elution, is plotted versus k'_1, assuming that different volumes of solvent, between zero and five column volumes, are needed for column regeneration. Fig. 3 clearly shows that the definition of the cycle time influences considerably the production rate in isocratic elution.

The production achieved is largest when there is no need for column regeneration — i.e., when the

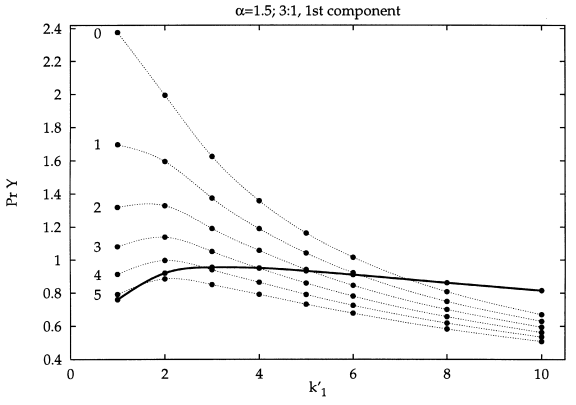

Fig. 3. Plot of the optimum value of the objective function (PrY) against the retention factor of the less retained component. The solid line gives the value of (PrY) in displacement chromatography when six column volumes of solvent are needed for column regeneration. The dashed line gives the value of (PrY) in isocratic elution chromatography when the amount of solvent needed for column regeneration changes between 0 and 5 column volumes.

[1]The production rate for that configuration is shown in Fig. 6c with identical cycle time definitions for the two modes.

cycle time is simply determined by the retention time of the more retained component. In this particular case, displacement chromatography (which always requires regeneration by six column volumes of solvent) performs better than isocratic elution only if the retention factor, k_1' is larger than 7. However, as the regeneration need of isocratic elution approaches that of displacement chromatography, the displacement mode begins to outperform isocratic elution for decreasing values of the retention factors. Finally, displacement chromatography performs better than isocratic elution at any retention factor when six column volumes are needed for the regeneration of the column (this issue will be further discussed in Fig. 6).

It is important to note that any of the modifications of the cycle time definition just discussed cause no change in either the optimum experimental conditions or the chromatogram obtained under these conditions. The optimum values of the plate number and the loading factor — and accordingly, the throughput, the production per cycle and the recovery yield — remain constant, regardless of the definition of the cycle time. Only the value of the production rate is affected by the change in the actual value of the cycle time resulting from the use of a different definition. This is illustrated in Fig. 4, in which are plotted the optimum chromatograms calculated for $k_1'=4$. The solid line corresponds to displacement chromatography. The different broken lines show the elution chromatograms obtained with the different definitions of the cycle time used also in Fig. 3 and just discussed. These optimum chromatograms match quite well together and the figure should be considered as an illustration of the uncertainty of the numerical calculations made during the whole optimization routine and of their effect on the optimum band profiles.

4.3. Comparison of the performance of different modes

Note that the band profiles shown in Fig. 4 are plotted against the absolute time and not the reduced time. This illustrates the fact that a comparison of the performance of the two modes of preparative separation is not straightforward because the actual optimum experimental conditions are quite different:

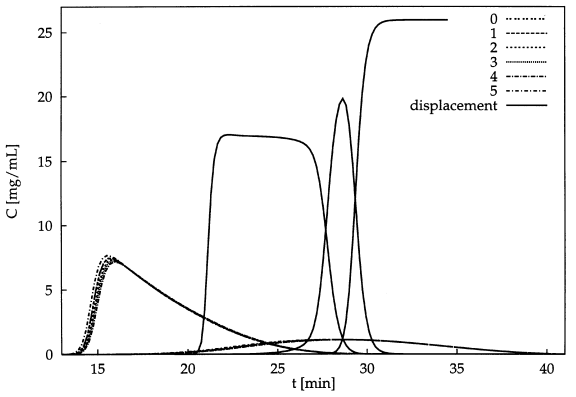

Fig. 4. Optimum chromatograms whose data are plotted in Fig. 3 at $k_1'=4$ and $\alpha=1.5$. The solid line is the optimum displacement chromatogram when six column volumes of solvent are needed for column regeneration. The column length is $L=10$ cm. The optimum conditions are: $L_f=24.5\%$, $N=747$, $C_d=26.0$ mg/ml, and $\mathrm{Pr}Y_{max}=0.952$. As a consequence, the other optimum experimental conditions are: $d_p=9.2$ μm, $\nu=98.9$, $u=1.07$ cm/min, $Y=85\%$ and $\mathrm{Pr}=1.12$. The other lines show the optimum chromatograms calculated in isocratic elution when the amount of solvent needed for column regeneration changes between 0 and 5 column volumes. The optimum conditions are: $L_f=16.3\%$, $N=273$ and $\mathrm{Pr}Y_{max}=0.732$. As a consequence, the other optimum experimental conditions are: $d_p=12.4$ μm, $\nu=240$, $u=1.93$ cm/min, $Y=80\%$ and $\mathrm{Pr}=0.91$ with 6 column volumes for regeneration.

although the column length is the same in both cases, 10 cm, the hold-up times are 5.18 min in elution and 9.35 min in displacement chromatography, leading to quite different values of the reduced time for the same value of the absolute time. Although the retention times of the elution and displacement chromatograms in Fig. 4 are not very different, there is a major difference between these two chromatograms. It arises from the higher plate number that displacement chromatography requires as compared to elution. In this instance, $N=747$ for displacement chromatography and $N=273$ for isocratic elution. Accordingly, because the column is operated at the maximum possible flow-rate (with an inlet pressure of 125 atm, see earlier) and the plate number can be altered by changing either the particle size or the column length, the flow-rate is almost twice as high in elution ($F_v=0.253$ cm^3/min) than in displacement chromatography ($F_v=0.142$ cm^3/min). In turn, this difference between the flow-rates influences strongly the cycle time because, during regeneration, the

mobile phase flow-rate cannot be higher than the flow-rate determined by the maximum pressure drop (the effect of the feed component concentration on the viscosity [24] is neglected). Therefore, in this case, column regeneration will take much longer time in the displacement mode than in isocratic elution.

Conversely, however, there are other phenomena that have more beneficial effects in displacement than in elution chromatography: the former mode is operated with a higher loading factor and shorter retention times. For example, in the case illustrated in Fig. 4, the loading factor is approximately 50% higher in the displacement mode ($L_f = 24.5\%$) than in isocratic elution ($L_f = 16.3\%$) while the breakthrough time of the displacer is about 66% of the analytical retention time of the more retained component in isocratic elution. Another well-known advantage of displacement chromatography can also be seen in Fig. 4: the band profiles are more compact, resulting in a higher concentration of the collected fractions.

A previous study of the optimization of the experimental conditions in overloaded gradient elution preparative chromatography [15] was recently carried out, using the same objective function and cycle time definition as in this study. The results of this investigation can be summarized as follows:

1. The recovery yield achieved under optimum conditions is the same in gradient and in isocratic elution.
2. The optimum loading factor is higher in gradient elution than in isocratic elution, because the band compression diminishes the tag-along effect of the more retained component. Accordingly, the average concentration of the collected fractions and the production rate are higher in gradient than in isocratic elution.
3. There is no need for a high column efficiency in gradient elution because the average retention factor is higher than the optimum value during the separation.
4. The optimum gradient steepness depends mostly on the elution order. It is higher for the purification of the less retained component than for that of the more retained one.

One of the important findings of previous optimization studies is the existence of an optimum value of the retention factor for which the objective function is maximum. While the discussion of the dependence of the objective function on the retention factor is simple in elution and displacement chromatography, it is not straightforward in gradient elution because the retention factor changes continuously during a gradient elution separation. The following transformation is proposed to include gradient elution into a general comparison of the performance of the various modes of chromatography. In gradient elution, the retention time of a band under linear conditions (i.e., assuming so-called analytical injection) is given by [18].

$$t_R = t_0\left(1 + \frac{1}{G}\ln(1 + k_0' G)\right) \tag{16}$$

In the case of a Langmuir-type isotherm, the analytical retention time coincides with the elution time of the diffuse rear of nonlinear band profiles. Therefore, Eq. (16) can be used to estimate the time required to completely elute the material from the column. We can reformulate Eq. (16) as

$$t_R = t_0(1 + k_g') \tag{17}$$

where $k_g' = 1/G\ \ln(1 + k_0' G)$ is the gradient retention factor that can be used for the characterization of solute retention in gradient elution, in much the same way as the classical retention factor, k', is used in isocratic separations.

To compare the performance of gradient elution with those of isocratic elution and displacement chromatography, k_g' was calculated from the initial retention factor and the gradient steepness. The maximum value obtained for the objective function was then plotted against k_g'. The applicability of the gradient retention factor k_g' for performance comparison is demonstrated in Fig. 5. The solid lines are the profiles of the two component bands in an optimized isocratic elution, shown for a retention factor of $k_1' = 4.62$. Two quite different gradient separations are also shown. Their common feature is to have the same gradient retention factor, $k_g' = 4.62$. Because of the rather different values of the initial retention factors and the gradient steepness of these two gradient elution experiments, the band profiles obtained under optimum conditions (dotted and dashed lines, respectively) are quite different. However, very similar optimum values of the objective

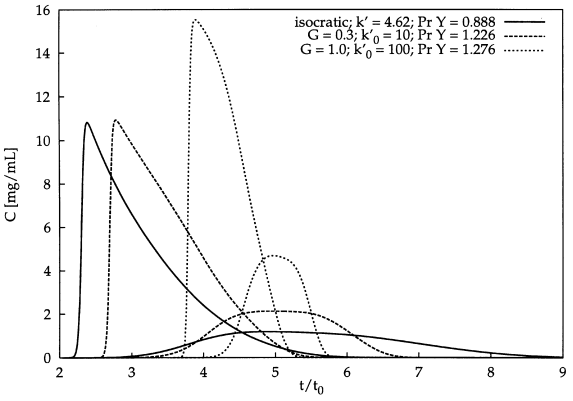

Fig. 5. Comparison of the band profiles for optimum separations carried out by isocratic elution (solid line) and gradient elution (dashed and dotted lines). The gradient retention factor in each of the gradient elution separations is equal to the retention factor in isocratic elution (4.62). For $G=0$ (isocratic elution), $L_f=25.0\%$ and $N=209$. The maximum value of the objective function was $\mathrm{Pr}Y_{max}=0.888$. The other optimum experimental conditions were: $d_p=13.3$ μm, $\nu=294$, $u=2.21$ cm/min, $Y=77\%$ and $\mathrm{Pr}=1.155$. For $G=0.3$ (isocratic elution), $L_f=26.1\%$ and $N=135$. The maximum value of the objective function was $\mathrm{Pr}Y_{max}=1.226$. The other optimum experimental conditions were: $d_p=15$ μm, $\nu=420$, $u=2.80$ cm/min, $Y=68\%$ and $\mathrm{Pr}=1.79$. For $G=1.0$ (isocratic elution), $L_f=27.6\%$ and $N=124$. The maximum value of the objective function was $\mathrm{Pr}Y_{max}=1.276$. The other optimum experimental conditions were: $d_p=15.3$ μm, $\nu=450$, $u=2.93$ cm/min, $Y=63\%$ and $\mathrm{Pr}=2.03$.

function are obtained for the two separations: $(\mathrm{Pr}Y)_{max}=1.226$ when $G=0.3$ and $k_0'=10$, $(\mathrm{Pr}Y)_{max}=1.276$ when $G=1.0$ and $k_0'=100$, a difference of less than 4%. Both values are about 40% higher than the maximum product $(\mathrm{Pr}Y)_{max}=0.888$ obtained for isocratic elution with a retention factor equal to the two gradient retention factors. This result demonstrates that the gradient retention factor characterizes sufficiently well the retention in preparative gradient elution chromatography to allow meaningful comparison of the performances achieved with the different modes.

Fig. 6 summarizes the results of a comparison of the maximum performance of the three modes of chromatography for a separation factor $\alpha=1.5$, for different mixture compositions and elution orders. One can conclude that the dependence of the separation performance on the retention factor is most significant in gradient elution. Displacement chromatography is the mode which is the least sensitive to changes of the retention factor. While the particular experimental set-up (mixture composition, elution order, separation factor, etc.) selected arbitrarily may vastly influence the separation performance, we can conclude that either gradient elution or displacement chromatography generally outperforms isocratic elution in all cases in which this last method requires extensive column regeneration (with five column volumes or more). On the other hand, when the isocratic separation does not require column washing, all three modes give similar results. In the specific calculations carried out for this work, a gradient steepness that results in a gradient retention factor no larger than $k_g'=5$ is advantageous. The maximum performance of gradient elution was found for values of k_g' slightly below 3 for the purification of the less retained component and slightly above 3 for the purification of the more retained component. Displacement chromatography appears to be the method of choice if the retention factor (or the gradient retention factor) is larger than 5. Displacement chromatography is more attractive than isocratic elution for retention factors in excess of 2 when important washing is needed for column regeneration. If a cleaner feed can be used, elution becomes more attractive in a much wider range of retention factors (see Fig. 3).

The same phenomenon is also observed for more difficult separations. In Fig. 7, isocratic elution and displacement are compared for a separation factor $\alpha=1.2$. The calculations show that the performance of displacement chromatography is less sensitive to changes of the retention factor than that of elution and that displacement chromatography becomes the more advantageous mode when $k'\geq 2$.

The choice of a uniform cycle time definition — with the requirement of six column volumes of solvent for the regeneration of the column in all cases — is arbitrary and might seem pessimistic in the case of isocratic elution. To study the consequences of this choice, the comparison of the performance of the three modes of preparative chromatography shown earlier in Fig. 6, in the case of $\alpha=1.5$, was also carried out with different values of the volume of solvent required for column regeneration. The results are summarized in Fig. 8. The narrow broken lines correspond to different volumes required for the regeneration, from 0 to 6 column

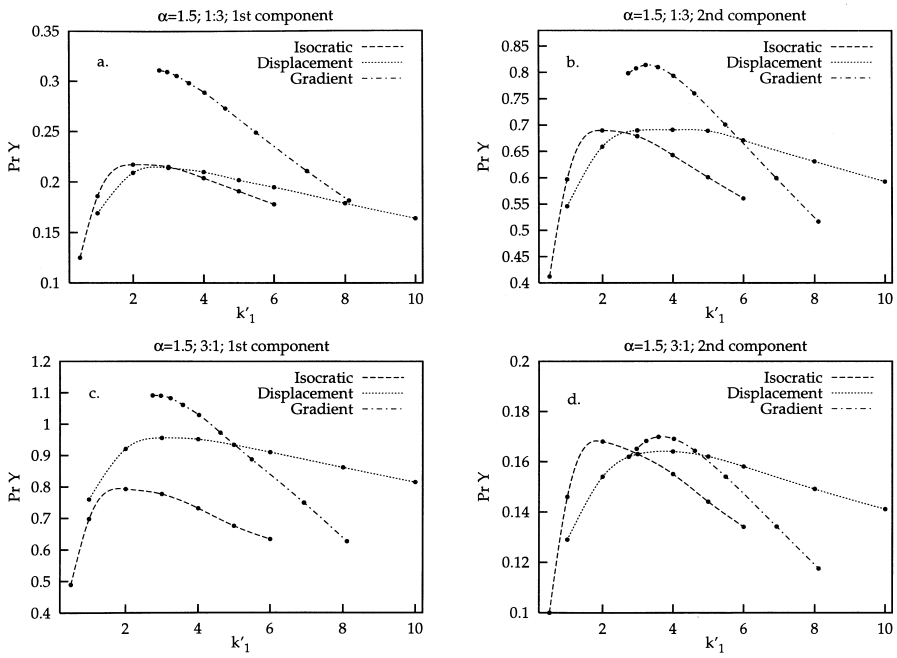

Fig. 6. Comparison of the performance of isocratic elution, gradient elution, and displacement chromatography for different mixture compositions and elution order at $\alpha = 1.5$.

volumes. The results show that isocratic elution outperforms the other two modes when there is no need for column regeneration, for instance in the case of an impurity-free isocratic separation. As the time needed for column regeneration increases in isocratic elution, the other two modes of separation become more and more attractive.

A similar trend is observed in Fig. 9, corresponding to a more difficult separation, with $\alpha = 1.2$. In this figure isocratic elution is compared to displacement chromatography for the purification of either component of a binary mixture of relative concentration 3:1. At this separation factor — and regardless of the elution order — displacement gives a higher value of the objective function whenever k'_1 exceeds 6, even if no column regeneration is required in isocratic elution. Because the performance of displacement chromatography is rather insensitive to changes in the retention factor, it always becomes the most advantageous mode at values of the high retention factor.

Figs. 8 and 9 illustrate the considerable influence of the solvent volume needed for column regeneration on the performance achieved at small retention factors. At $k'_1 = 1$ for instance, the performance (i.e., the value of PrY) of isocratic elution is about 3.5-times higher if there is no need for column regeneration than if six column volumes are required. The gain in performance is a factor of approximately 2.5 at $k'_1 = 2$ and only 1.4 at $k' = 10$. This phenomenon arises from the larger contribution of the retention time to the cycle time observed at higher retention factors. Correspondingly, the contribution of the regeneration time to the cycle time decreases. Accordingly, the solvent volume needed for column regeneration must be determined very carefully when working at small retention factors.

The optimum retention factor afforded by the new objective function is somewhat higher than the one obtained when the production rate itself was optimized [16]. The definition of the cycle time also influences the optimum retention factor. Fig. 3 illustrates how, in the case of isocratic elution, the optimum value of k'_1 shifts toward higher retention factors as the solvent volume needed for column regeneration increases. The optimum retention factor is always higher in displacement chromatography than with the other two modes. It was found to be

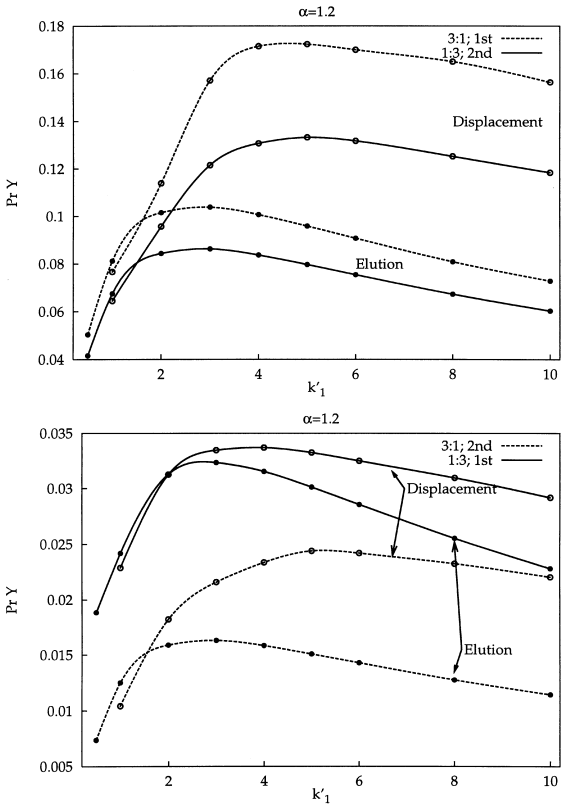

Fig. 7. Comparison of the performance of isocratic elution and displacement chromatography for different mixture compositions and elution orders at $\alpha = 1.2$.

$k_1' = 3$–4 for $\alpha = 1.5$ and $k_1' = 4$–5 for $\alpha = 1.2$. The strong dependence of the value of the optimum retention factor on the separation factor is illustrated in Fig. 10. The optimum value decreases from $k_1' = 4.5$ to $k_1' = 1.7$ when the separation factor improves from $\alpha = 1.1$ to $\alpha = 1.8$, regardless of the elution order or of the feed composition. It has always been known in chromatography that separation requires retention. It is not surprising that, whatever the criterion, easy separations require less retention than difficult ones.

5. Conclusions

This study confirms previous results [13] that there are only two critical parameters in the optimization of a preparative separation by elution chromatography, the loading factor and the column efficiency (assuming that it is independent of the concentration). In gradient elution and displacement chromatography, there is one more parameter, the gradient steepness or the displacer concentration, respectively. This observation simplifies markedly the solution of optimization problems. Furthermore, the optimum parameters, the optimum values of the amount injected and the column efficiency, the corresponding values of the recovery yield and the production per cycle, and the other experimental parameters of the separation are independent of the definition of the cycle time.

Some of the results of this study are different from those of previous analyses based on the same models and might even seem to contradict some of their conclusions [8]. For instance, we reported previously that the optimum retention factor was rather small in isocratic overloaded elution, seldom higher than $k_1' = 1$, that the optimum retention factor was around $k_1' = 2$ in the displacement mode and that the former mode outperforms the latter in a wide range of experimental conditions (i.e., of separation and retention factors) [4,8,16]. These earlier conclusions, however, were based on the results of calculations performed with a different objective function and a different definition of the cycle time. In the present study, the recovery yield and the production rate are maximized simultaneously. As a consequence of this change in objective function, experimental conditions under which the recovery yield is poor become heavily penalized, even if they give a high production rate. The optima of the new objective function, Pr*Y*, were always found for such combinations of column design and operating parameters that the recovery yield was between 70 and 90%, while the maximum production rate was often associated with values of the recovery yield lower than 50%. Moreover, previous calculations assumed much shorter cycle times than the more realistic one defined in this work. As seen in Fig. 3, the definition of the cycle time significantly influences the value of the optimum retention factor, besides the performance of the separation method. Still, although larger than previously estimated, the optimum values of the retention factor are low compared to those generally used in applications. This suggests that significant gains could often be achieved by operating prepara-

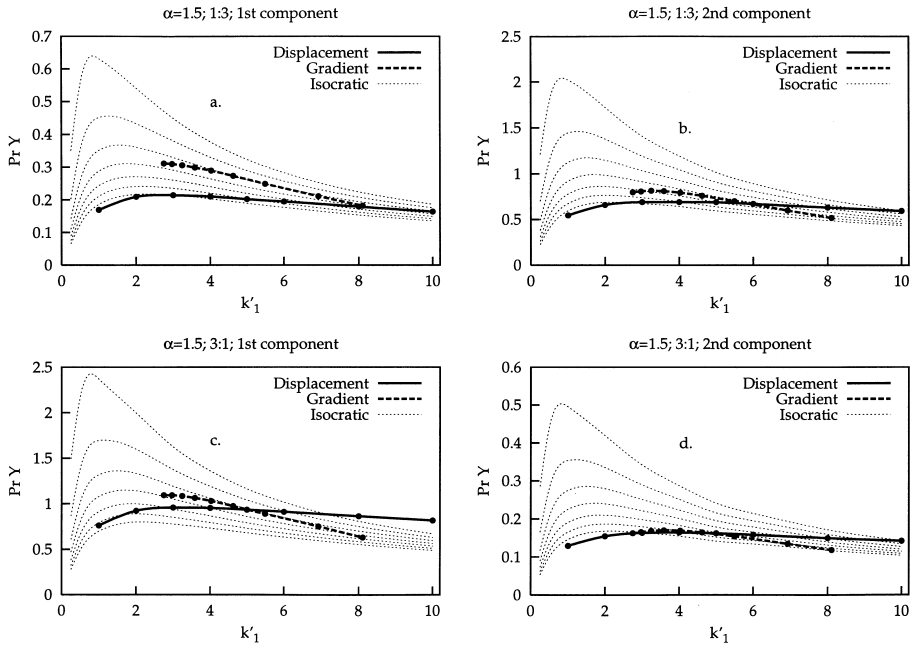

Fig. 8. Comparison of the performance of isocratic elution, gradient elution, and displacement chromatography for different mixture compositions and elution order at $\alpha = 1.5$. The narrow broken lines give the performance of isocratic elution (downwards) when solvent of 0, 1,· · · 6 column volumes are required for column regeneration.

tive chromatography with phase systems affording values of the retention factors lower than conventionally adopted.

The new uniform definition of the cycle time used here has two advantages. First, it allows a more reasonable comparison of the three modes of separation, although, as a general rule, the volume of solvent required to wash and regenerate the column after a batch separation will always be larger in displacement chromatography than in elution and often larger in gradient elution than in isocratic elution. Second, it allows an illustration of the importance of the feed purity in preparative separations and of the performance loss caused in the elution mode by the need for column regeneration. It even permits a quantitation of this loss. The comparisons made earlier (Figs. 6–9) show that either gradient elution or displacement outperforms isocratic elution when the feed contains significant amounts of strongly retained impurities, making column regeneration needed at the end of each cycle. By contrast, isocratic overloaded elution seems to be the method of choice in the cases when column regeneration is significantly faster than with the other two modes, is not required, or can be performed during the cycle. In isocratic or gradient elution, a pre-column can be placed on-line with the main column and properly washed during the second, longer part of the cycle, e.g., through backflushing. The use of such implementation schemes or of other approaches for the pre-purification of the feed can permit large improvements in the production rate.

For the purpose of optimizing overloaded gradient elution at least, the gradient retention factor is a more significant parameter than the gradient steepness because the former incorporates also the retention factor at the initial mobile phase composition. As long as the gradient retention factor remains unchanged, the characteristics of a separation are insensitive to the individual values of the gradient steepness and of the retention factor at the initial mobile phase composition. This allows some useful flexibility in adjusting the experimental conditions of a separation by gradient elution. The optimum performance of gradient elution are obtained when the gradient retention factor is in the range $k'_g = 3–4$.

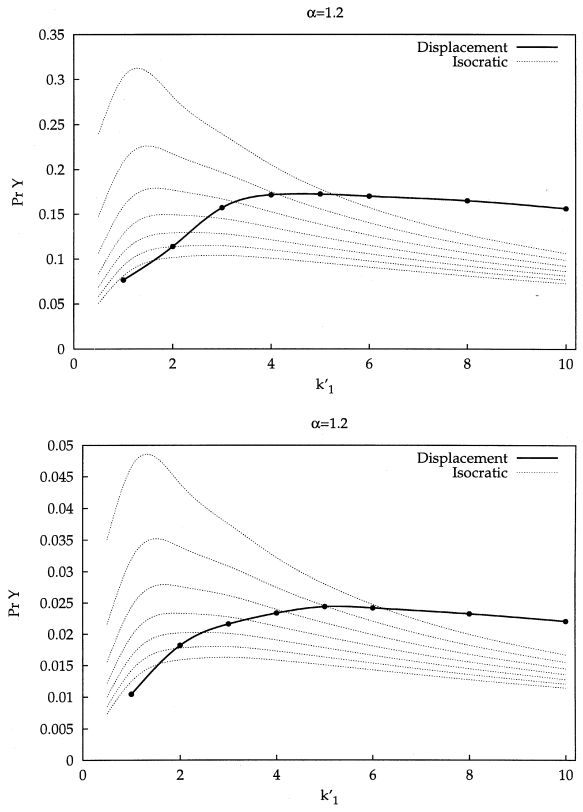

Fig. 9. Comparison of the performance of isocratic elution and displacement chromatography for different elution orders at $\alpha =$ 1.2, relative mixture composition 3:1. The narrow broken lines give the performance of isocratic elution (downwards) when solvent of 0, 1,· · · 6 column volumes are required for column regeneration.

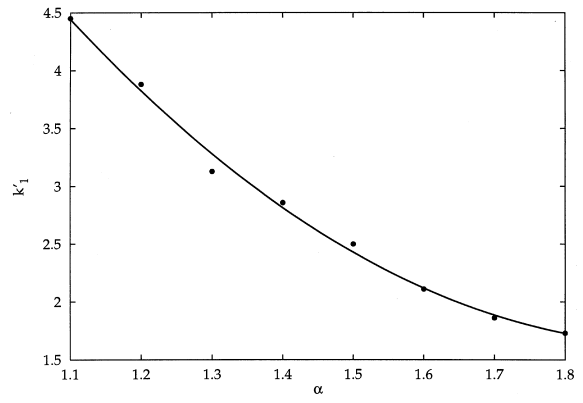

Fig. 10. Optimum value of the retention factor plotted against the separation factor in isocratic elution chromatography.

However, gradient elution is the mode for which the performance is most sensitive to changes of the retention factor. A significant loss of production rate will be observed if the gradient steepness is not high enough and the value of k'_g is outside this range.

Usually both gradient elution and displacement chromatography have higher optimum loading factors than isocratic elution. Besides the increased feed load, gradient elution requires less efficient columns than the other two modes, hence uses shorter columns packed with larger particles. Accordingly, the column may be operated at higher flow-rate in gradient elution, which further shortens the cycle time. The combination of both effects explains the better performance of gradient elution compared to isocratic elution. The latter one explains a higher performance of gradient elution compared to displacement chromatography.

Although this was not investigated in this study, previous results clearly show that the concentration of the collected fractions is significantly higher in the displacement mode than in elution [16]. In displacement chromatography, the isotherms and the concentration of the displacer determine the concentration while the initial feed concentration is not essential as it is in elution. Because of axial dispersion, the larger the value of the retention factor, the lower the concentration of the fractions collected in the elution mode. On the contrary, in the displacement mode, increasing the retention factor yields more concentrated fractions [16].

These different effects can be discussed separately for the purpose of explaining the relative advantages and drawbacks of the modes considered. However, optimization is a self-consistent process which cannot be resolved in separable effects. The performance of each mode of preparative chromatography is the result of the combination of all those effects. It must be emphasized that the optimization of chromatography looks formidable because it is a nonlinear problem and because of the large number of parameters one faces when looking at the characteristics of columns used for large-scale separations. With the help of theory, this problem can be considerably simplified by reducing the number of key parameters to two major ones in elution, three in gradient elution and displacement chromatography. The other parameters are then derived as the solution of simpler

optimization problems (e.g., how to design a column giving a certain number of plates? How to achieve a certain value of k'_g?). When the experimental and column design parameters of the separation are carefully selected, the production rate of a preparative separation can be increased significantly while assuring a high recovery yield.

Finally, an important result of this work must be underlined. The choice of the most advantageous mode to perform a given separation relies mostly on the actual values of the retention factors of the two components to be separated. There is probably much work to pursue along this line. Optimizing the retention factor, when possible, would bring important improvements in the degree of performance achieved. However, this work neglects the actual economy of separation processes. Even if elution gives a lesser degree of performance and a lower production rate than gradient elution and displacement, it may turn out to be the less costly solution because it does not cause the obvious additional problems of solvent recovery.

Symbols

a	First coefficient of the Langmuir isotherm
b	Second coefficient of the Langmuir isotherm
C	Mobile phase concentration of the solute
C^0	Injected concentration,
D_a	Apparent axial dispersion coefficient
D_m	Solute molecular diffusivity
d_p	Average particle diameter of the stationary phase
F	Phase ratio
F_v	Mobile phase flow-rate
G	Gradient steepness
H	Height equivalent to a theoretical plate
h	Reduced plate height
k'	Retention factor at infinite dilution
k'_g	Gradient retention factor at infinite dilution
k_0	Specific column permeability
L	Column length
L_f	Loading factor
N	Number of theoretical plates
n_c	Number of Craig cells
Pr	Production rate
q	Equilibrium stationary phase concentration of the solute
q_s	Isotherm saturation capacity
S	Solvent strength parameter
S_A	Column cross-section area,
t	Time
t_0	Hold-up time
t_c	Cycle time
t_G	Gradient time
t_R	Retention time
u	Mobile phase linear velocity
V_s	Sample volume
x	Reduced column length
Y	Recovery yield
z	Column length
α	Separation factor
ΔP	Pressure drop
η	Mobile phase viscosity
ν	Reduced mobile phase velocity
τ	Reduced time
ε	Total porosity of the column
φ	Modifier volume fraction

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