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Comparison of the performances of overloaded elution and displacement chromatography for a given column

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ABSTRACT

Using the semi-ideal model, chromatograms were calculated for the separation of a binary mixture on a given column by displacement and elution chromatography. The optimum conditions for the maximum production rate with or without a recovery yield constraint were determined using the simplex algorithm. Mixtures of relative retention 1.20 and 1.70 and relative composition 1:3 and 3:1 were considered. The maximum production rates achieved in both modes of chromatography are close. In all instances studied the ratio of the production rates of the two modes was between 0.5 and 2. Without a yield constraint, for the cases studied the recovery yield achieved in overloaded elution is much higher than that achieved in displacement chromatography. However, the concentration of the fraction collected in the displacement mode is one to two orders of magnitude higher than that of the fraction collected in the elution mode. The choice between the modes of operation will depend largely on the comparative economics of displacer recovery and fraction concentrations.

INTRODUCTION

Most practical applications of preparative chromatography, whether for research and development or for production purposes, are carried out in the overloaded elution mode. However, it has been suggested that the displacement mode would permit much larger throughputs and the production of more concentrated fractions [1–3]. The major difficulties encountered in the use of the displacement mode are (i) the greater complexity of method development, (ii) the need to select a proper displacer and to optimize its concentration, (iii) the fact that displacement chromatography does not permit production with total recovery, (iv) difficulties in recording individual band profiles by direct detection, (v) the need to regenerate the column at the end of each run before the next one can be started and (vi) the high cost of the displacer. It has been stressed that these disadvantages would fade if the method

gained recognition. Moreover, attention has been focused on a comparison of the production rates which can be achieved by either methods and on the product concentration [4–6], rather than on the issues listed above.

Unfortunately, the discussion of the relative merits of each mode has remained academic and qualitative, as there are few data available to permit a detailed, quantitative comparison of the performances obtained with the two methods. Liao *et al.* [4] and Viscomi *et al.* [5] presented experimental data obtained with the same column, for the same mixture, in overloaded elution and displacement. They concluded that the latter gave a superior performance. We note that in the former paper [4], however, the production rate achieved in displacement was no more than twice as large as the production rate in elution, neglecting regeneration. More important, in both papers, no effort was made to optimize the experimental conditions, such as mobile phase composition, amount of sample and flow-rate, particularly in the elution mode. Based on the ideal model of chromatography, assuming 99.99% purity, the production rate, yield and enrichment factor have been calculated for a single case [6]. It was shown that displacement chromatography gives a higher yield for both components and a 10-fold higher concentration in the collected fractions compared with elution. The production rates are of the same order of magnitude. As the ideal model does not permit optimization of the velocity, these results are approximate. We are of the opinion that a valid comparison between the two methods must be based on the maximum production rate achieved in the two modes under the same set of constraints (production purity and, possibly, recovery yield).

Ideally, this comparison should be done experimentally. Calculations may help, however, in orienting the selection of the experiments to perform and in reducing their number. We have already shown that the individual band profiles of the components of a binary mixture can be predicted accurately, provided that the equilibrium isotherms of the two components are known and can be represented accurately by a model [7,8]. We have used the semi-ideal model to calculate the individual band profiles in overloaded elution [9–11] and in displacement chromatography [12]. The results of these calculations permit an easy determination of the production rate and recovery yield for any product purity required. The influence of the various experimental parameters on the production rate and yield at constant product purity has been studied [12–14].

Using a simplex optimization method, the optimization of experimental conditions in overloaded elution has been investigated [15]. It was shown that the column should be operated at high mobile phase velocities, *i.e.*, at velocities which are one to two orders of magnitude higher than the velocity at which the column efficiency is maximum. The highest production rate is obtained for a large sample size, leading to strongly overlapping bands, with a recovery yield of the order of 60%. Finally, the optimum column length, for a given particle size, corresponds to a limiting column efficiency for a very small sample size (*i.e.*, under linear conditions) which provides only a moderate resolution of the two peaks, of the order of unity and sometimes even lower.

The results of this simplex optimization have been confirmed by those of a more fundamental approach using the closed-form solution of the ideal model in the case of Langmuir competitive isotherms and correcting for the finite column efficiency

[13,14,16]. Recently, we have been able to calculate the optimum experimental conditions for the maximum production rate of the second component of a binary mixture with constraints on recovery yield [16]. These results, however, cannot be extended to the first component.

In an earlier publication we discussed the influence of the experimental parameters on the production rate and yield of products at a stated purity in displacement chromatography [12]. In a separate study, we determined the individual profiles of the components of a binary mixture in displacement chromatography and discussed the agreement between experimental results and numerical calculations [17].

In this paper, we compare the performance of a given column operated in overloaded elution and in the displacement mode for the separation of the components of a binary mixture. In each instance we determine first the optimum experimental conditions for the maximum production rate of the second component at a given purity. Then we compare the values of the production rates obtained by the two methods. In the second part, we add a recovery yield constraint and repeat the same calculations.

THEORY

The approach

The individual band profiles of the two components in either overloaded elution or displacement were calculated using the method described by Rouchon *et al.* [18], implemented by Guiochon and co-workers [9,19] and discussed by Czok and Guiochon [8,20]. The difference between the calculations performed for the two modes of chromatography lies in the boundary conditions after the injection of a rectangular plug of the binary mixture has been made. In elution, the stream of mobile phase is resumed, whereas in displacement a solution of displacer in the mobile phase is introduced into the column until the displacer front appears at the column exit. No attempt has been made to model the regeneration of the column, which would require some additional assumptions.

Once the individual profiles have been calculated, an integration program permits the determination of the cutting points at a specified purity. Then, the amount produced, the recovery yield and the production rate can be calculated. For the calculation of the production rate, the cycle time in overloaded elution is assumed to be equal to $\Delta t_c = t_{c,2} - t_{c,1}$, where $t_{c,1}$ is the time when the concentration of the first component is $1 \cdot 10^{-6}$ mg/ml and $t_{c,2}$ is the time when the concentration of the second component is $1 \cdot 10^{-6}$ mg/ml. In displacement chromatography, the cycle time is the breakthrough time of the displacer front. This last assumption certainly favors displacement chromatography. It seems difficult, in spite of the ingenuity of separation chemists, to imagine schemes permitting column regeneration which would take much less time than the separation of a single batch. However, other assumptions regarding the regeneration time would be arbitrary.

The simplex algorithm was described by Dose [21]. The modified simplex algorithm [22] was extended to handle approaches to parameter-value boundaries (*e.g.*, maximum inlet pressure) by randomly placing test points along the simplex trial vector and searching for the outermost trial point which satisfies all the user's parameter-value criteria, that is, the outermost trial point allowed. The user may

specify limits on other than explicit parameters, although these indirect criteria generally depend on the parameter values themselves. It is prudent to check first any criteria depending directly on the parameter values to ensure that the parameters can safely be used to compute other, indirect criteria.

In this work, only displacer concentrations of 300 mg/ml or less were allowed. Displacer concentrations and loading factors were constrained to be positive. The yield constraints described under Results and Discussion were handled as indirect constraints, requiring a separate simulation for each test of this criterion. This last requirement increased the computational time but greatly simplified the management of the optimization. Parameter starting values were chosen at opposite ends of the permitted parameter space, and parameter values at convergence were independent of the starting parameter values. Convergence was declared when the standard deviation of the test points' parameter values decreased below a prescribed level; for the displacer concentration and loading factor these levels were 1 mg/ml and 0.1%, respectively. Convergence generally required 16–20 simplex cycles.

Statement of column properties

The production rate is calculated for a column of specified properties. This column was assumed to be 25 cm long and packed with 20-μm particles. The ratio d_p^2/L is 16 (where d_p = average particle diameter in μm and L = column length in cm) [13]. In the last study, the column efficiency being insufficient, we made the calculations assuming that the particle size was 10 μm, whereas the other column characteristics remained unchanged. The column porosity is 0.80, the mobile phase viscosity is 1 cP and the diffusion coefficient of all compounds in the mobile phase is $D_m = 1 \cdot 10^{-5}$ cm^2/s. Throughout this work, the mobile phase velocity, the loading factor and the production rate per unit cross-section surface area are used. The values of the loading factor refer to the second component and are denoted accordingly $L_{f,2}$. The loading factor is the ratio of the amount of component injected to the column saturation capacity for this compound.

The column efficiency is assumed to be given by the Knox equation [23]:

$$h = \frac{2}{v} + v^{0.33} + 0.10v \tag{1}$$

where h is the reduced plate height ($h = H/d_p$, H the actual height equivalent to a theoretical plate) and v is the reduced velocity ($v = ud_p/D_m$). With the values selected for the particle diameter and the diffusion coefficient, $v = 200u$ in this work. The numerical values selected for the coefficients of the Knox equation correspond to a well packed column, with a packing material having reasonably fast mass-transfer characteristics.

In all calculations, we have assumed that the equilibrium isotherms of the three compounds (the two components of the sample and the displacer) are given by the competitive Langmuir isotherm:

$$q_i = \frac{a_i C_i}{1 + \sum_{j=1}^{3} b_j C_j} \tag{2}$$

where q_i is the concentration of the ith compound in the mobile phase at equilibrium with concentrations C_j (j = 1–3) of the three other compounds, and a_i and b_i are numerical coefficients. The numerical values of the coefficients a_i and b_i of the isotherms are 23 and 0.0183 ml/mg, respectively, for the first component and 60 and 0.0215 ml/mg, respectively, for the displacer. For the second component, they are 23α and 0.0197 ml/mg, respectively, where α is the relative retention of the two components of the sample at infinite dilution. The respective values of the retention factor for the three components are 5.75, 5.75α and 15. We studied two series of mixtures, with α = 1.20 and 1.70, respectively (hence capacity factors k'_2 are 6.90 and 9.77). The values of the relative retentions of the second component and the displacer are 2.17 and 1.53, respectively.

We studied the column performance in the two operation modes using mixtures of two different compositions, 1:3 and 3:1, where this ratio represents the relative amounts of each component.

We selected a value of 98% for the required degree of purity of the product, assuming further that there should be 1% of the first component and 1% of the displacer as impurities in the product prepared by displacement. Requiring less displacer in the product seemed unrealistic in view of the shape of the displacer front. The required movement of the cut-point would have resulted in much reduced production rates by the displacement mode. Calculations were carried out without a yield constraint. In other calculations we required recovery yields of 60 and 90%. The first value was chosen because under some sets of experimental conditions a 90% yield was very difficult to achieve with 20-μm particles. The value of 90% was chosen as it is the practical maximum which can be achieved in displacement chromatography.

Finally, the calculations were carried out by optimizing the loading factor at constant mobile phase velocity, then repeating the process at increasingly large values of this velocity in overloaded elution. In displacement chromatography, the loading factor and the displacer concentration were optimized at constant mobile phase velocity and the process was repeated for increasing values of the mobile phase velocity. The highest value of the velocity corresponds to the maximum inlet pressure, assumed to be 125 atm (*i.e.*, 1750 p.s.i.); it is 2 cm/s, with v = 400. For the 10-μm particle column, the maximum velocity is 0.5 cm/s, with v = 50. A solubility limit of 300 mg/ml was imposed on the displacer concentration.

RESULTS AND DISCUSSION

As the optimum conditions for maximum possible production rate that we have derived may seem unexpected to those used to carrying out displacement chromatography under conventional conditions, we give first a schematic presentation of guesses which would lead to the optimum in the case of a 1:3 binary mixture with α = 1.70, having a reduced velocity of 100. Then we discuss the results of the simplex optimization for the two chromatographic modes and compare their performances.

Optimization path in displacement chromatography
The optimization process starts with experimental conditions corresponding to formation of a nearly isotachic train at the column exit (see Fig. 1). On the chromatograms, the two vertical dot-dashed lines indicate the positions of the cutting
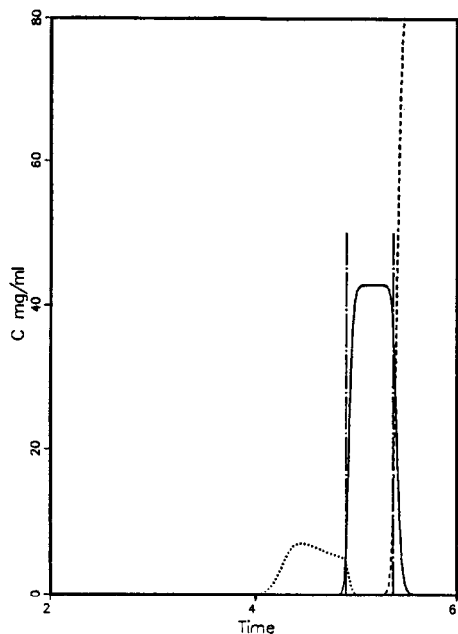
Fig. 1. Calculated displacement chromatogram used as the starting point of a simplex optimization. Column length, 25 cm; particle size, 20 μm; column porosity, 0.8; mobile phase viscosity, 1 cP; diffusion coefficient of solutes, $1 \cdot 10^{-5}$ cm/s; mobile phase reduced velocity, 100; equilibrium isotherms, see text; $\alpha = 1.70$; $k'_2 = 9.78$; mixture composition, 1:3; loading factor for the second component, 5%; displacer concentration, 85 mg/ml. Solid line, profile of the second component band; dashed line, profile of the first component; dotted line, profile of the displacer; vertical dot-dashed lines, cutting points for a product containing 1% displacer and 1% first component. Recovery yield: 93.1%. Production rate: 1.6 mg/cm$^2 \cdot$ s. Time in min.

times for production of the second component at the required degree of purity. The dotted line corresponds to the profile of the first component, the solid line to the profile of the second and the dashed line to the displacer front.

The most natural action to take in order to increase the production rate is to increase the sample size. Fig. 2 shows the displacement chromatogram recorded with a sample size four times as large as for Fig. 1. The isotachic train is far from being formed, but the recovery yield has hardly decreased (by only 1%) so the production rate has quadrupled. We may try now to increase the displacer concentration to achieve the formation of an isotachic train. This would decrease the elution time of the displacer front (i.e., the cycle time) and thus increase production rate. Fig. 3 shows the chromatogram obtained with the same sample size as in Fig. 2 but with a displacer concentration twice as large. The cycle time decreases from 323 to 218 s while the yield increases by nearly 2% and the production rate by 40%.

A further increase in both the sample size and the displacer concentration results in a certain increase in the production rate, but at the cost of a lower recovery yield, 60% (Fig. 4). Since at this column length and with that sample size it is not possible to form an isotachic train, a very wide mixed zone remains between the pure bands of the two components. Finally, the simplex converges towards the optimum conditions of
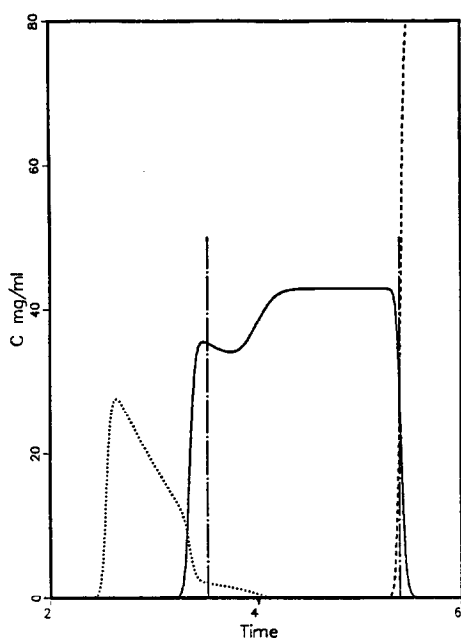
Fig. 2. Calculated displacement chromatogram obtained during a simplex optimization. Same conditions as for Fig. 1, except loading factor for the second component = 20%. Recovery yield: 92%. Production rate: 6.5 mg/cm^2 · s. Time in min.
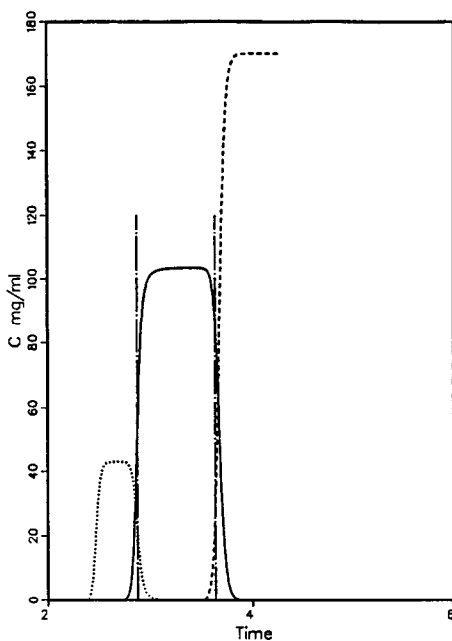
Fig. 3. Calculated displacement chromatogram obtained during a simplex optimization. Same conditions as for Fig. 2, except displacer concentration = 170 mg/ml. Recovery yield: 93.7%. Production rate: 9.1 mg/cm^2 · s. Time in min.

the displacement chromatogram shown in Fig. 5a. Compared with the initial conditions (Fig. 1), the displacer concentration has been multiplied by 3.5, resulting in a decrease in the displacer front elution time from 323 to 144 s and the sample size has been multiplied by 13.5. While the throughput has increased nearly 30-fold, the production rate has increased by nearly one order of magnitude and the recovery yield has decreased 3-fold.

As seen in Fig. 5a, the optimum conditions for maximum production rate are sensitive to the degree of product purity required. The tail of the first component band profile touches the front of the displacer, so there is no totally pure fraction of second component eluted from the column. A further increase in the displacer concentration fails to generate a chromatogram closer to an isotachic train or to increase the production rate (Fig. 6). The two component bands are compressed into an increasingly narrow retention space, their resolution deteriorates and the yield drops rapidly, as found by the simplex algorithm.

Comparison of performance in overloaded elution and displacement

The optimum conditions for maximum possible production rate in overloaded elution correspond to the chromatogram shown in Fig. 5b. Although the throughput is much smaller than in displacement chromatography, the production rate at this flow
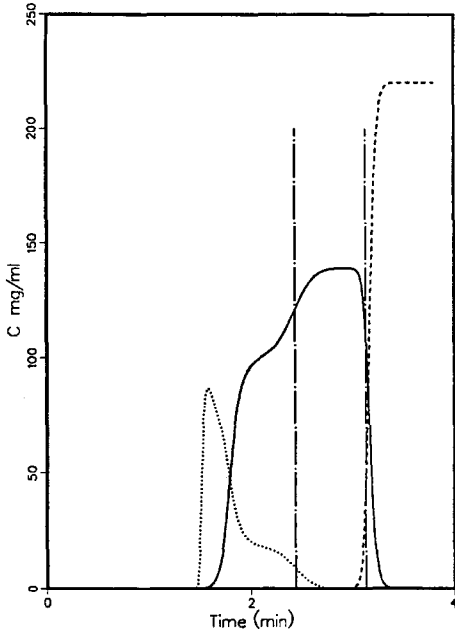
Fig. 4. Calculated displacement chromatogram obtained during a simplex optimization. Same conditions as for Fig. 3, except loading factor for the second component = 40% and displacer concentration = 220 mg/ml. Recovery yield: 60%. Production rate: 12.6 mg/cm^2 · s.
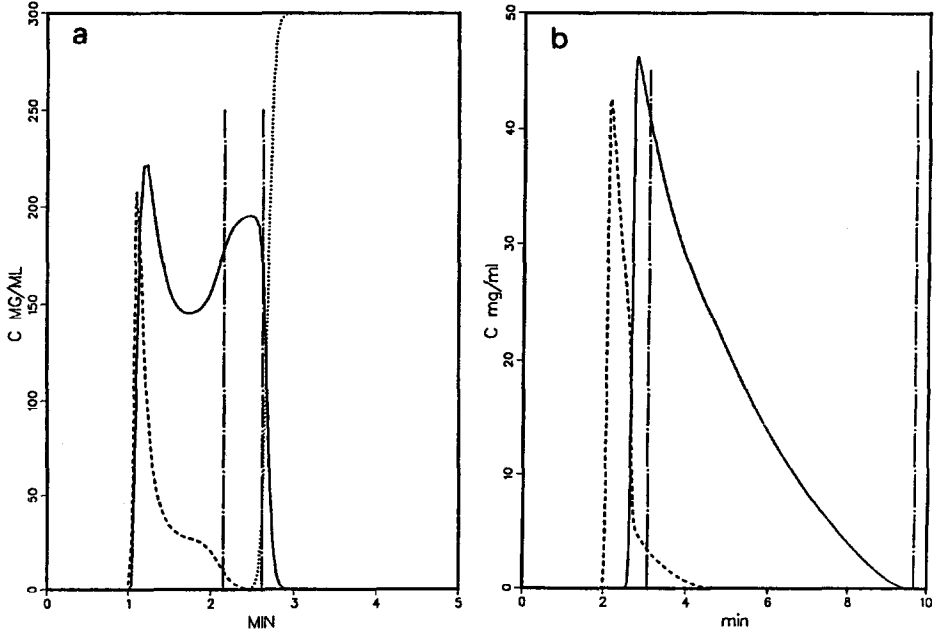

Fig. 5. Optimum chromatograms corresponding to maximum possible production rate in (a) displacement and (b) elution. (a) Calculated displacement chromatogram at the simplex optimum. Same conditions as for Fig. 4, except loading factor for the second component = 67.4% and displacer concentration = 300 mg/ml. Recovery yield: 34%. Production rate: 13.4 mg/cm^2 · s. Concentration of the collected fraction: 198 mg/ml. (b) Calculated overloaded elution chromatogram obtained at the simplex optimum. Same conditions as for Fig. 1, except no displacer and loading factor for the second component = 26.9%. Recovery yield: 84%. Production rate: 5.9 mg/cm^2 · s. Concentration of the collected fraction: 14 mg/ml.
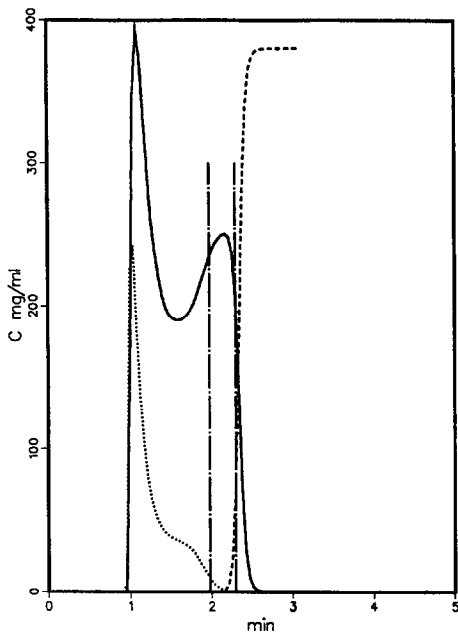
Fig. 6. Calculated displacement chromatogram obtained during a simplex optimization. Same conditions as for Fig. 5a, except loading factor for the second component = 80% and displacer concentration = 380 mg/ml. Recovery yield: 25%. Production rate: 12.0 mg/cm^2 · s. Concentration of the collected fraction: 264 mg/ml.

velocity ($v = 100$) is only 2.3 times smaller, because the recovery yield is much higher, 84%, compared with only 34% in displacement. On the other hand, the concentration of the collected fraction of the second component at the required degree of purity (amount of pure product divided by fraction volume) is much larger in displacement (198 mg/ml) than in overloaded elution (14 mg/ml).

Optimum conditions were determined at increasing values of the reduced velocity for the same mixture (composition 1:3, $\alpha = 1.70$). The results obtained are summarized in Fig. 7. Fig. 7a is a plot of the maximum production rate *versus* the mobile phase reduced velocity. The numbers adjacent to each symbol give the concentration of the collected fraction. Fig. 7b (bar graph) is a comparison of the recovery yields in the two modes for the different values of the reduced velocity.

In the range of velocities investigated, the production rate increases monotonically with increasing velocity. As the separation factor is large, the separation is easy and it is possible to trade efficiency (hence a decrease in yield) for an increase in the production rate. Under linear conditions, the column efficiency needed to separate the two components with a resolution of unity is only 115 theoretical plates ($k'_2 = 9.78$, $\alpha = 1.70$). For a reduced velocity of 400, the column efficiency is still 265 plates, thus permitting a resolution of 1.5 between the analytical peaks. As shown previously, the production rate in overloaded elution increases with increasing flow velocity until the limiting resolution drops to nearly unity [13,15]. This is because more is gained by reducing the cycle time (hence increasing the throughput) than is lost by reducing the
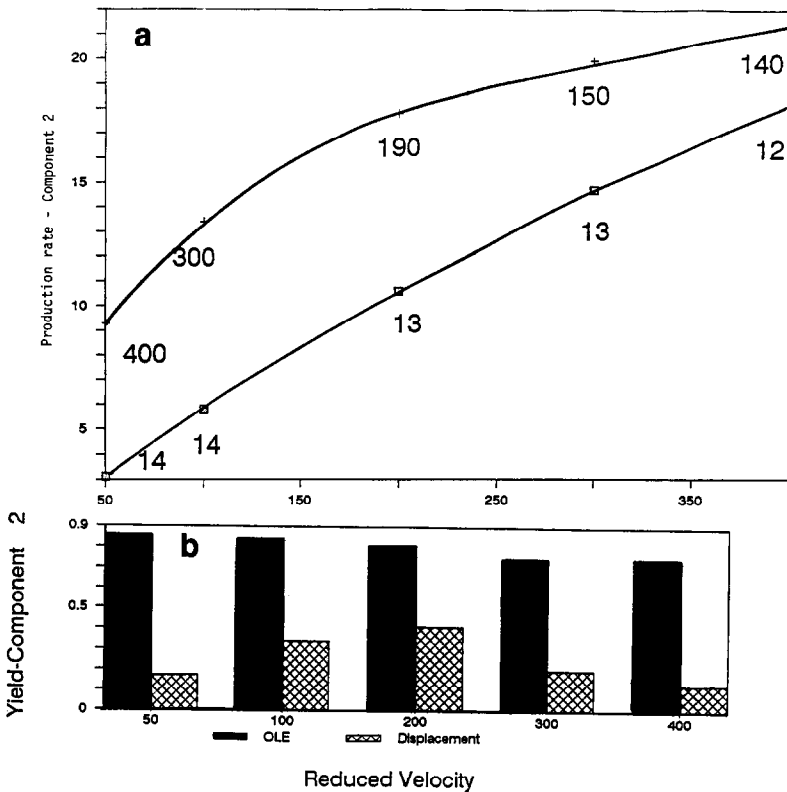
Fig. 7. Comparison of the performances of (+) displacement and (□) elution for maximum possible production rate in the separation of a certain mixture on a given column. Column and mixture as for Fig. 1. (a) Plot of maximum production rate of second component *versus* reduced velocity of the mobile phase. Figures by the symbols on the lines are concentrations of the collected fraction. (b) Recovery yield of second component.

column efficiency (hence reducing the yield), provided that a significant degree of resolution can be maintained. Using methods described elsewhere [13,16], we can calculate that the maximum possible production rate with a maximum pressure of 125 atm and a column packed with 20-μm particles would be achieved with a 15-cm column operated at a reduced velocity of 660, with a loading factor of 20%. However, the production rate would be only 20% higher than that achieved with the 25-cm column used here.

In this example, the production rate achieved by displacement always exceeds the production rate achieved by overloaded elution, but by a factor which decreases rapidly with increasing flow velocity. The ratio of the production rates is 3 for $v = 50$, 1.55 for $v = 200$ and 1.2 for $v = 400$. The recovery yield in overloaded elution is 86% at low velocities. It decreases slowly with increasing velocity, to a value of 75% for $v = 400$. In displacement, the yield has a maximum of 41% for $v = 200$. However, it is only about 17% at $v = 50$ and 12% at $v = 400$. These values are unacceptably low in

practice. Therefore, it is suggested that one should accept a lower production for a higher yield.

The significant advantage of the displacement mode is in the higher concentration of the collected fractions. In this example, the ratio of the concentration of the products obtained in the two modes decreases monotonically with increasing velocity, from 28 for $v = 50$ to 12 for $v = 400$.

Influence of a yield constraint

In view of the very poor recovery yield achieved in displacement chromatography under the optimum experimental conditions for maximum possible production rate for a given column, we performed the same calculations as described above but with the addition of a yield constraint. We now assume a required yield of 90% for the second component. At a reduced velocity $v = 100$, the chromatograms at the maximum production rates are shown in Fig. 8 for (a) the displacement and (b) the overloaded elution mode. As expected, as the recovery yield for maximum production rate in overloaded elution was 85% without a yield constraint, there is little difference between the elution chromatograms in Figs. 5b and 8b (the loading factors are 26.9% and 24.8%, respectively). In contrast, the displacement chromatograms in Figs. 5a and
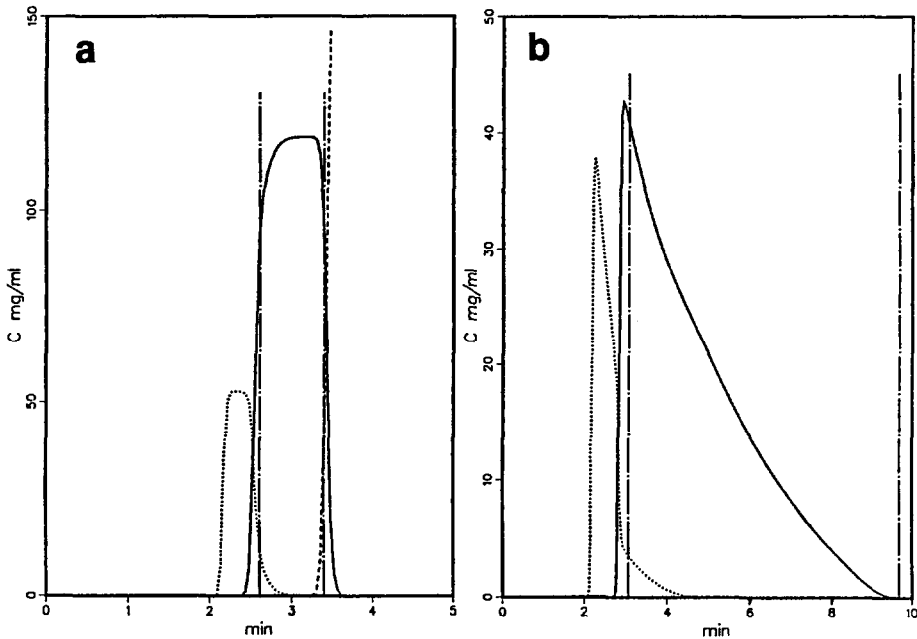

Fig. 8. Optimum chromatograms corresponding to maximum production rate in (a) displacement and (b) elution with a recovery yield of 90%. (a) Calculated displacement chromatogram obtained as the result of a simplex optimization. Same conditions as for Fig. 5a, except loading factor for the second component = 24.7% and displacer concentration = 191 mg/ml. Recovery yield: 90%. Production rate: 11.3 mg/cm$^2 \cdot$ s. Concentration of the collected fraction: 118 mg/ml. (b) Calculated overloaded elution chromatogram obtained as the result of a simplex optimization. Same conditions as for Fig. 1, except no displacer and loading factor for the second component = 24.8%. Recovery yield: 90%. Production rate: 5.9 mg/cm$^2 \cdot$ s. Concentration of the collected fraction: 14 mg/ml.

8a are very different. In Fig. 8a, an isotachic train is nearly formed. This is consistent with the previous observation (Figs. 1 and 3) that the recovery yield for an isotachic train is of the order of 92–94%. Accordingly, the sample size in Fig. 8a is much smaller than that in Fig. 5a and the displacer concentration is lower. The displacer front and the first component fronts are eluted later in Fig. 8a than in Fig. 5a. The concentrations of the collected fractions are the same in overloaded elution, with or without a yield constraint. In the displacement mode the concentration is much higher without a yield constraint than for a 90% yield.

The data in Fig. 9 permit a comparison between the performances of the two modes with a recovery yield of 90% at increasing velocity. Fig. 9a is a plot of the maximum production rate *versus* the reduced velocity for overloaded elution and displacement chromatography. The numbers on each curve give the optimum loading factor (elution mode) or the optimum loading factor and displacer concentration (displacement). The optimum loading factors are very close in the two modes. The bar graph (Fig. 9b) gives the concentration of the recovered fractions. This concentration is nearly constant at 15 mg/ml in overloaded elution. In displacement chromato-
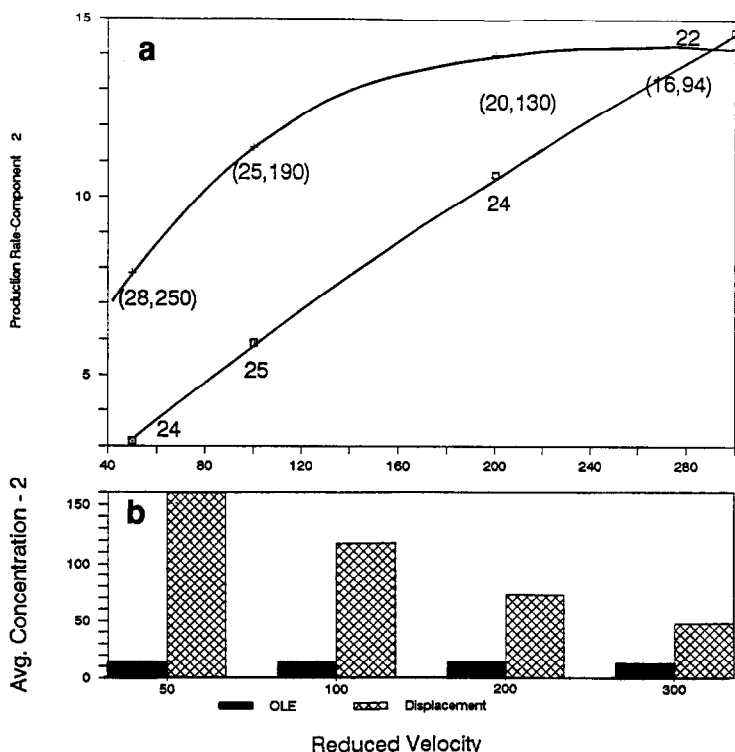

Fig. 9. Comparison of the performance of (+) displacement and (□) elution for the maximum production rate in the separation of a certain mixture ($\alpha = 1.70$) on a given column, with a recovery yield of 90%. Column and mixture as for Fig. 1. (a) Plot of production rate of second component *versus* reduced velocity of the mobile phase. Figures by the symbols on lines are loading factors and, in the case of displacement, displacer concentrations. (b) Concentration of the collected fraction (mg/ml).

graphy, the concentration of the collected fractions decreases with increasing velocity, from 160 mg/ml for $v = 50$ to 50 mg/ml for $v = 300$. Thus, the concentration enrichment provided by displacement, compared with overloaded elution, decreases from above 11 to about 3.5 in the useful range of velocities.

In overloaded elution, the production rate increases almost linearly with increasing velocity in the range investigated ($v = 50$–300). We have shown independently that for a 20-cm column having the same plate-height equation (eqn. 1), the optimum reduced velocity is 388, corresponding to an efficiency of 225 theoretical plates, assuming a 95% yield [16]. These results are in excellent agreement. In contrast, we see in Fig. 9 that the production rate in displacement chromatography reaches a plateau for reduced velocities between 200 and 300 (the maximum is very broad). The production rate in the elution mode exceeds that in the displacement mode for reduced velocities above 290.

Influence of relative retention

We performed a similar study for a binary mixture of the same composition (1:3) but with a relative retention of 1.2. The same column was used. Only the isotherm for the second component was changed. The results of the calculations are summarized in Fig. 10. Fig. 10a is a plot of the maximum possible production rate in both modes *versus* the reduced velocity. The figures on the curves are the concentrations of the
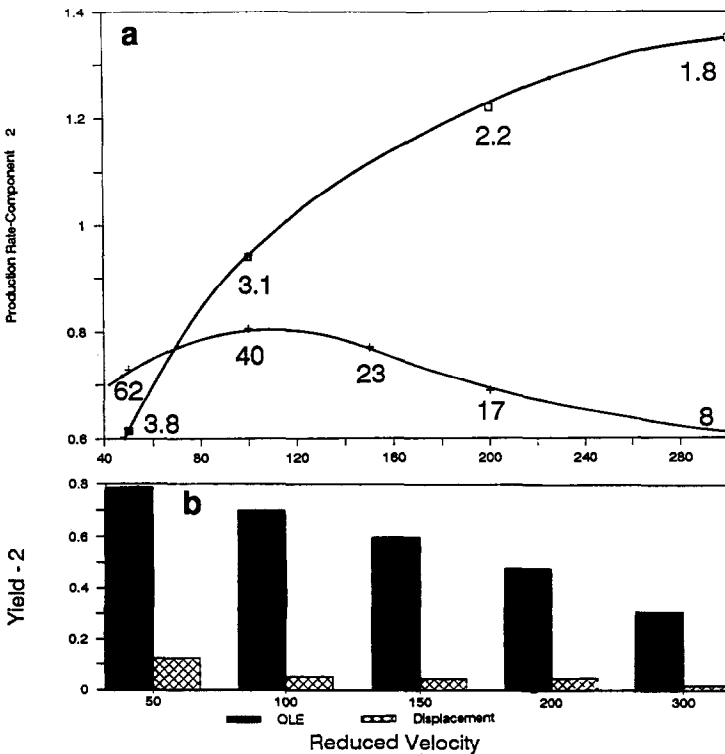

Fig. 10. Same as Fig. 7, but for a mixture with $\alpha = 1.20$ and $k'_2 = 6.90$.

collected fractions. Fig. 10b gives the recovery yields achieved in the two modes.

In this instance, the production rate in displacement is almost always smaller than that in elution. Only at very low velocities ($v < 70$) does displacement give a larger production rate than elution. The production rate in displacement has a maximum for $v \approx 100$ and decreases slowly at higher velocities. The production rate in elution increases until $v = 300$, showing a trend towards a broad maximum which should take place in the range between 350 and 500. For a reduced velocity of 300, the column efficiency is 342 theoretical plates. The resolution of the two components ($\alpha = 1.20$, $k'_2 = 6.90$) under analytical conditions would be only 0.67. The concentrations of the recovered fractions are nearly ten times lower with the new feed ($\alpha = 1.20$) than with the previous one ($\alpha = 1.70$). The recovery yields in the displacement mode are uniformly very poor and, in practice, unacceptable. The recovery yields in elution are good at low velocities (above 70% for reduced velocities below 100) but decrease with increasing velocity, to 40% at $v = 300$.

In practice, higher values of the recovery yield are required. Fig. 11 shows the chromatograms calculated under the optimum conditions found by the simplex program for (a) the displacement mode and (b) the elution mode with a required yield
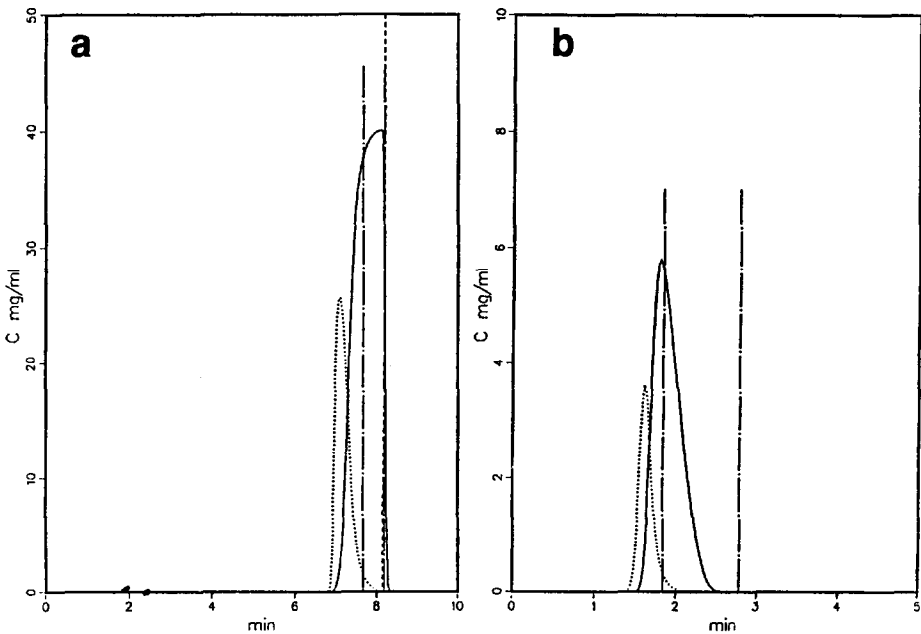

Fig. 11. Optimum chromatograms corresponding to maximum production rate in (a) displacement and (b) elution with a recovery yield of 60%. Same as Fig. 8, except $\alpha = 1.20$ and recovery yield = 60%. (a) Calculated displacement chromatogram obtained as the result of a simplex optimization. Same conditions as for Fig. 8a, except reduced velocity = 50, loading factor for the second component = 6.0% and displacer concentration = 136 mg/ml. Recovery yield: 60%. Production rate: 0.62 mg/cm^2 · s. Concentration of the collected fraction: 41 mg/ml. (b) Calculated overloaded elution chromatogram obtained as the result of a simplex optimization. Same conditions as for Fig. 1, except no displacer, reduced velocity = 300 and loading factor for the second component = 2.4%. Recovery yield: 62%. Production rate: 1.2 mg/cm^2 · s. Concentration of the collected fraction: 1.5 mg/ml.

of 60%. The optimum loading factors are much lower than in the previous case (Fig. 8). The overloaded chromatogram is as expected. The displacement chromatogram is nearly isotachic. The displacement chromatogram presented (Fig. 11a) was calculated for the optimum velocity giving the maximum production rate with a 60% yield ($v = 50$), while the elution chromatogram presented (Fig. 11b) was calculated at a velocity above the optimum velocity ($v = 240$). The optimum loading factor in elution is 3.2%.

Fig. 12 shows the performance data with both modes in the same format as before. Compared with the data in Fig. 10 (no yield constraint), the production rates and the optimum velocities are lower. Elution gives a production rate higher than displacement for reduced velocities above 50 and, eventually, permits a maximum production rate twice as large as displacement, but with an optimum reduced velocity nearly five times as large. The collected fractions are much more concentrated in displacement chromatography than in elution. Under the optimum conditions for each mode, the fractions obtained by displacement ($v = 50$) are nearly 100 times more concentrated than those in elution ($v = 240$).

Influence of feed composition

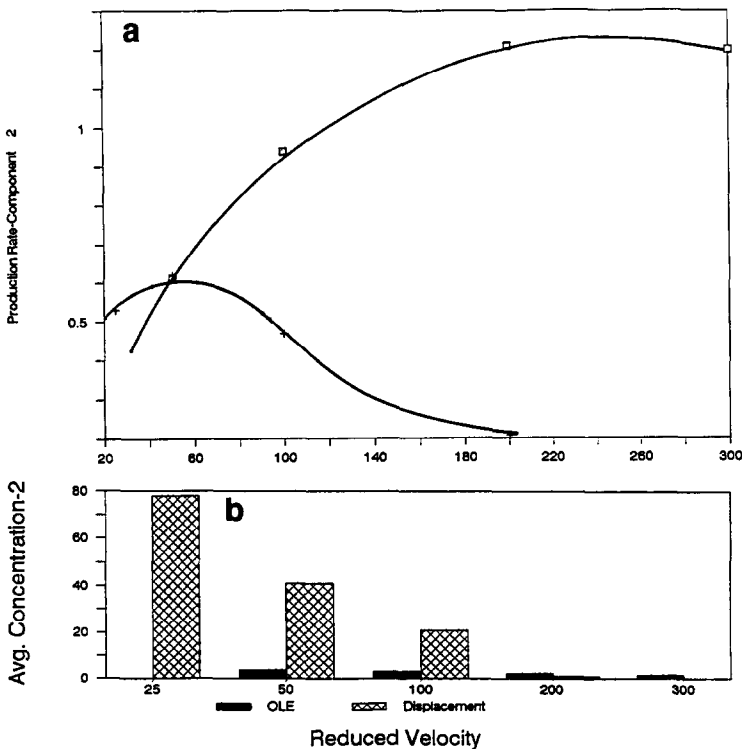Finally, in order to study the effect of the feed composition on the performance

Fig. 12. Comparison of the performance of (+) displacement and ($\square$) elution for the maximum production rate in the separation of a certain mixture ($\alpha = 1.20$) on a given column, with a recovery yield of 60%. Column and mixture as for Fig. 11. (a) Plot of production rate of second component *versus* reduced velocity of the mobile phase. (b) Concentration of the collected fraction (mg/ml).

achieved in the two modes of chromatography, using the same column, we studied a 3:1 binary mixture. In this instance there is no displacement effect in overloaded elution, but a significant tag-along effect [9]. In the displacement mode, the relative importance of the boundary layers, between the first and the second components or between the second component and the displacer, is larger. As a result, we found that it was difficult to obtain reasonable yields for this injection composition, with a relative retention of 1.20 and a 20-μm particle column. Therefore, we assumed a particle diameter of 10 μm for these calculations. The chromatograms corresponding to the optimum conditions (displacement) and to a reduced velocity of 50 (elution) are shown in Fig. 13. The reduced velocity of 50 corresponds to the maximum inlet pressure of 1700 p.s.i.

The production rate in the displacement mode is maximum for a mobile phase reduced velocity between 35 and 50. The production rate in the elution mode increases with increasing reduced velocity over the whole range studied and passes the production rate in displacement for a reduced velocity slightly below 50. The concentrations of the collected fractions again are much higher in displacement than in elution by more than one order of magnitude.

Fig. 14 compares the performance of both modes. The results are similar to those in Fig. 12 and the same conclusions can be drawn.
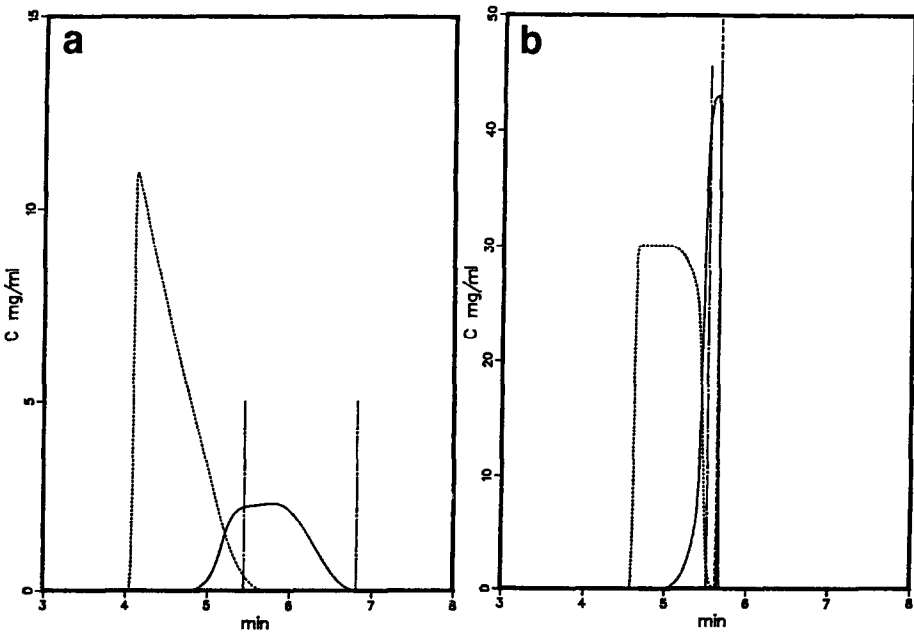

Fig. 13. Same as Fig. 11, except composition of the mixture = 3:1. (b) Calculated displacement chromatogram obtained as the result of a simplex optimization. Same conditions as for Fig. 8a, except mixture composition = 3:1, particle size = 10 μm, reduced velocity = 40, loading factor for the second component = 1.8% and displacer concentration = 105 mg/ml. Recovery yield: 60%. Production rate: 0.36 mg/cm^2 · s. Concentration of the collected fraction: 51 mg/ml. (a) Calculated overloaded elution chromatogram obtained as the result of a simplex optimization. Same conditions as for Fig. 1, except mixture composition = 3:1, no displacer, particle size = 10 μm, reduced velocity = 50 and loading factor for the second component = 1%. Recovery yield: 62%. Production rate: 0.45 mg/cm^2 · s. Concentration of the collected fraction: 1.5 mg/ml.
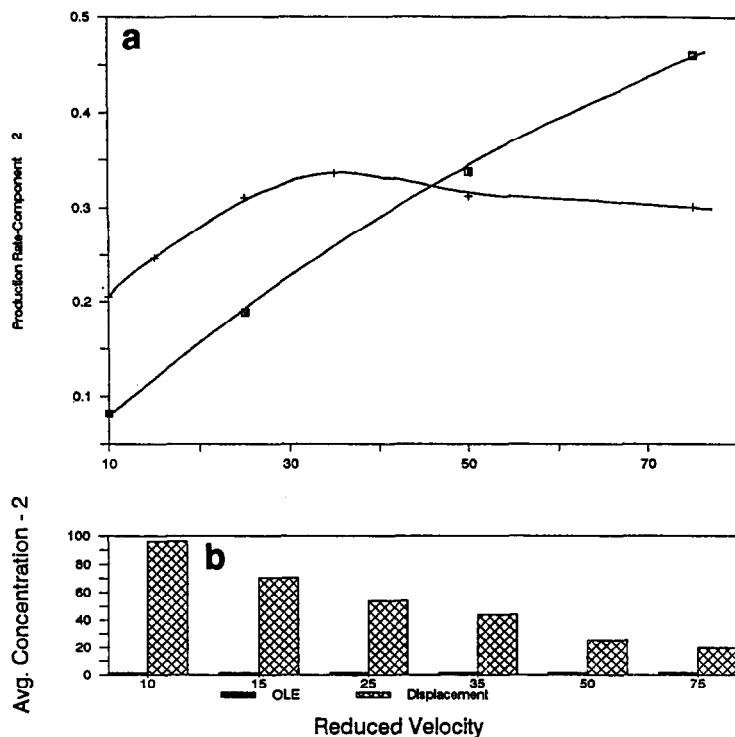
Fig. 14. Comparison of the performance of (+) displacement and (□) elution for the maximum production rate in the separation of a certain mixture ($\alpha = 1.20$) on a given column, with a recovery yield of 60%. Column and mixture as for Fig. 13. (a) Plot of production rate of second component *versus* reduced velocity of the mobile phase. (b) Concentration of the collected fraction (mg/ml).

CONCLUSION

Many of the conclusions of this work rest on an implicit assumption made in this study. It has been assumed that the only limitation to the use of high mobile phase velocities in displacement chromatography results from the flow velocity dependence of the column efficiency and the relationship between this efficiency and the width of the mixed layers between bands in displacement chromatography. There are surprisingly few results in the literature on this problem. The results by Cramer and Subramanian [24] support this assumption, whereas two isolated results by Frenz *et al.* [2] and Cardinali *et al.* [25] are less clear.

Another basic assumption made in this work is that the optimum column lengths for maximum production rates in displacement and elution are not very different. Based on the results of other work, the column chosen for this work is slightly too long for the 1:3 mixture (optimum length 15 cm) and slightly too short for the 3:1 mixture (optimum length 33 cm) [13,16]. With a column of optimized length, the production rate would be higher than that calculated here. However, we did not attempt to optimize the column length in this work.

Finally, we assumed that the parameters of the chromatographic system used to perform the separations are the same in displacement and in overloaded elution chromatography. The coefficients of the adsorption isotherms used in the two cases are the same. The conventional wisdom seems to be that displacement should be carried out under conditions where the feed components are much more strongly retained than ordinarily would be the case for elution chromatography. However, it does not seem that the column saturation capacity changes much with the mobile phase composition, except in those rare cases where the conformation of the adsorbed molecule is substantially changed. Then, the amount of feed which can be loaded in the column before each run remains approximately independent of the retention of the feed components in the weak solvent which fills the column before the displacement is started. Thus, in most instances, the production rate in displacement chromatography depends only weakly on the retention. For both modes of chromatography, the production rate increases with increasing column saturation capacity, but we have little way of controlling this capacity.

Our results demonstrate clearly that the optimum conditions for maximum production rate in the displacement mode (with or without a yield constraint) correspond to a non-isotachic displacement train. This was to be expected, as once an isotachic train is formed it propagates without any further improvement of the separation. A significant decrease in the cycle time is obtained by accepting experimental conditions for which the isotachic train is not fully developed.

Our results lead to two important conclusions which are unexpected in view of what has been written in the past regarding the different modes of preparative chromatography. The first conclusion is that elution tends to give much better recovery yields than displacement. This stems directly from the need to consider non-isotachic trains for maximum possible production rates. The second is that the production rate in displacement chromatography is never much larger than in elution even when the maximum production rate without a yield constraint is considered. If a yield constraint is imposed, the production rates achieved are close or elution permits a higher production rate. Only a certain range of experimental conditions have been investigated but they are typical of most current applications. The ratio of the maximum production rate in displacement and elution are significantly larger than unity only at high relative retentions, when no yield constraint is imposed. Even in this case, the ratio rarely exceeds two, making it improbable that better production rates can ever be achieved in practice with displacement than with elution, as time has to be allowed for column regeneration.

Finally, the only major advantage of displacement over elution is in the much higher concentration of the fractions which are produced. The gain is one order of magnitude, which may be highly significant in the economy of some processes as it could permit a similar reduction in the volume of solvent to be evaporated in order to isolate the pure product. In many instances, however, difficulties will be experienced in displacement chromatography because of solubility problems. The displacer concentrations contemplated in this work are very high. Choosing a proper displacer is not easy in an academic study. In an industrial environment, the product cost and the solubility problem further complicate the choice. If the product has a limited solubility in the mobile phase, it is not possible to exceed this value in the fraction, which adds another constraint to the development of the method and reduces the extent of the advantage of displacement over elution.

It is not possible to close a discussion involving the practical application of displacement in preparative chromatography without addressing column regeneration. Our results show that this operation cannot be accelerated much by using high flow velocities, as has been suggested, as the optimum velocities are a large fraction of the maximum velocity permitted by a realistic pressure constraint. In reversed-phase chromatography a strong solvent is not necessarily a strongly retained compound; it merely has to be a good solvent of the sample component and the displacer. Flushing the column with pure acetonitrile or methanol followed by re-equilibration with the mobile phase can permit rapid column regeneration. In reversed-phase chromatography, however, good displacers tend to be high-molecular-weight compounds, which are much more expensive than regular solvents. In normal-phase chromatography, the displacer must be more polar than the most polar sample component, so small polar molecules can be used. Then, regeneration would require a volume of mobile phase equal to the retention volume of the displacer under linear conditions with the carrier as mobile phase. This could require a large volume of solvent and extensive purification of the fraction for recycling of the displacer.

As a conclusion, it seems to us at this stage that the choice between the displacement and elution modes for preparative chromatographic applications depends much on a comparison of the economics of downstream processing of the dilute fractions containing the purified component and of displacer recovery, which includes column regeneration and treatment of the eluate collected during this stage of the process. On this topic there is little help that academic theorists can offer, except to suggest that the choice of the chromatographic mode depends much on the specifics of each case.

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