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## EFFECT OF MOBILE PHASE FLOW-RATE ON THE RECOVERIES AND PRODUCTION RATES IN OVERLOADED ELUTION CHROMATOGRAPHY

### A THEORETICAL STUDY

SAMIR GHODBANE and GEORGES GUIOCHON\*

\**Department of Chemistry, University of Tennessee, 575 Buehler Hall, Knoxville, TN 37996-1600 (U.S.A.) and Oak Ridge National Laboratory, Analytical Chemistry Division, Oak Ridge, TN 37831-6120 (U.S.A.)*

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#### SUMMARY

The effect of mobile phase flow-rate on the recoveries and production rates in overloaded elution liquid chromatography was investigated from a theoretical standpoint. Numerical solutions of the semi-ideal model of non-linear chromatography were calculated for simulated columns, using a classical form of the HETP equation. Production rates and recoveries were derived for several mixtures with different relative retentions. It is generally observed that the recoveries decrease with increasing mobile phase flow-rate but that the production rates pass through a maximum which is reached at very high velocities. In the cases studied here, the optimum flow-rate corresponds to the efficiency for which the analytical resolution between the two compounds separated is around unity or even slightly lower.

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#### INTRODUCTION

The explosive growth of the biotechnological and pharmaceutical industries in the last 10 years has generated tremendous needs for large amounts of highly pure compounds. Thus, preparative chromatography has arisen as a powerful separation and purification tool. In these applications, the ultimate goal is to recover the largest possible amount of a given compound at the highest possible degree of purity while minimizing the investment and operational costs. So far, most of the applications have been developed by analytical chemists, organic chemists, biochemists or chemical engineers. Each group has brought along its expertise but also its biases. It turns out that most preparative chromatographic units are still operated far below their optimum capacities, because the phenomena underlying the separation process are poorly understood. Consequently, it is still impossible to predict the optimum experimental conditions for a preparative separation<sup>1</sup>.

An overall solution to this optimization problem will be obtained by establishing a theoretical framework taking all of the effects of finite concentration into account. Such a model would correctly predict the elution profiles of the components of a mixture and would relate the experimental parameters of a chromatographic separation to these band profiles. It would then permit the calculation of the

performance of a given column. This would be an essential tool in the determination of the optimum experimental conditions necessary to achieve maximum production<sup>1</sup>.

The fundamental theory of non-linear chromatography can serve as a basis for the development of this approach<sup>1-3</sup>. This theory is based on a study of the solutions of the system of partial differential equations obtained by writing a mass balance and a kinetic equation for each component of the mixture of interest. It may be assumed that the mobile phase is not adsorbed on the stationary phase<sup>4</sup>. In most modes of chromatography, the kinetics of mass transfer between phases are extremely fast and, as a first approximation, it has been assumed that instantaneous equilibrium is achieved between the mobile and stationary phases. This last assumption implies that: the kinetic equations are replaced by the equilibrium isotherms and the molecular diffusion coefficients are set equal to zero.

The remaining system of partial differential equations is the ideal model of chromatography. It has been widely studied in the past and its shortcomings are well known<sup>5,6</sup>. It cannot be solved analytically.

Recently, a numerical solution based on a finite difference method was described and used to solve the system of partial differential equations discussed above in a variety of boundary conditions<sup>2,3,7</sup>. In this method, the continuous  $(z, t)$  plane is replaced by a  $(z, t)$  grid which is characterized by a space,  $\delta z$ , and a time,  $\delta t$ , increment. The proper selection of the finite space increment  $\delta z$ , set equal to the height equivalent to a theoretical plate of the column (HETP),  $H$ , and of the time increment ( $\delta t = 2H/u$ ) allows for the introduction of a numerical diffusion term which simulates the column efficiency and has exactly the same role as the apparent diffusion coefficient, which accounts for the kinetic effects and axial diffusion<sup>8</sup>. The profiles obtained are very realistic and quantitatively compare very well with those generated experimentally for a single compound<sup>9</sup>.

In order to achieve the highest production possible, it is essential to optimize the column length ( $L$ ), the mobile phase flow-rate ( $u$ ), the diameter of the particles ( $d_p$ ) and the column efficiency. These parameters are interrelated. In this paper, we shall investigate only the effect of flow-rate on the recoveries and production, keeping the column length and particle size constant.

The mobile phase flow-rate is an important part of the separation process and has a very prominent effect on the column efficiency. In chromatography, packed beds are used. Because the packing structure is complex, the flow pattern is very complicated and it has never been possible to relate exactly the band spreading to the column characteristics and the flow-rate. It is known that the optimum mobile phase flow-rate is a function of the particle size, the column dimensions and the molecular diffusion of the solute in the mobile phase. The decrease in column efficiency resulting from the use of a high flow-rate of the mobile phase is often neglected in preparative liquid chromatography. As band profiles rapidly broaden and become less symmetrical when the sample size is increased, users tend to operate at high flow-rates in an attempt to increase production, on the basis that as the apparent column efficiency drops dramatically, it is not necessary to be concerned about this more moderate source of band broadening. As a result, however, the apparent diffusion coefficient increases, which leads to smooth band flanks and to increasing band interactions, *i.e.*, to increasing overlap of the bands. The resolution between bands and, consequently, the recoveries decrease. This leads us to the following questions: to what extent does

TABLE I  
PARAMETERS OF THE LANGMUIR ISOTHERMS

Component	<i>a</i>	<i>b</i>
1	23	2.38
2	25	2.56

the reduction in the injection cycle compensate for the decrease in yield?; and does the production per unit time (production rate) or per unit volume of mobile phase increase with increasing flow-rate? This paper aims at answering these questions.

#### DESCRIPTION OF THE PROBLEM

To illustrate our point, let us investigate the preparative separation of a binary mixture on a 25-cm long column. The relative retention of the two compounds (equal to 1.09) and their relative concentrations (1:3) are kept constant throughout. The average size of the porous particles ( $d_p$ ) making up the stationary phase is  $20 \mu\text{m}$ . The diffusion coefficient of both solutes,  $D_m$ , in the mobile phase is  $0.7 \cdot 10^{-5} \text{ cm}^2/\text{s}$ . The two solutes compete for the sites available on the surface. Their equilibrium adsorption isotherms are both of the Langmuir type and they slowly diverge with increasing solute concentration. They are of the general form

$$q_i = \frac{a_i c_i}{1 + b_1 c_1 + b_2 c_2} \quad (1)$$

where  $a_i$  and  $b_i$  are the Langmuir coefficients for compound  $i$  (see Table I).

Although there is serious doubt that such a simple relationship could be exact in any real situation, this is also, on a qualitative basis, the most general type of behavior observed in liquid chromatography. We have also assumed a typical relationship between the column plate height and the flow-rate. For the sake of simplicity, it is expressed using the reduced plate height ( $h$ ) and velocity ( $\tau$ ) introduced by Giddings<sup>10</sup>:

$$h = \frac{H}{d_p} \quad (2)$$

$$\tau = \frac{u d_p}{D_m} \quad (3)$$

The HETP equation we have assumed is

$$h = \frac{1.5}{\tau} + \tau^{0.33} + 0.03\tau \quad (4)$$

This equation is classical<sup>11</sup> and corresponds to a good column with a minimum reduced plate height of 2.024 and an optimum reduced velocity of 2.735<sup>12</sup>. A plot of  $h$  versus  $\tau$  is shown in Fig. 1.

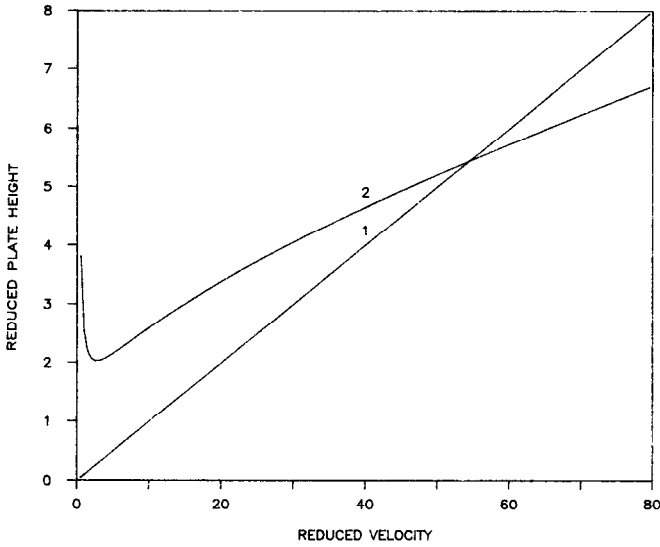


Fig. 1. Plots of column reduced plate height *versus* reduced flow velocity: (1) equation used by Knox and Pyper<sup>14</sup>; (2) eqn. 4.

For each set of simulations, we proceeded as follows. We start with a value of  $\tau$  between 1 and 300 (see Table II) and from eqn. 4 determine the reduced plate height. The plate height  $H$  is then calculated using eqn. 2. This, in turn, gives the time and space increments of the  $(z, t)$  grid, defined above, on which the numerical problem is solved. Eqn. 3 allows for the determination of the mobile phase flow-rate  $u$ . Consequently, the retention time for an unretained solute,

$$t_0 = \frac{L}{u} \quad (5)$$

and the retention time at infinite dilution ( $c_i \rightarrow 0$ ) for the two components of the mixture,

TABLE II  
PARAMETERS USED IN THE SIMULATION

$\tau$	$u$ (cm/s)	$h$	$N$	$R_s$
1	0.0035	2.53	4941	1.36
2.735	0.0096	2.024	6173	1.52
20	0.07	3.362	3718	1.18
40	0.14	4.615	2708	1.01
50	0.175	5.166	2420	0.95
100	0.35	7.58	1645	0.79
200	0.7	11.845	1055	0.63
300	1.05	15.68	797	0.55

$$t_{r_i} = \frac{L}{u} (1 + k'_i) \quad (6)$$

are determined.

As  $H$  and  $t_{r_i}$  are known, the apparent diffusion coefficient,  $D_a$ , for a given set of experimental conditions is derived through the Einstein equation:

$$D_a = \frac{HL}{2t_{r_i}} \quad (7)$$

where  $L$  is the column length and  $t_{r_i}$  the average retention time of the two components of the mixture of interest at infinite dilution.

At this point, all the parameters needed to undertake a series of simulation are known. Those used are listed in Table II.

## RESULTS AND DISCUSSION

The amount of solute eluted from a column is the integral of the concentration *versus* the volume of solvent flowing out from the column, and the peak area is the integral of the detector signal *versus* time. The ratio of the sample size to the peak area is thus equal to the flow-rate. This relationship, which holds for concentration-sensitive detectors, holds here also, as the natural time unit for our calculations is inversely proportional to the mobile phase flow-rate (see the above description of the time increment). This requires proper adjustment of the peak area introduced into the calculation, depending on the value of the flow-rate selected. To avoid any misunderstanding, the sample size is given here exclusively as a fraction of the column saturation capacity<sup>13</sup>, which is equal to  $a/b$  (see eqn. 1). To avoid any problem, this ratio is the same for both compounds.

### Recoveries

The column efficiency decreases with increasing flow-rate over most of the range investigated. We may therefore expect that the yield, at constant sample size and purity, will follow the same trend as the column efficiency. This effect can be clearly seen by comparing the profiles in Fig. 2a and b. As the injection period increases with decreasing flow-rate, the production rate, which is the amount of a given product (1 or 2) recovered at a certain degree of purity per unit time, will depend on a combination of two different, opposite effects, the yield, which decreases with increasing flow-rate, and the period, which increases. The aim of this work is to determine whether there is an optimum and where it is, in order to maximize the production rate.

As a general rule, the recovery decreases with increasing mobile phase flow-rate (see Figs. 3 and 4). The dependence of the recovery on the flow-rate, *i.e.*, on the efficiency, is stronger for the first component (see Figs. 3a and 4a) than for the second (see Figs. 3b and 4b). The recoveries tend to be higher for the second component at low or moderate column loadings but lower at high loadings. Between 80 and 15%, the recovery decreases linearly with increasing logarithm of sample size (fraction of column saturation capacity,  $\log X$ ) for the first component over most of the range

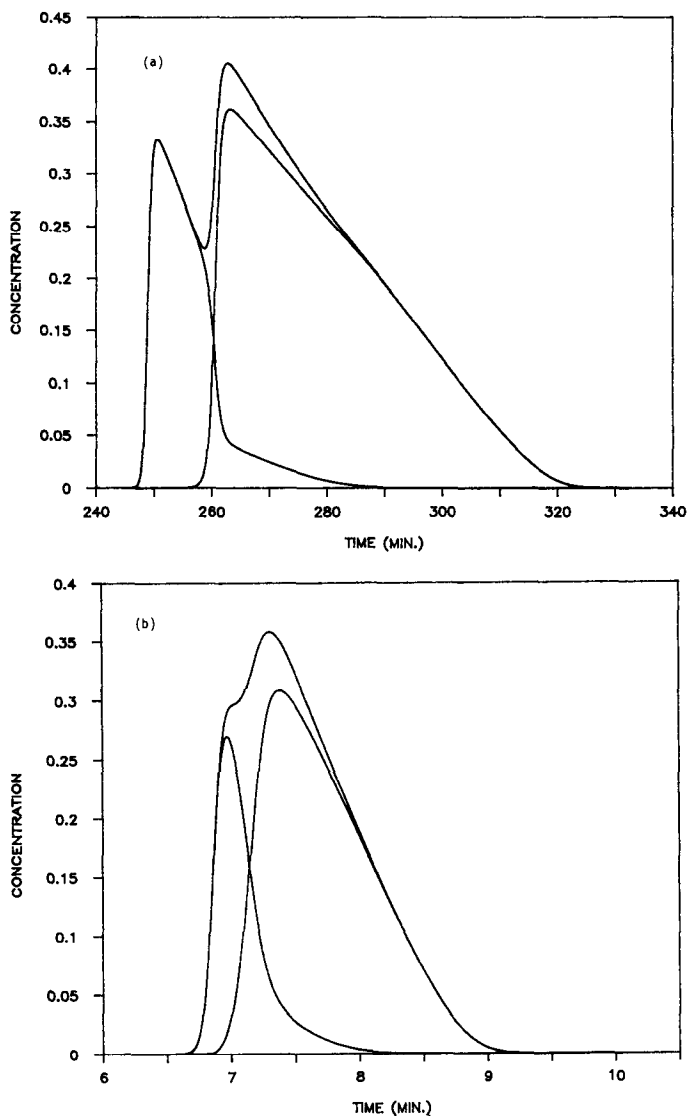


Fig. 2. Chromatograms of a sample amount corresponding to 2.5% of column saturation capacity for a 1:3 mixture ( $\alpha = 1.09$ ) and (a)  $\tau = 2.735$  ( $u = 0.0096$  cm/s) (b)  $\tau = 100$  ( $u = 0.35$  cm/s).

studied. For the second component, the plot of the recovery *versus* the logarithm of  $X$  has a strong S-shape (see Figs. 3b and 4b).

At  $\tau = 100$  ( $u = 0.35$  cm/s) and for a required purity of 98%, the recovery of the lesser retained component of the mixture is poor even at low sample sizes (the "analytical" resolution is only 0.79) and is only 25% for a 1.5% column saturation capacity sample [ $\log(X) = -1.8$ ]. At  $\tau = 2.735$  ( $u = 0.0096$  cm/s, *i.e.*, the efficiency optimum flow-rate, the recovery exceeds 80% for the same sample size, *i.e.*, it is more than three times larger (see Fig. 3a). The corresponding figures for the second component are 42% and 59%, respectively (see Fig. 3b).

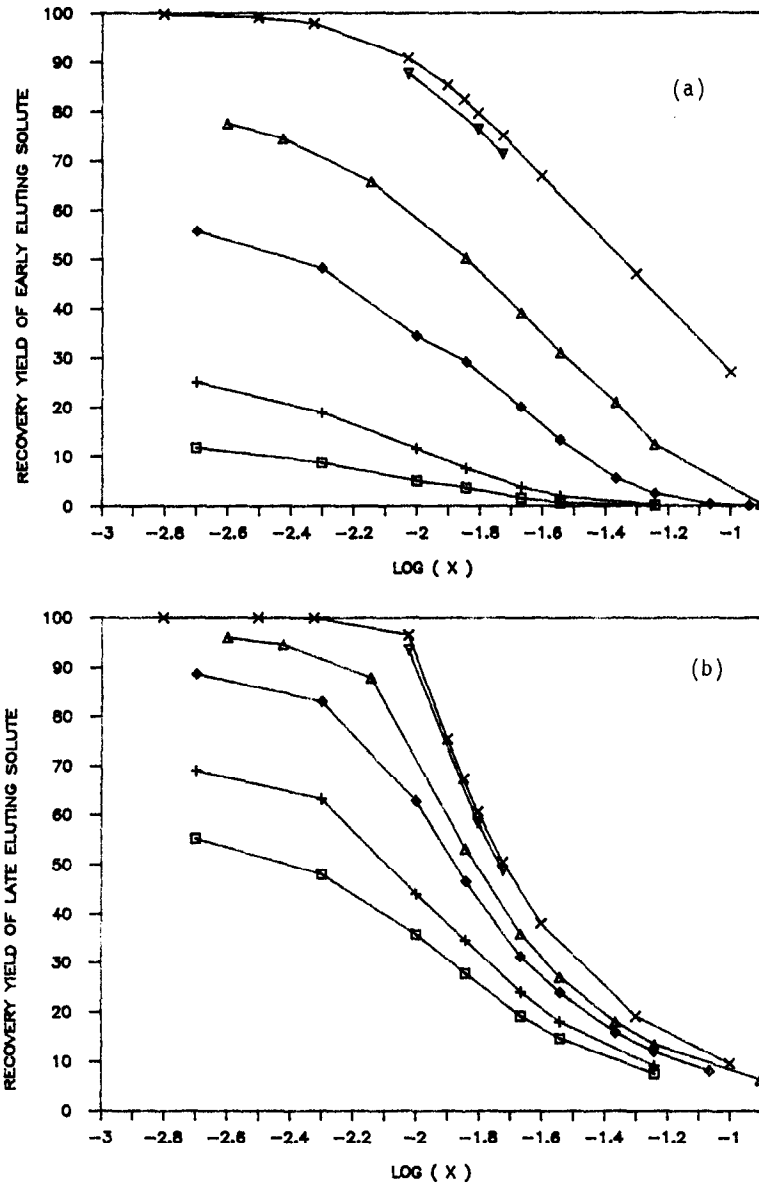


Fig. 3. Plots of the recovery (98% purity) versus logarithm of sample size (fraction of column saturation capacity) ( $\alpha = 1.09$ ) for (a) component 1 and (b) component 2.  $u$ :  $\square = 1.05$ ;  $+$  = 0.7;  $\diamond = 0.35$ ;  $\triangle = 0.175$ ;  $\times = 0.0096$ ;  $\nabla = 0.0035$  cm/s.

For a required purity of 95% and a 5% column saturation capacity sample [ $\log(X) = -1.3$ ], the yields are about 25 and 29% at  $\tau = 100$  and 2.735, respectively, for the second component (see Fig. 4b), and 15 and 52%, respectively, for the first component (see Fig. 4a).

As mentioned above, simulations were run using a value of the mobile phase

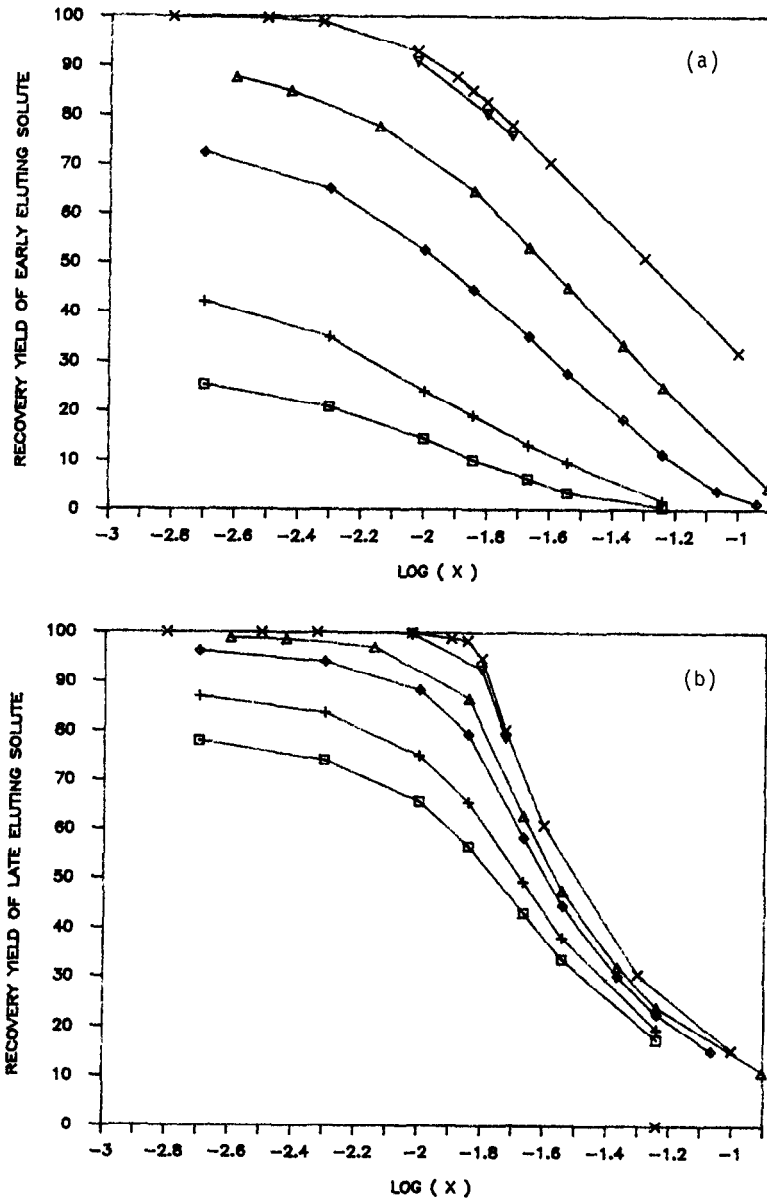


Fig. 4. As Fig. 3 for a purity of 95%.

flow-rate below the minimum of the Van Deemter curve ( $\tau = 1$ , see Table II). As expected, in all instances the recoveries were lower than those obtained for the optimum flow-rate ( $\tau = 2.735$ ) but higher than those obtained at  $\tau = 20$  ( $u = 0.07$  cm/s), confirming that the origin of the influence of the flow-rate on the recovery lies in its effect on the column efficiency (see Figs. 3 and 4).



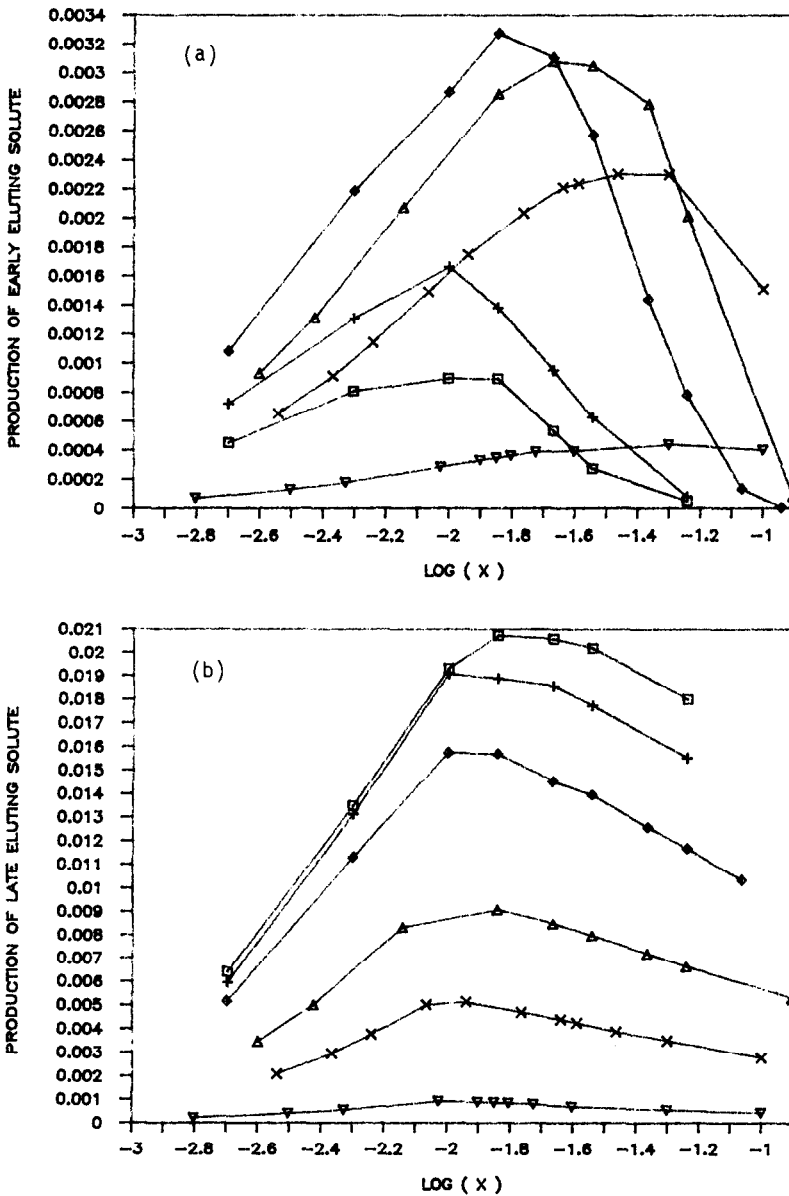


Fig. 5. Plots of the production rate (mol/cm<sup>3</sup>/min) (98% purity) versus logarithm of sample size (column saturation capacity) ( $\alpha = 1.09$ ) for (a) component 1 and (b) component 2.  $u$ :  $\square = 1.05$ ;  $+$  = 0.7;  $\diamond = 0.35$ ;  $\triangle = 0.175$ ;  $\times = 0.07$ ;  $\nabla = 0.0096$  cm/s.

### Production rates

As the production rate is the product of a factor that decreases with increasing flow-rate (the recovery) and on that increases (the injection frequency), all production rates pass through a maximum as expected from the above discussion (see Figs. 5 and 6). In general, these figures show that the production rates increase rapidly with

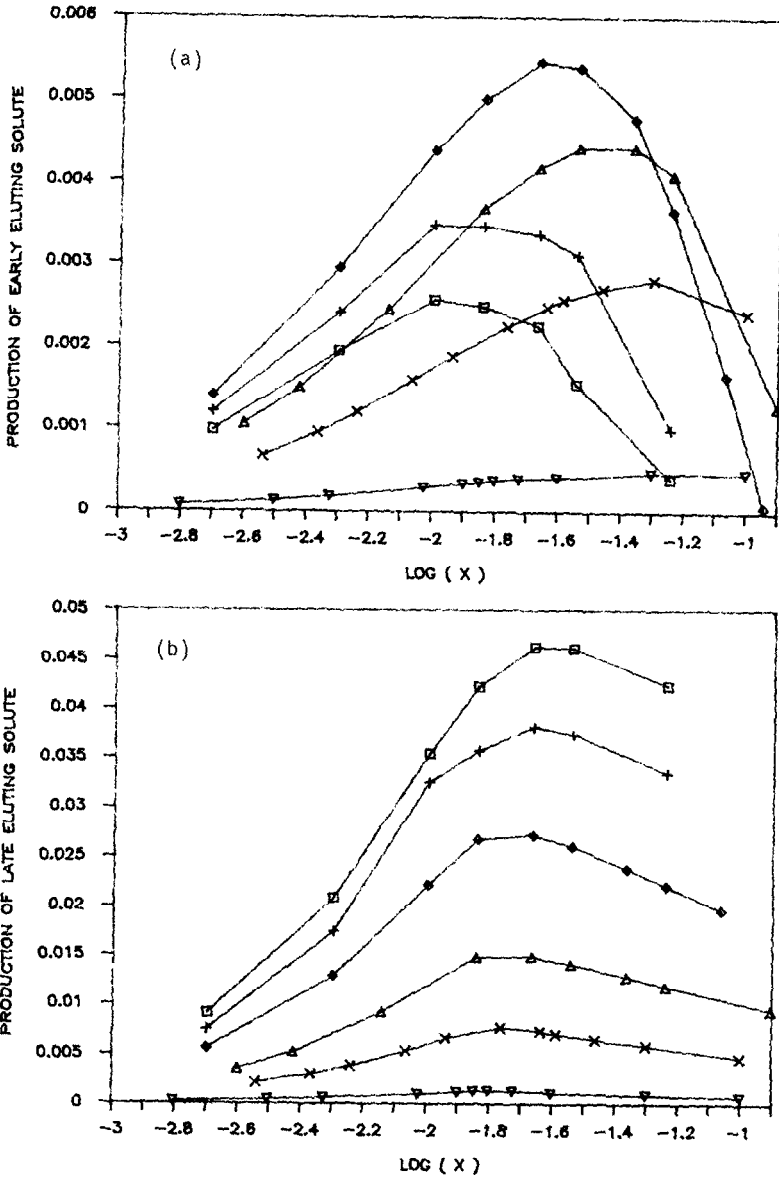


Fig. 6. As Fig. 5 for a purity of 95%.

increasing flow-rate at constant load per cycle [except for compound 1, at large loading factors and velocities (Figs. 5a and 6a) or at high purity]. The production rates are higher for component 2 than they are for component 1, which is normal as the concentration of component 2 is three times higher than that of component 1. The production rates under a given set of experimental conditions are not in this ratio of 1:3, however. Also, the production rates increase with decreasing purity requirements (compare Figs. 5a and 6a on the one hand and Figs. 5b and 6b on the other). When the

required purity decreases from 98 to 95%, the maximum production rate of compound 1 observed increases from 0.0033 to 0.0055 mol/cm<sup>3</sup>/min.

It can also be seen that for a given value of the required purity, the optimum sample size for maximum production increases with decreasing mobile phase flow-rate for the early eluting component of the mixture (Figs. 5a and 6a). This is no longer true for the more retained compound (Figs. 5b and 6b). In the latter instance, the optimum sample size appears to be independent of the mobile phase flow-rate (loading factor *ca.* 1.2%). It is remarkable that the maximum production rate increases with increasing flow-rate up to values of the reduced velocity in excess of 100. As the viscosity of a water-methanol mixture is of the order of 1 cP, and with a column permeability factor of about 1000 for a 25-cm long column packed with 20- $\mu$ m particles, the pressure drop for a reduced velocity of 100 would be of the order of 22 atm, which, although reasonable for preparative applications, could easily be exceeded if needed.

Figs. 5a and 6a are strikingly different from Figs. 5b and 6b. For both a 98% and 95% purity of the recovered first compound, there is an optimum flow-rate above which the production rate starts to decrease. For a purity of 98%, for example, the optimum production rate increases from  $u = 0.0096$  cm/s to 0.35 cm/s, where it is maximum and equal to 0.0033 mol/cm<sup>3</sup>/min. It then decreases for higher flow-rates. It is interesting that the maximum possible production rates, at both 98% and 95% purity, are reached for a flow-rate at which the "analytical" efficiency between the bands of the two compounds has dropped below 1 (see Table II), which is a small value. This is even more true for component 2, for which the production rate optimum velocity has yet to be reached at a reduced velocity of 300 ( $u = 1.05$  cm/s).

Clearly, there are two different optimum velocities, one for each component of the mixture. It is quite possible that the optimum flow velocity depends also on the relative concentration ratio of the mixture under consideration.

#### *Comparison with the prediction of a simpler model*

In this last section, we compare our predictions with those made by Knox and Pyper<sup>14</sup>. Their derivation suggests that the optimum throughput is determined exclusively by the minimum number of theoretical plates,  $N^*$ , required to provide "adequate" preparative resolution for the components of interest, *i.e.*, to achieve the separation when the tail of the early eluting component and the leading front of the late eluting one just start to touch. This plate number corresponds to the largest sample size for which the recovery is still complete. Under such optimum conditions, Knox and Pyper<sup>14</sup> have shown that the thermodynamic contribution to the band width has become identical with twice the kinetic contribution. Assuming triangular shape bands, the critical plate number is given by<sup>14</sup>

$$N^* = 16 \left( \frac{t_{r_2}}{t_{r_2} - t_{r_1}} \right)^2 = 16 \left( \frac{1 + k'_2}{k'_2 - k'_1} \right)^2$$

The optimization procedure recommended by Knox and Pyper<sup>14</sup> is thus as follows:

- (i) determine the critical number of plates,  $N^*$ , for the required separation using eqn. 8;
- (ii) compare it with the maximum column efficiency,  $N_{\max}$ ;
- (iii) if  $N_{\max} \leq 3N^*$ , operate the column under the optimum flow-rate and inject

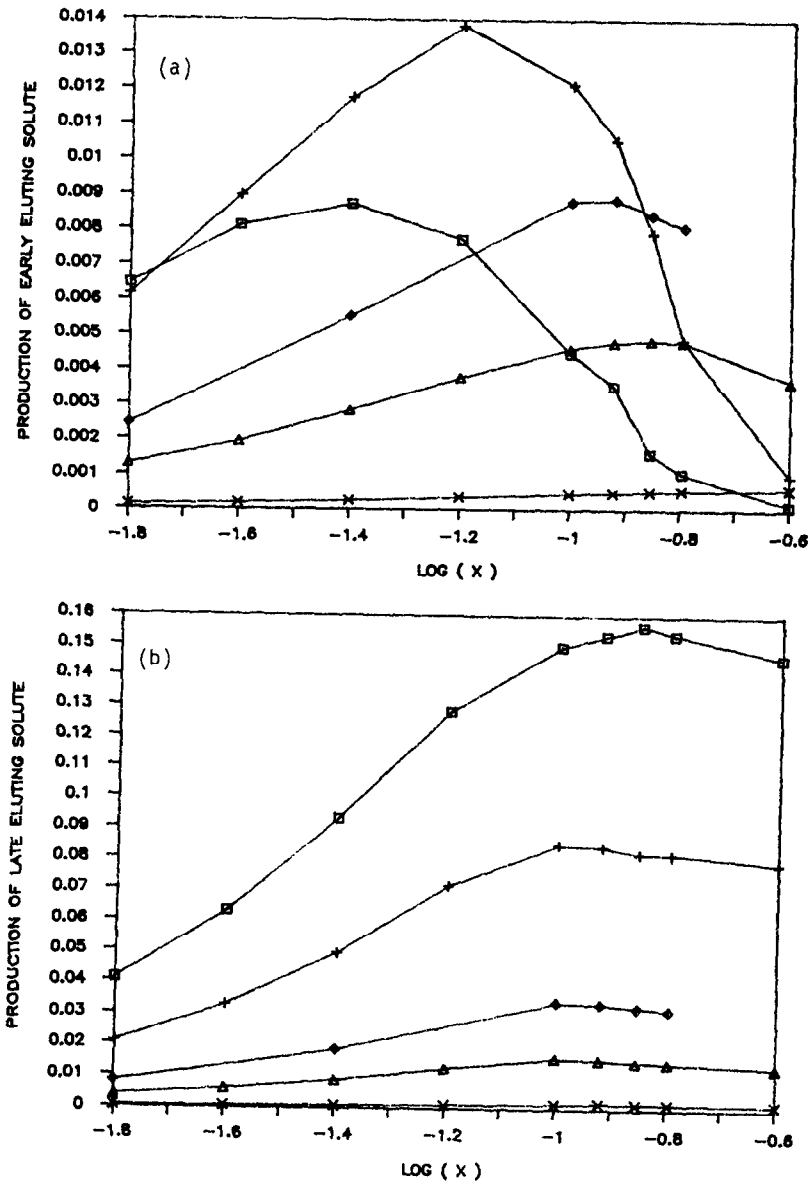


Fig. 7. Plots of the production rate versus logarithm of sample size ( $\alpha = 1.25$ ): (a) component 1 collected at 99% purity and (b) component 2 collected at 95% purity.  $N$ :  $\square = 275$ ;  $+$  = 538;  $\diamond = 1034$ ;  $\triangle = 1614$ ;  $\times = 3102$  plates.

the sample amount required to achieve a resolution of 1 as described in the previous paragraph;

(iv) if  $N_{\max} > 3N^*$ , operate the column at a high flow-rate at which the efficiency  $N$  ( $N < N_{\max}$ ) is just equal to  $3N^*$  and load the column as described under (iii). In most practical situations, this requires high or very high velocities, much larger than that

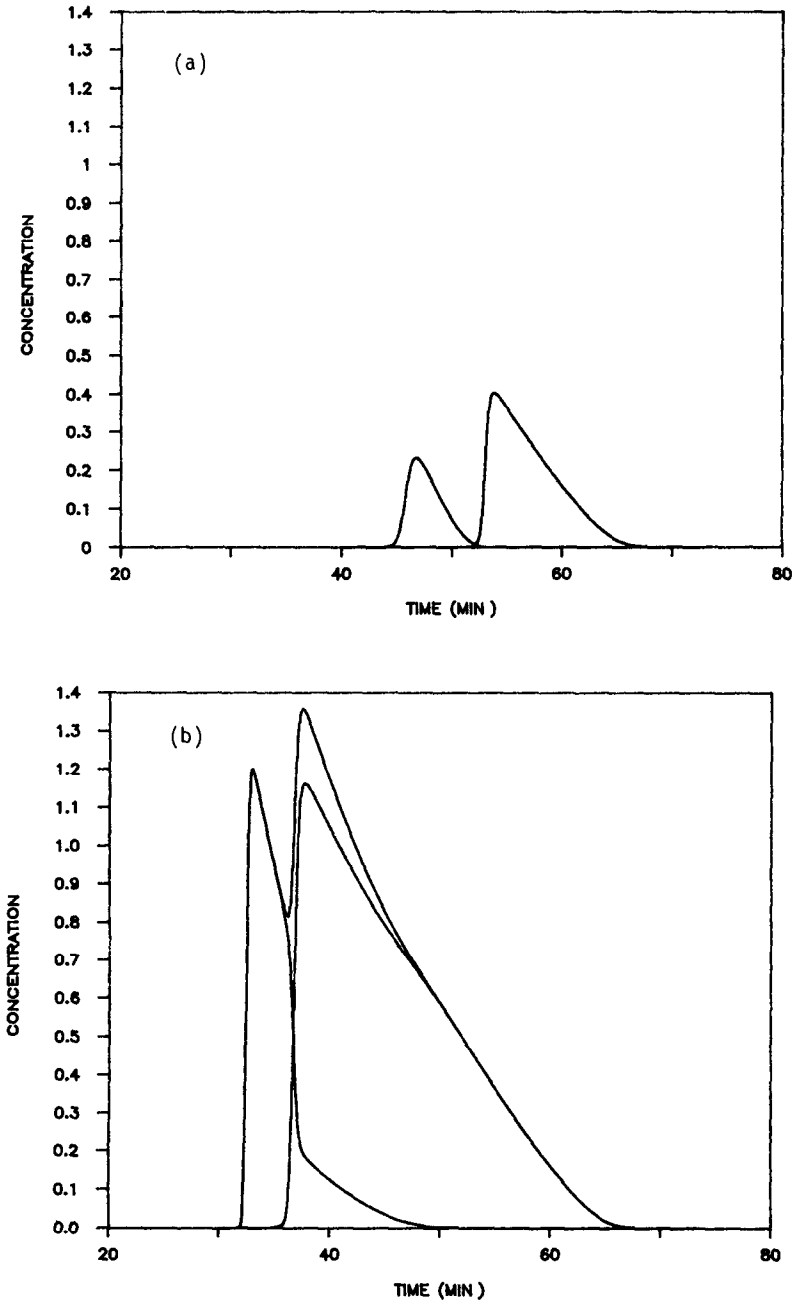


Fig. 8. Chromatograms of a 1:3 mixture ( $\alpha = 1.25$ ) on a 1614-plate column under optimum conditions according to (a) Knox and Pyper<sup>14</sup> and (b) our model.

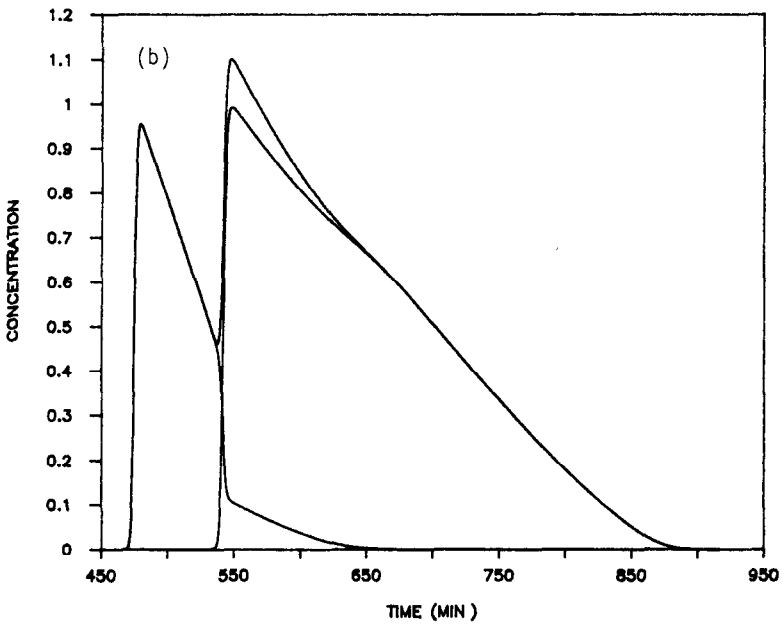
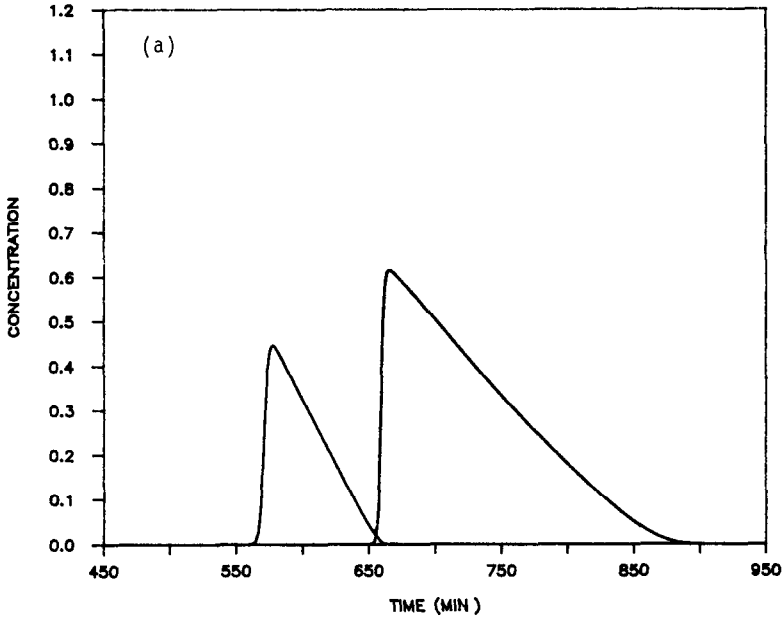


Fig. 9. As Fig. 8 for a 3102-plate column.

corresponding to the minimum of the Van Deemter curve. Hence the characteristics of the pumping system may limit the performance and force operation at sub-optimum conditions.

The results discussed in the previous two sections correspond to the separation of a pair of compounds ( $\alpha = 1.09$ ) which requires a large critical plate number,  $N^* = 3364$ . This is more than a third of the efficiency (6173 plates) of the column used for the previous simulation. Accordingly, we are under conditions which are sub-optimum according to Knox and Pyper<sup>14</sup>. Our result that maximum production is not achieved at the flow-rate for which the column efficiency is maximum is at variance with their conclusions. To check (iii) further and also (iv), we carried out other series of calculations for a much easier separation.

We now consider a 1:3 binary mixture with a relative retention of 1.25 and  $k'_2 = 6.25$  on a 25-cm long column packed with 40- $\mu\text{m}$  particles. The critical plate number,  $N^*$ , is now equal to 538 plates only ( $\tau = 195.5$ ). The corresponding number for the analytical separation is therefore 1614 plates ( $\tau = 27.5$ ). Hence, according to Knox and Pyper<sup>14</sup>, one should operate the column at a linear velocity corresponding to  $N = 3N^* = 1614$  to reach the maximum throughput. The reduced plate height equation remains the same (eqn. 4). As a result, the coordinates of the Van Deemter minimum are the same as those used above. Accordingly, at the linear velocity corresponding to the minimum HETP, the column efficiency is 3102 plates ( $\tau = 2.735$ ). The reduced velocity recommended by Knox and Pyper<sup>14</sup> would thus be 27.5 (Fig. 1).

A numerical study of the variation of the production rate of the two compounds as a function of the sample load shows that the production increases rapidly with increasing throughput and continues to do so even for throughputs larger than those suggested by Knox and Pyper<sup>14</sup> (see Fig. 7a and b), despite the appearance and growth of an interference between the two bands, *i.e.*, although the yield decreases. Further, the maximum production rate observed at a given flow-rate increases with increasing flow-rate, up to very large values of the flow-rate and, accordingly, to very low values of the efficiency. The column should be operated at flow-rates much higher than that corresponding to the minimum of the Van Deemter curve, sometimes even higher than that corresponding to an analytical resolution between the bands of units. As observed previously, the maximum production for component 2 keeps increasing with increasing flow-rate, whereas it passes through a maximum for component 1, at least for a 99% requested purity. This maximum seems to occur for an analytical resolution close to 1, *i.e.*, in this instance between  $N = 538$  ( $R_s = 1.25$ ) and  $N = 275$  plates ( $R_s = 0.89$  and  $\tau = 497.3$ ).

Finally, Figs. 8a and 9a show the two bands under conditions for which the production rates should be maximum, according to Knox and Pyper<sup>14</sup>, for  $N = 1614$  and 3102 plates, respectively. They correspond to loads of 1.6 and 4% of the total column saturation capacity, *i.e.*,  $\log(X) = -1.8$  and  $-1.4$ , respectively. For the sake of illustration, the chromatograms corresponding to the maximum production as predicted by our model for  $N = 1614$  and 3102 plates are shown in Figs. 8b and 9b, respectively. These figures confirm that one must allow for the two bands to interfere a great deal in order to achieve maximum production.

It is interesting to pursue a quantitative comparison between our results and those predicted by Knox and Pyper<sup>14</sup>. They calculated the maximum throughput under their optimum conditions from the following equation (eqn. 62 in ref. 14):

$$\log[\varphi T(\text{m/s})] = -0.64 - 1.58 \log N^* \quad (9)$$

with  $\varphi = [k'_2/(1 + k'_2)]^2 (b_2/4a_2)$ . In the numerical case discussed here, this gives  $\varphi = 1.9 \cdot 10^{-5} \text{ m}^3/\text{mol}$ . Their expression relating the throughput  $T = Q/t_0$  to the "practical" throughput  $PT = Q/[t_0(k'_2 + 2)]$  is then used to arrive to the following expression:

$$\log[PT(\text{mol}/\text{m}^2/\text{s})] = 3.16 - 1.58 \log N^* \quad (10)$$

The expected "practical" throughput is therefore equal to  $0.00042 \text{ mol}/\text{cm}^2/\text{min}$  for a yield of 100% at this velocity. According to our simulation, the expected throughput is equal to  $0.0012 \text{ mol}/\text{cm}^3/\text{min}$  with a loading factor of 1.6% (Fig. 6a). For a 25-cm long column with  $\varepsilon = 0.8$ , this corresponds to a production rate of  $0.0060 \text{ mol}/\text{cm}^2/\text{min}$ . Comparison between these two values must be made with caution. First, it is satisfactory, given the differences in approaches over which we elaborate below, that they agree within a factor 15. This may explain part of the difference. Secondly, Knox and Pyper used a simplified plate equation,  $h = Ct$  (see eqn. 50 in ref. 14), in their work whereas we used a more realistic plate equation (see eqn. 4). As a result, the value of the reduced velocity used in our simulation is 1.4 times lower than that used by Knox and Pyper for the same reduced column efficiency (see Fig. 1). Accordingly, had we used the same plate height equation in our calculations, we would have obtained a production rate 1.4 times higher, *i.e.*, 0.0084, which is to be 20 times greater than the Knox and Pyper expected practical throughput (0.00042). There are several possible explanations for this difference. We must emphasize that our value corresponds to a yield of 99% and theirs to 100%. Also, Knox and Pyper<sup>14</sup> neglected the effect of the displacement of the first component by the second<sup>15,16</sup>. The latter results in a concentration of the first eluting solute in the mobile phase, *i.e.*, a narrowing of its band<sup>17</sup>. This, in turn, allows for the amount of the mixture required to reach the optimum production rate at a unit yield larger than when the two solutes do not interfere and explains in part why our predicted value is higher than theirs. The remainder of the difference may lie in the nature of the assumptions used in their model, *viz.*, triangular peak shape and optimum throughput achieved when the kinetic dispersion is half the thermodynamic dispersion.

In practice, if a certain loss in the recovery is accepted, a 5–10-fold gain in production can be achieved by injecting large samples and making the proper fraction cuts. Figs. 8b and 9b show the chromatograms obtained with the injections giving maximum productivity under the flow-rate recommended by Knox and Pyper<sup>14</sup>. Fig. 10a and b show the chromatograms corresponding to the maximum production we could achieve for the first (purity 99%) and the second compound (purity 95%), respectively.

## CONCLUSION

The main conclusion is that an increase in the mobile phase flow-rate far beyond the values conventionally used in analytical HPLC leads to an important increase in production rate of a preparative column. The recoveries always increase with increasing column efficiency and are maximum at the optimum column flow-rate.



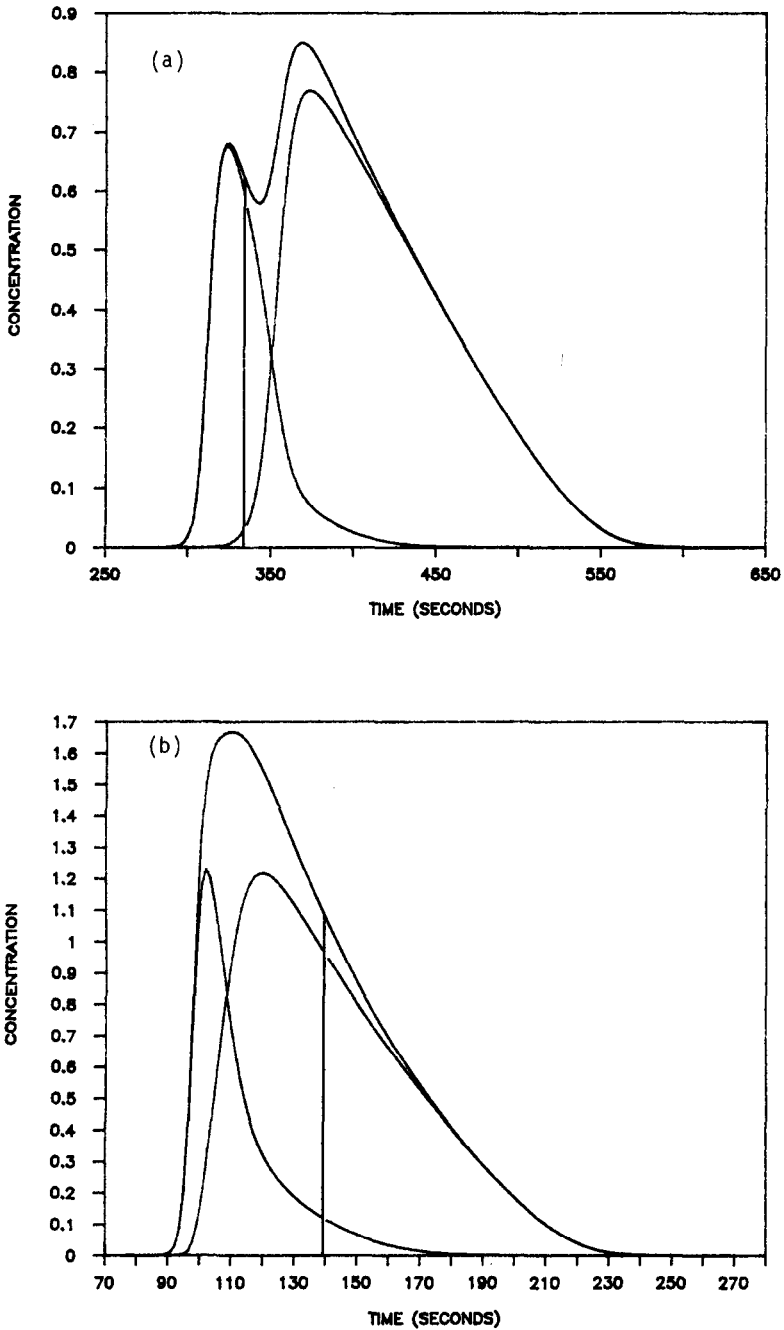


Fig. 10. Chromatograms of a 1:3 mixture ( $\alpha = 1.25$ ) corresponding to the maximum achievable production rate for: (a) component 1 collected at 99% purity ( $N = 538$  plates) and (b) component 2 collected at 95% purity ( $N = 275$  plates).

On the other hand, the number of injections per unit time increases with increasing flow-rate as retention times are inversely proportional to the flow-rate. Accordingly, there must be a production rate optimum flow-rate. This turns out to be true in the general case but this optimum flow-rate is much higher than the efficiency optimum flow-rate. It corresponds to an analytical resolution of about 1 for component 1 for highly pure fractions and less than 1 for 95% purity. By accepting the loss of recovery associated with the use of strongly interfering bands, it is possible to improve the production rates of a preparative chromatograph by nearly an order of magnitude. The relative maximum production rates of compounds 1 and 2 are not in the ratio of their proportions in the injected mixture, however, and these maximum rates are reached for different linear velocities and different amounts injected.

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