Journal of Chromatography, 605 (1992) 175–191
Elsevier Science Publishers B.V., Amsterdam

CHROM. 24 200

Step elution in preparative liquid chromatography

S.-G. Hu, D. D. Do and Md. M. Hossain

Department of Chemical Engineering, University of Queensland, Brisbane, Queensland 4072 (Australia)

(First received October 15th, 1991; revised manuscript received March 24th, 1992)

ABSTRACT

Preparative liquid chromatography with the step elution, in which a sample is loaded into a column at higher binding strength and then eluted at lower binding strength, was numerically simulated with a detailed rate equation model. The model takes into account film mass transfer resistance, pore diffusion, axial dispersion and local equilibrium. The step elution mode was compared with the isocratic elution mode in terms of the preparative performance for a binary mixture having a constant or variable separation factor. The effects of various parameters, such as step height, sample size, solute concentration, feed composition and mobile phase flow-rate, were examined.

INTRODUCTION

Preparative or production-scale liquid chromatography is fundamentally different from analytical chromatography in its purpose. In all preparative and production applications of chromatography, the goal is to separate a desired product with a specified degree of purity and at the lowest cost. Consequently, it is important to seek conditions for the optimum preparative performance, other than conditions for the best resolution between the solute bands.

Fundamental studies of preparative liquid chromatography have been published. These reports discuss theoretically the effects of parameters such as relative retention [1,2], feed composition [3], concentration overload [4], sample size and volume [5], mobile phase flow-rate [6] and column efficiency [7,8]. These investigations allow a better understanding of non-linear preparative liquid chromatography carried out in the isocratic elution mode.

The elution strategy is also important in the optimization of preparative chromatography. Although the linear gradient elution mode is widely used in analytical and preparative chromatography where the components display a broad range of retentivity, only a few reports have described fundamental studies on the gradient elution mode in preparative liquid chromatography. Computer simulations based on the Craig distribution model have been used to examine preparative separations with linear gradient elution for heavily overloaded conditions [9–11]. Antia and Horváth [12] compared isocratic elution with linear gradient elution under preparative conditions. Cox and Snyder [13] reported experimental studies to show that displacement effects occur in the preparative gradient elution chromatography of proteins. Apart from linear gradient elution, non-linear gradients, such as stepwise elution [14–16], are also employed in chromatographic separations in order to take the advantage of the simple apparatus and operating procedure. However, the performance of the preparative liquid chromatography with step elution has not been well understood.

In this paper, we present a comparison of step elution with isocratic elution as a function of sample size on the basis of production rate and yield at a specified purity. In step elution, a sample is loaded into the column with a certain modulator level, such

Correspondence to: Dr. D. D. Do, Department of Chemical Engineering, University of Queensland, Brisbane, Queensland 4072, Australia.

0021-9673/92/$05.00

as salt concentration and pH, so that the binding strength of the solute on the adsorbent in the loading stage is high, and then eluted with a buffer solution having another modulator level to reduce the binding strength. The high loading capacity, the strong compression effect and the short elution time in the step elution mode lead to an improvement in the preparative performance of overloaded liquid chromatography. A detailed rate equation model was used in this work to investigate the preparative performance under the step elution conditions. The effects of various parameters such as step height, sample size, solute concentration, feed composition and mobile phase flow-rate were studied by means of numerical simulation.

THEORY

Model description

We consider a chromatographic process involving a binary mixture taking place in a column of length L. The following assumptions are made in formulating the model: the porous solid adsorbent is spherical and uniform in size; the process is isothermal with no concentration gradient in the radial direction of the column; the pore diffusivity and film mass transfer coefficient are constant; local equilibrium is assumed between the adsorbed species and the free species in the porous particle; and modulator molecules do not adsorb on the stationary phase.

By defining the non-dimensional variables and parameters as in Table I, the following dimensionless equations for solute k can be obtained via mass balance.

The governing equation for the bulk fluid phase is given by

$$\sigma_b(k)\frac{\partial Y_b(k)}{\partial \tau} = \eta(k)\left[\frac{1}{Pe}\cdot\frac{\partial^2 Y_b(k)}{\partial \xi^2} - \frac{\partial Y_b(k)}{\partial \xi}\right] - 3\psi(k)\left.\frac{\partial Y(k)}{\partial X}\right|_1 \quad (1)$$

The initial condition and boundary conditions for eqn. 1 are

$$\tau = 0; \quad Y_b(k) = 0 \quad (2)$$

$$\xi = 0; \quad \frac{1}{Pe}\cdot\frac{\partial Y_b(k)}{\partial \xi} = [Y_b(k) - Y_b^0] \quad (3)$$

$$\xi = 1; \quad \frac{\partial Y_b(k)}{\partial \xi} = 0$$

where

$$Y_b^0 = \begin{cases} 0 & \tau > \tau_{inj} \\ 1 & \tau \leqslant \tau_{inj} \end{cases} \quad (4)$$

TABLE I

DEFINITIONS OF DIMENSIONLESS VARIABLES

$$Y(k) = \frac{C_k}{C_k^0};\ Y_\mu(k) = \frac{C_{\mu k}}{C_{\mu s,k}};\ Y_b(k) = \frac{C_{bk}}{C_k^0};\ \xi = \frac{Z}{L};\ X = \frac{r}{R_0}$$

$$\tau = \frac{t}{t_0};\ t_0 = \frac{L}{V_f}\frac{\sigma_t}{\sum_{l=1}^{NC} C_l^0};\ \sigma_t = [\varepsilon_b + (1-\varepsilon_b)\varepsilon_p]\sum_{l=1}^{NC} C_l^0 + [1-\varepsilon_b(1-\varepsilon_p)]\sum_{l=1}^{NC} C_{\mu s,k}$$

$$\sigma(k) = \frac{(1-\varepsilon_b)\varepsilon_p C_k^0}{\sigma_t};\ \sigma_\mu(k) = \frac{(1-\varepsilon_b)(1-\varepsilon_p)C_{\mu s,k}}{\sigma_t};\ \sigma_b(k) = \frac{\varepsilon_b C_k^0}{\sigma_t}$$

$$\psi(k) = \frac{(1-\varepsilon_b)\varepsilon_p D_{p,k} C_k^0}{\frac{V_f}{L}\sum_{l=1}^{NC} C_l^0};\ \eta(k) = \frac{C_k^0}{\sum_{l=1}^{NC} C_l^0};\ Bi(k) = \frac{k_f R_0}{\varepsilon_m D_{pk}};\ Pe = \frac{LV_f}{D_{ax}}$$

where τ_{inj} is the dimensionless time duration of sample injection.

The governing equation for the particle phase is given by

$$\sigma(k)\frac{\partial Y(k)}{\partial \tau} + \sigma_\mu(k)\frac{\partial Y_\mu(k)}{\partial \tau} = \psi(k)\frac{1}{X^2}\cdot\frac{\partial}{\partial X}\left[X^2\frac{\partial Y(k)}{\partial X}\right] \tag{5}$$

where

$$\frac{\partial Y_\mu(k)}{\partial \tau} = \sum_{l=1}^{NC}\frac{\partial Y_\mu(k)}{\partial Y(l)}\cdot\frac{\partial Y(l)}{\partial \tau}$$

and the term $\partial Y_\mu(k)/\partial Y(l)$ is determined from the isotherm model, described in the next section.

The initial condition and boundary conditions for eqn. 5 are given by

$$\tau = 0; \quad Y(k) = Y_\mu(k) = 0 \tag{6}$$

$$X = 0; \quad \frac{\partial Y(k)}{\partial X} = 0 \tag{7}$$

$$X = 1; \quad \frac{\partial Y(k)}{\partial X} = Bi(k)[Y_b(k) - Y(k)]$$

The film mass transfer coefficient, k_f, used to calculate the Biot number and axial dispersion coefficient, D_{ax}, used to calculate the Peclet number are estimated with the following correlations [17,18]:

$$k_f = (2 + 1.45Re^{0.45}Sc^{1/3})D_{AB}/d_p \tag{8}$$

$$D_{ax} = V_f d_p/(0.2 + 0.11Re^{0.48}) \tag{9}$$

where Sc is the Schmidt number ($Sc = \mu/\rho D_{AB}$), and Re is the Reynolds number ($Re = d_p V_f/\mu$).

In step elution, the rectangular front of elution buffer will be gradually deformed owing to the dispersion. This effect must be taken into account in the model. The mass balance equations for the modulator are basically similar to those for solute except that the term $\partial Y_\mu(k)/\partial \tau$ in eqn. 5 is equal to zero because of the assumption that the modulator molecules do not adsorb on the stationary phase. Unlike analytical chromatography, heavily overloaded elution chromatography generally behaves like the frontal operation in the loading stage. Jandera and Guiochon [19] have observed that the strength of the sample solvent significantly affects the band profile in preparative liquid chromatography. Therefore, the initial conditions of the feed solution, such as pH and ionic strength, play an important role in the preparative performance. For the sake of definition, in this work we assume that the conditions of the feed solution were adjusted prior to injection so that the conditions are the same as those in the initial buffer solution. Accordingly, the initial and boundary conditions for modulator may be written as

$$\tau = 0; \quad Y_m = Y_m^0 \tag{10}$$

$$\xi = 0; \quad Y_m = \begin{cases} Y_m^0, & 0 \leqslant \tau \leqslant \tau_{inj} \\ Y_m^e, & \tau > \tau_{inj} \end{cases} \tag{11}$$

$$\xi = 1; \quad \frac{\partial Y_m}{\partial \xi} = 0 \tag{12}$$

where Y_m is the dimensionless concentration of the modulator and Y_m^0 and Y_m^e are the modulator concentrations in initial and elution buffers, respectively.

Adsorption isotherm

The competitive Langmuir isotherm is the most commonly used isotherm in describing multi-component equilibria. Experimental data for some binary systems are also in reasonable agreement with the predictions from the Langmuir isotherm [12]. For simplicity, therefore, the competitive Langmuir isotherm is chosen as an isotherm model in this work. For multi-component adsorption, the non-dimensional form of the Langmuir isotherm is given by

$$Y_\mu(k) = \frac{\lambda(k)Y(k)}{1 + \sum_{l=1}^{NC}\lambda(l)Y(l)} \tag{13}$$

where $\lambda(k)$ is defined as

$$\lambda(k) = C_k^0 b(k) \tag{14}$$

In order to incorporate the effects of modulator concentration into the isotherm model, the following assumptions are proposed: the saturation capacity, $C_{\mu s}$, is not affected by the modulator concentration; the parameters $b(k)$ are independent of each other; and the types of interaction involved are only electrostatic and hydrophobic.

Depending on the nature and properties of both the solute and stationary phase, various correlations

of $b(k)$ *versus* the modulator concentration can be expected. For the chromatographic systems based on hydrophobic and electrostatic interactions, Melander *et al.* [20] proposed a comprehensive relationship between the retention factor [$= C_{\mu s,k}b(k)(1 - \varepsilon_b)/\varepsilon_b$] and salt concentration. By separating the $C_{\mu s,k}$ from $b(k)$ and lumping $C_{\mu s,k}$ into the α term in their equation, the following equation can be obtained to express the dependence of $b(k)$ on the modulator concentration, as a Langmuir competitive isotherm with a constant saturation capacity is assumed in this work [20]:

$$\log b(k) = \alpha(k) - \beta(k)\log C_m + \gamma(k)C_m \quad (15)$$

where $\beta(k)$ and $\gamma(k)$ are the electrostatic and hydrophobic interaction parameters, respectively. The parameter $\alpha(k)$ is a constant encompassing all characteristic system parameters and C_m is the molar concentration of the modulator. It is noted that the same form of eqn. 15 was used in the modelling of gradient elution presented by Gu *et al.* [21] and a simplified form was applied by Antia and Horváth [12] in their simulations of linear gradient elution. Although the assumption of the independence of $C_{\mu s}(k)$ on modulator concentration has some experimental support [13], it would be not expected to be valid over a wide range of conditions in multiple solute systems as discussed by Antia and Horváth [12]. However, the influence from the dependence of $C_{\mu s}$ on the modulator concentration can be included in the effect of separation factor as defined by

$$S_f = \frac{b(l)C_{\mu s,l}}{b(k)C_{\mu s,k}} \quad (16)$$

As the separation factor is the foremost factor characterizing the chromatographic separation, the effect of a variable S_f should be equivalent to the effect of a $C_{\mu s}$ dependent on the modulator concentration. Generally, S_f varies with the modulator concentration. In some special cases where the solutes are similar in the molecular structure, S_f may be assumed to be constant. In this study, simulations were carried out for systems with both constant and variable separation factors with changing modulator concentration.

Method for numerical solution

Owing to the non-linearity involved, there is no analytical solution possible for the model equations. Approximate solution by efficient numerical techniques is the only feasible alternative. The model equations are discretized with the method of orthogonal collocation on finite elements [22]. The resulting ordinary differential–algebraic equation set was then solved by using the software DASSL [23].

Definitions of parameters

The load factor, L_f, is given by the ratio of the amount of reference solute in the sample fed into the column to the saturation capacity of the column for that solute. The lesser retained solute A is chosen as the reference in this work.

$$L_f = \frac{V_f C^0 t_0 \tau_{inj}}{L(1 - \varepsilon_b)C_{\mu s}} \quad (17)$$

The production rate, P_k, is the amount of solute k recovered per run at the specified degree of purity per unit column cross-section area divided by the cycle time τ_c, which extends from the start of the run to the point where the last component completes its elution. For simplicity, the following dimensionless form was used for result presentation:

$$P_k = \frac{V_f C_k^0 \int_{\tau_{1,k}}^{\tau_{2,k}} Y_e(k)\mathrm{d}t}{V_r C_r \tau_c} \cdot 100 \quad (18)$$

where $Y_e(k)$ is the outlet concentration of solute k at time τ, $\tau_{1,k}$ and $\tau_{2,k}$ are cutting points between which solute k is collected at the desired purity, V_f is the superficial velocity, V_r is a reference superficial velocity, C_k^0 is the concentration of solute k in the feed solution and C_r is the reference concentration. The parameter P_k so defined can be used for comparing the results obtained under different V_f and C_k^0.

It should be pointed out that an actual cycle time τ_c would include any wash and regeneration steps. The influence of column regeneration in step elution on the production rate will be discussed in the next section.

The recovery yield, RY_k, is the fraction of the solute k fed into the column that is recovered at the column outlet as a product with a specified degree of purity. A purity of 98% is chosen in this work.

$$RY_k = \frac{1}{\tau_{inj}} \int_{\tau_{1,k}}^{\tau_{2,k}} Y_e(k)\mathrm{d}\tau \quad (19)$$

TABLE II

VALUES OF PARAMETERS IN EQN. 15 FOR SIMULATION

Parameter		Constant S_f			Variable S_f	
		$S_f = 2.41$	$S_f = 1.41$	$S_f = 1.35$	Divergent	Convergent
$\alpha(k)$	A	−3.68	−3.65	−3.03	−3.73	−3.74
	B	−3.30	−3.5	−2.9	−3.38	−3.65
$\beta(k)$	A	3.0	3.0	2.6	3.0	3.0
	B	3.0	3.0	2.6	3.0	2.7
$\gamma(k)$	A	3.0	3.0	1.84	3.09	2.55
	B	3.0	3.0	1.84	3.0	2.6

The enrichment factor, E_k, is defined as the product concentration of solute k divided by its concentration in the feed:

$$E_k = \frac{1}{(\tau_{2,k} - \tau_{1,k})} \int_{\tau_{1,k}}^{\tau_{2,k}} Y_e(k) d\tau \quad (20)$$

RESULTS AND DISCUSSION

Based on the model presented above, the preparative performance in the different elution modes was simulated for various conditions. The parameter values used for simulations are listed in Tables II and III unless mentioned otherwise in the discussion or stated in the figure captions. Parameter values for $\alpha(k)$, $\beta(k)$ and $\gamma(k)$ were selected by reference to some experimental data with proteins [20]. However, these parameters could take any other values, depending on the modulator type and the unit of variables. As the objective of this work was to investigate the effect of the difference in binding strength between the loading stage and elution stage, the important parameter is the binding strength [$b(k)$], which is calculated from eqn. 15. The value of $b(k)$ in this work covers a wide range of 0.02–50. For the parameters given in Table II and the range of modulator concentration (0.5–2.0 M) used in simulations, the binding strength is increased with an increase in the modulator concentration, and also the solute A is the lesser retained component in the binary mixture.

Comparison for the system with constant S_f

Effects of sample size and step height. The simulation results for the system with a constant separation factor of 2.41 are shown in Fig. 1. In the insets in Fig. 1, the values following the hollow symbols are the molar concentrations of the modulator in isocratic elution, while the ranges following the filled symbols are the variations of the modulator concentration in the step elution. The range is thereafter called the step height. The first number of the range is the modulator concentration in the initial buffer and the second is that of the elution buffer.

As shown in Fig. 1a and c, the production rates for both solutes in step elution are considerably higher than those in isocratic elution with a moderate degree of overload. It can also be seen from Fig. 1a and b that with step elution, the optimum load factor for the production rate of solute A is higher than that for solute B. The maximum production

TABLE III

PARAMETER VALUES USED IN SIMULATIONS

$R_0 = 25\ \mu m$	$C_r = 3\ kg/m^3$
$L = 25\ cm$	$\mu = 1.3 \cdot 10^{-3}\ kg/m \cdot s$
$\varepsilon_p = 0.5$	$\rho = 1100\ kg/m^3$
$\varepsilon_b = 0.4$	$D_{AB,k} = 6 \cdot 10^{-11}\ m^2/s$
$V_f = 5 \cdot 10^{-6}\ m/s$	$D_{p,k} = 3.2 \cdot 10^{-12}\ m^2/s$
$V_r = 5 \cdot 10^{-6}\ m/s$	$D_{bm} = 1.59 \cdot 10^{-9}\ m^2/s$
$C_A^0 = 3\ kg/m^3$	$D_{pm} = 3 \cdot 10^{-10}\ m^2/s$
$C_B^0 = 3\ kg/m^3$	$Pe = 2388$
$C_{\mu s,A} = 50\ kg/cm^3$	$Bi_k = 48$
$C_{\mu s,B} = 50\ kg/m^3$	$Bi_m = 11$

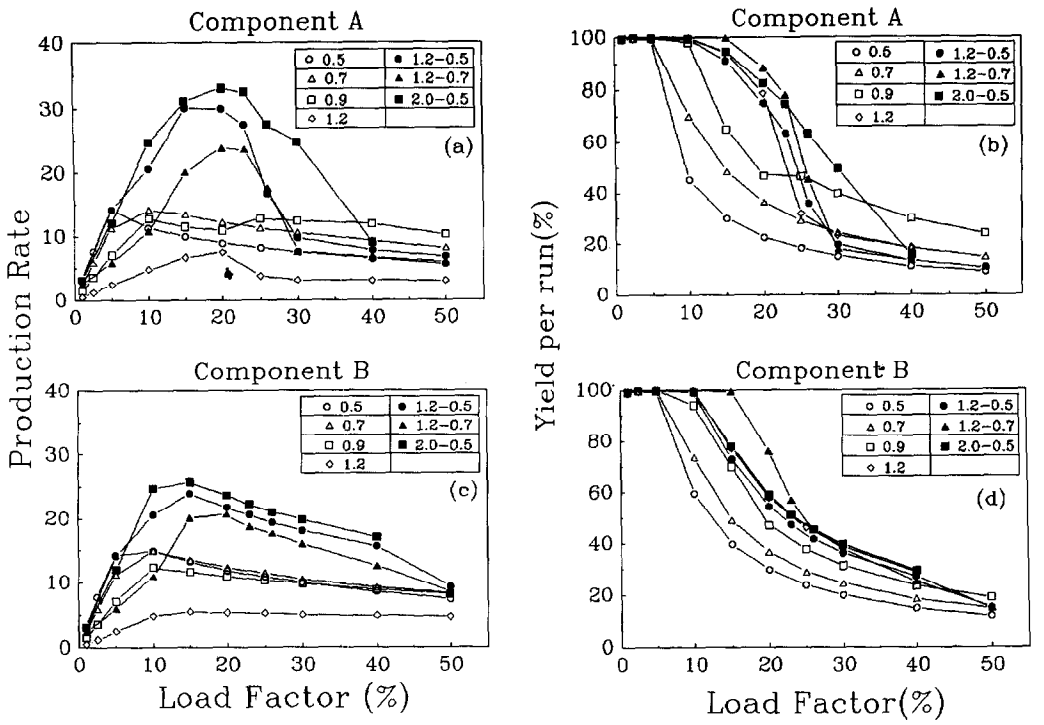

Fig. 1. Production rate and yield as a function of load factor obtained in step elution and isocratic elution. $S_f = 2.41$; 1:1 binary mixture.

rate of solute A is increased more than that of solute B by step elution.

It is also noted from Fig. 1a and c that the improvement in the production rate by using step elution depends greatly on the step height. For a given modulator concentration in the initial buffer, the maximum production rates for both solutes in step elution are decreased with an increase in the modulator concentration in the elution buffer, but the yield is improved to a certain extent as shown in Fig. 1b and d.

A higher initial binding strength allows a greater column loading capacity, which facilitates a larger sample size injected into the column and hence more product recovered in one run. Because of this, the higher the step height, the better is the production rate for the cases in Fig. 1. However, a high loading capacity does not imply a high production rate. It merely provides the possibility of obtaining more products in one run. This is because the production rate is also dependent on the operation time of the process. In isocratic elution, a higher loading capacity always implies a longer elution time. Step elution combines an initial high loading capacity with a short elution time, and this makes it an efficient elution mode in preparative chromatography.

Plots of enrichment factor (defined in eqn. 20) *versus* load factor are shown in Fig. 2. The product concentration of solute A in step elution is more than three times higher than that in isocratic elution for a load factor of 20%. It is clear that the sample size (load factor) favourable to the enrichment (about 20%) is consistent with that for the maximum production rate. Although solute B is not as highly concentrated as solute A in step elution, it is still higher than that in isocratic elution.

The favourable aspects of step elution can be further illustrated by comparing the bed profiles for the two elution modes (Figs. 3 and 4). During the loading stage (Figs. 3a and 4a), the bed profiles are the same for both elution modes because of the same conditions. Under overloaded conditions, the band development in the loading stage clearly resembles that in the frontal adsorption process, which is indicated by the roll-up peaks in Figs. 3a and 4a. In step elution, the difference in binding strength across

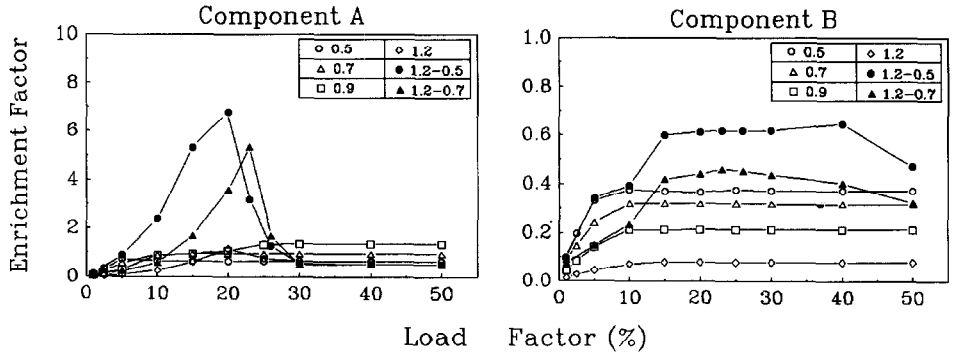

Fig. 2. Enrichment factor as a function of load factor in step elution and isocratic elution. S_f = 2.41; 1:1 binary mixture.

the front of the elution buffer results in the rear portion of both solute bands moving at a higher speed. Hence the bands of both A and B are strongly compressed, as shown in Fig. 3b. The high peak concentration, caused by the compression effect, results in a strong interaction between the bands of both solutes (Fig. 3b and c). The improvement in yield and enrichment with step elution is basically due to such enhanced interactions, especially the displacement effect [24]. On other hand, the "tag-along" effect [25] pulls forward the front of the solute B peak, and hence decreases its maximum concentration (Fig. 3b and c). In isocratic elution, solutes are eluted at the same binding strength as

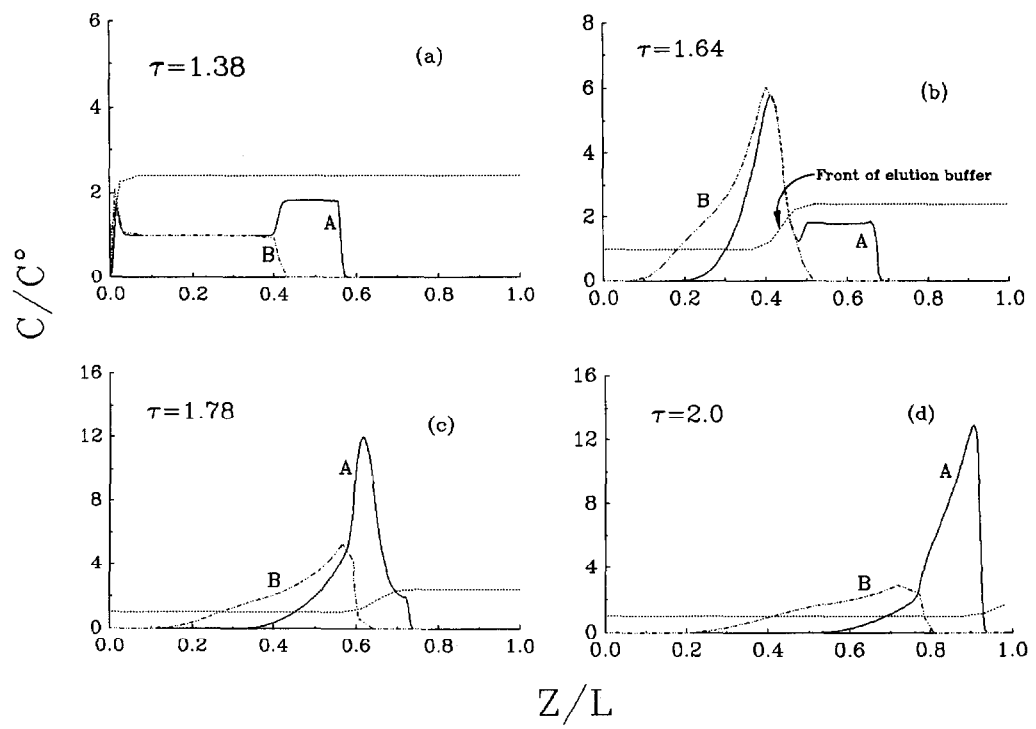

Fig. 3. Bed profiles for the step elution with 1.2–0.5 M variation range of modulator concentration. Load factor = 15%; S_f = 2.41; 1:1 binary mixture. Solid lines, solute A; dot-dashed lines, solute B; dotted lines, modulator.

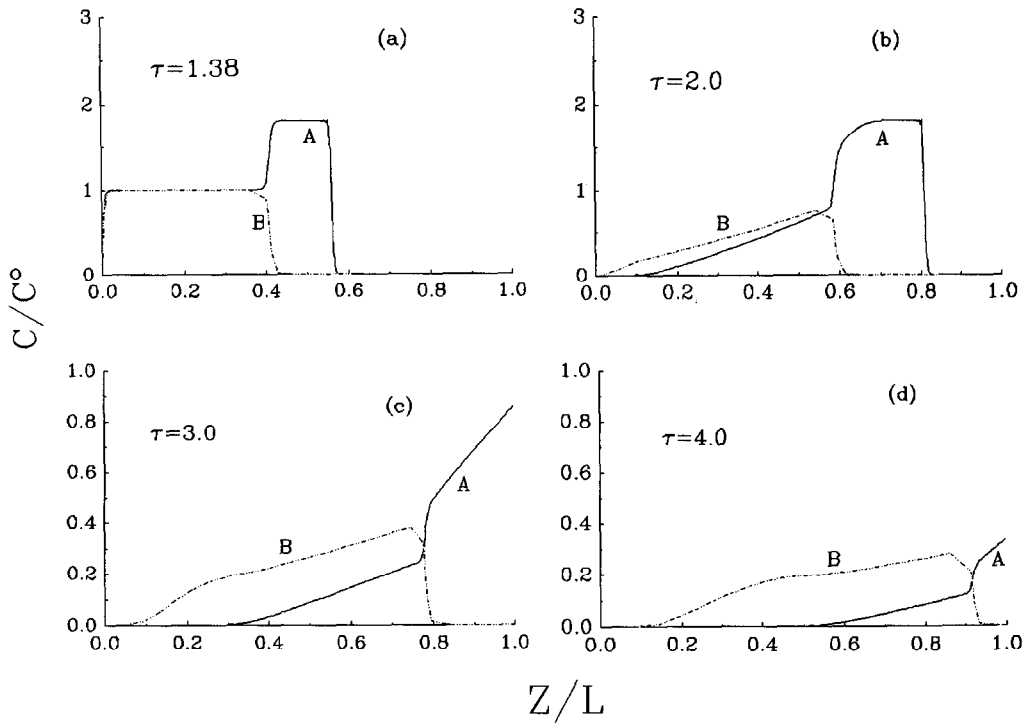

Fig. 4. Bed profiles for the isocratic under 1.2 M modulator concentration. Load factor = 15%; S_f = 2.41; 1:1 binary mixture. Solid lines, Solute A; dot-dashed lines, solute B.

that in the injection stage. The bed profiles in Fig. 4 show that more band spreading occurs as compared with that in Fig. 3, and the elution process takes a longer time to complete.

Unlike isocratic elution where the column is ready to introduce the next sample after the components of the previous sample have left the column, the column with step gradient elution must be returned to its initial conditions (equilibrated with the initial solvent) on completion of each gradient run. Therefore, the increase in production rate in step elution will be offset to some extent by the column regeneration. The regeneration time between successive runs depends largely on the physico-chemical mechanism governing the dependence of solute retention on the modulator. If no slow kinetics are involved in the regeneration process, the time for column re-equilibration is mainly controlled by the mass transfer of modulator between the mobile and stationary phases. This is true for the cases with a salt gradient in hydrophobic interaction and reversed-phase chromatography. As the diffusivity of modulators with small molecules is usually two orders of magnitude greater than those of proteins, the flow-rate used in the regeneration can be much higher than that in the elution stage. The regeneration can also be speeded up in some instances by using a reverse gradient [26]. The regeneration time for a system with a polar stationary phase, such as silica or alumina, could be as long as the elution time [27]. However, such polar packings are rarely used in the separation of proteins. It has been reported that the regeneration time for the reversed-phase chromatography of proteins is much less than the elution time and the initial solvent volume needed for the regeneration is less than the column volume [28]. If the initial solvent volume for regeneration is twice the column volume and the flow-rate for the regeneration is four times higher than that in the elution stage, then the regeneration time would be less than 20% of the elution time for the cases with a retention factor between 0.6 and 3. Because an actual increase in production rate by step elution is affected by the column regeneration, the advantage of step elution

is still significant only for the case where the production rate can be substantially increased by step elution. As we shall see later, the production rate in step elution with a dilute sample could be three times as high as that in isocratic elution. In such a case, step elution would be strongly favoured over isocratic elution even if the column regeneration time is as long as the elution time.

Effect of separation factor. So far our discussion is focused on a system with a separation factor of 2.41. Fig. 5 shows the results for a system with a lower separation factor of 1.41. The curves exhibit trends different from those observed in Fig. 1. The step height effect is significant only when the load factor is greater than 30%. The yield for the solute A also shows a different trend (Fig. 5b). The production rate of solute A (Fig. 5a) passes through a maximum and then decreases with increase in the load factor. Further, the plots in Fig. 5d show no difference in yields obtained by the different elution modes for the system with $S_f = 1.41$. Clearly, there is no significant advantage in using the step elution mode when the separation factor is low. Nevertheless, the situation can be improved for the system with dilute feed solution, as will be illustrated later.

With step elution, there exists a minimum for both the production rate and the yield of solute A, as shown in Fig. 5a and b. This phenomenon can be explained from the observations in Figs. 6 and 7. It is shown in Fig. 6 that the separation achieved during sample injection is not as good as that in Fig. 3 because of a weaker displacement effect resulting from a low value of S_f. The two peaks are simultaneously compressed at the earlier stage of the elution (the same as in Fig. 3), and then the peak of solute A becomes more concentrated owing to the displacement effect by solute B, while the peak of solute B exhibits a strong "tag-along" effect such that the front for solute B catches up with the front for solute A. This "catch-up" phenomenon only takes place for the case of a 20% load factor (Fig. 7b). In this case, the peak fronts are very close to each other as they approach the column exit. Therefore, no more time is available to allow further separation. However, for the case of a 10% load factor (Fig. 7a), a certain degree of separation is obtainable, although

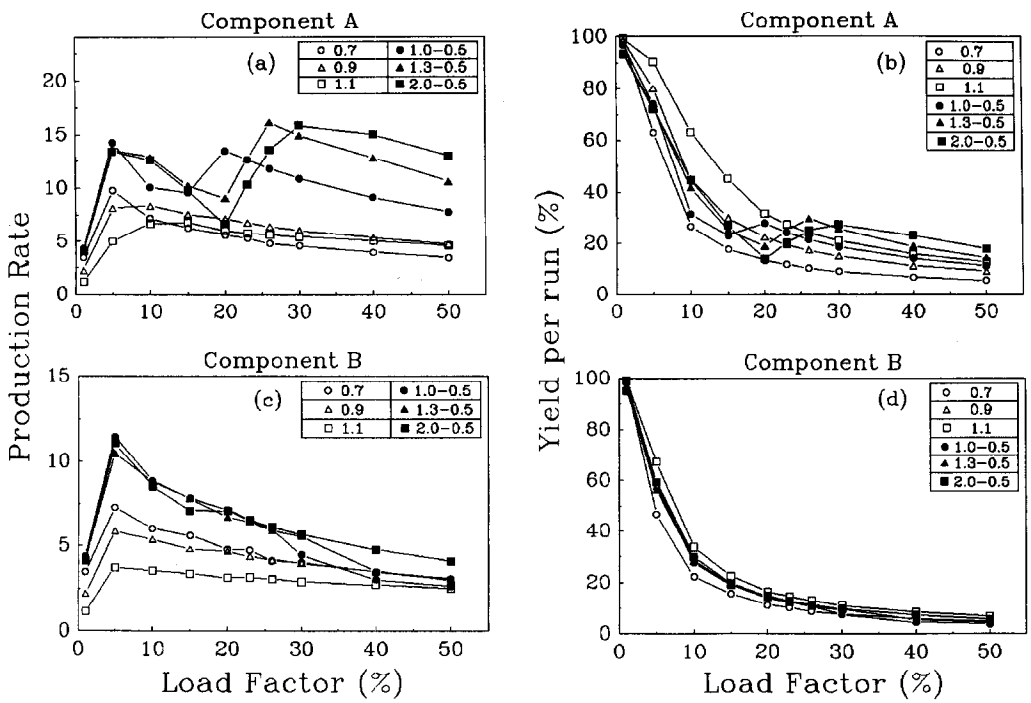

Fig. 5. Production rate and yield as a function of load factor in step elution and isocratic elution. $S_f = 1.41$; 1:1 binary mixture.

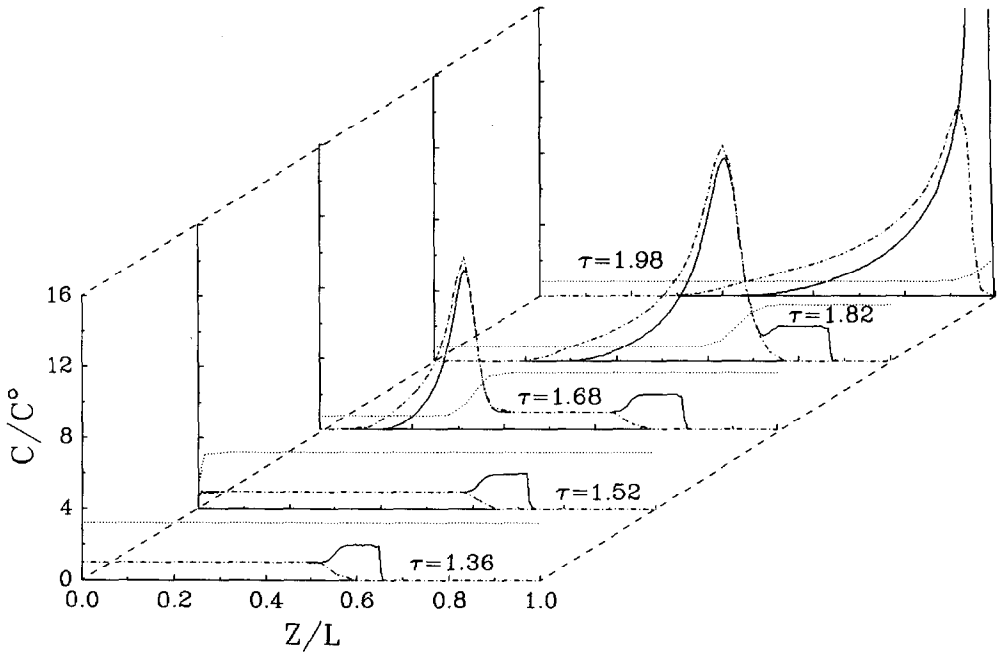

Fig. 6. Bed profiles for the step elution of 2.0–0.5 *M*. Load factor = 20%; S_f = 1.41; 1:1 binary mixture. Solid lines, solute A; dot-dashed lines, solute B; dotted lines, modulator.

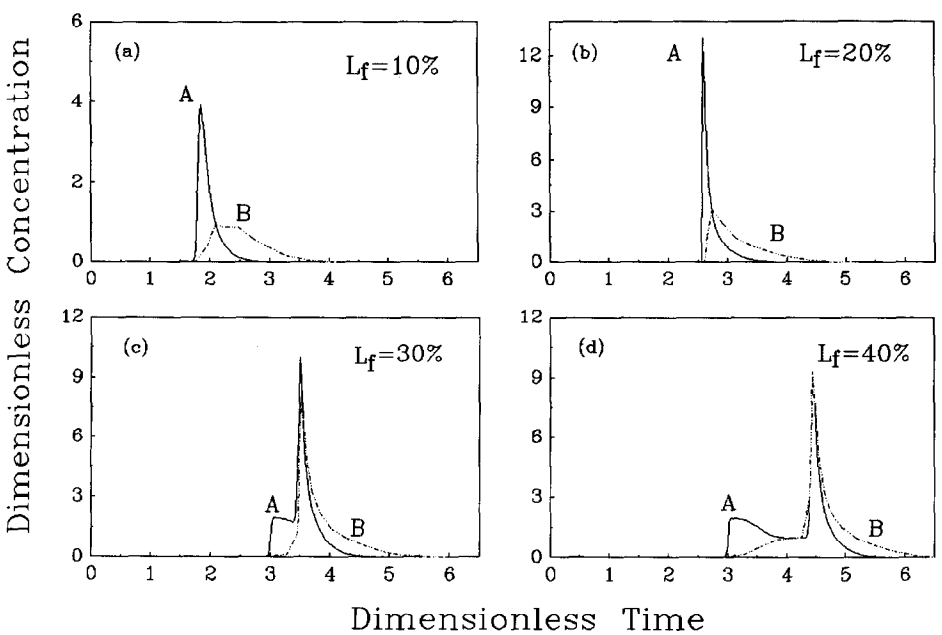

Fig. 7. Elution profiles under different load factors. S_f = 1.35; 1:1 binary mixture.

the fronts might have experienced to some extent the "catch-up" phenomenon. When the load factor is over 20%, the increase in production rate and yield for solute A is due to the result of the "roll up" peak generated by the displacement effect in the injection stage (Fig. 7c and d).

Effect of feed concentration. It is seen from Fig. 8 that step elution is particularly useful for increasing the production rate for the system with dilute feed solution (C_k^0 = 0.6 kg/m). This is also true for a system with a lower separation factor of 1.35, as illustrated in Fig. 9. The maximum production rates are nearly three times as high as those for isocratic elution. The optimum load factors for the maximum production rate (Figs. 8 and 9) are shifted to lower values compared with that in Fig. 1. When the separation factor is higher, the yield for both solutes in step elution with the optimum load factor is better than that in isocratic elution (see Fig. 8b).

It is obvious, by comparing Figs. 1 and 8, that a small volume of concentrated feed solution should be used whichever elution mode is applied. A similar conclusion was reported by Katti and Guiochon [5] in their analysis of preparative chromatography with isocratic elution. However, it is worth pointing out that process broths generally have low product concentrations and, hence, the application of step elution would be preferable. Further, the product can be more concentrated by step elution.

It is well known that for a dilute sample, application of the sample under low solvent strength (high retention) conditions leads to concentration of the sample at the top of the column, so that the band broadening associated with the sample volume is avoided. However, this practice is usually limited to the analytical application of liquid chromatography where the column is not overloaded. As shown in Figs. 8 and 9, the advantage of step elution can be seen only under overloaded conditions and the production rates for both elution modes are nearly the same with a low load factor. This also implies that effect of sample solvent strength is not significant in terms of preparative performance at small sample sizes.

Effect of feed composition. The simulation results for feed compositions (C_A^0/C_B^0) of 6:1 and 1:6 are

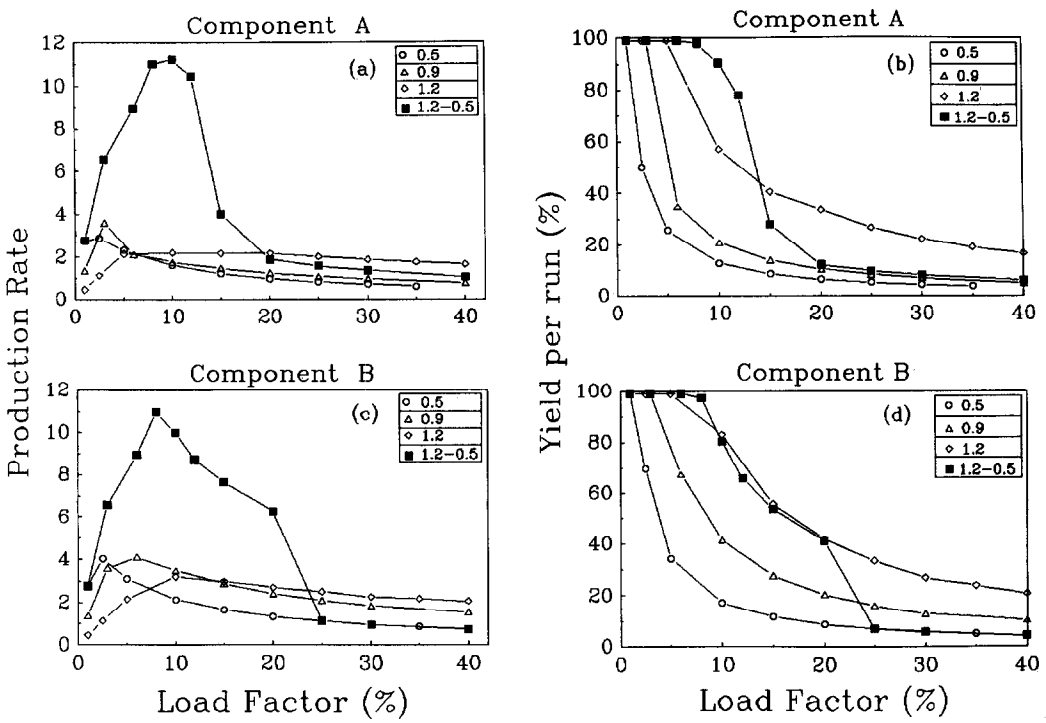

Fig. 8. Production rate and yield for the feed concentration of 0.6 kg/m³ in solute. S_f = 2.41; 1:1 binary mixture.

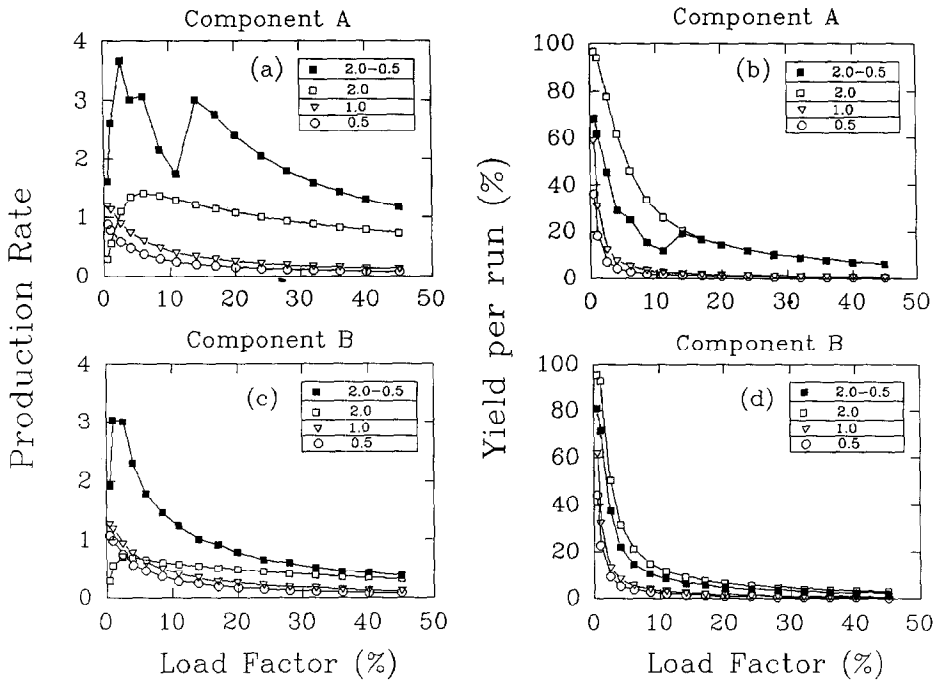

Fig. 9. Production rate and yield for the feed concentration of 0.6 kg/m^3 in solute. S_f = 1.41; 1:1 binary mixture.

shown in Figs. 10 and 11, respectively. It is seen that the improvement in the production rates with step elution is not influenced by the feed composition. The yield (with the optimum load factor for the maximum production rate) is the same as or just slightly lower than that in the corresponding isocratic elution. When the feed solution is rich in solute A (Fig. 10), the maximum production rate for solute A is increased whereas that for solute B is decreased, compared with those for the feed composition of 1:1, as shown in Fig. 1. If the feed solution is rich in solute B (Fig. 11), a trend opposite to that in Fig. 10 is observed.

Effect of mobile phase flow-rate. Simulation results for a superficial velocity of $5.0 \cdot 10^{-5}$ m/s are displayed in Fig. 12. The improvement in the production rate is still significant. It is obvious, by comparing Fig. 12 with Fig. 1, that the optimum sample size for maximum production rate for both solutes is decreased with an increase in the superficial velocity V_f, and the yield at $V_f = 5.0 \cdot 10^{-5}$ m/s is also reduced. Because of the low column efficiency resulting from the increase in V_f, the "catch-up" phenomenon occurs for the case with the step height of 2.0–0.5 M even though the separation factor S_f is 2.41 (Fig. 12a and b).

Comparison for system with variable V_f

Fig. 13 shows the simulation results for the system with a variable separation factor, in which the modulator concentration is varied from 1.3 to 0.5 M, and the corresponding S_f varies from 1.7 to 2.0. Although the separation factor with isocratic elution is higher than that in the loading stage of step elution with a step height of 1.3–0.5 M, it is seen that the curves show a general trend similar to Fig. 1. A significant improvement in the production rate of solute A can be achieved by step elution, whereas the improvement for solute B is relatively modest. The yield with the optimum load factor for the production rate is nearly the same as or better than the best case with isocratic elution.

Fig. 14 shows the simulation results for systems having convergent separation factors. In the case with the filled square symbol, the separation factor (S_f) varies from 1.55 to 1.06, corresponding to modulator concentrations from 1.3 to 0.5 M. For the case with the filled circle symbol, the modulator

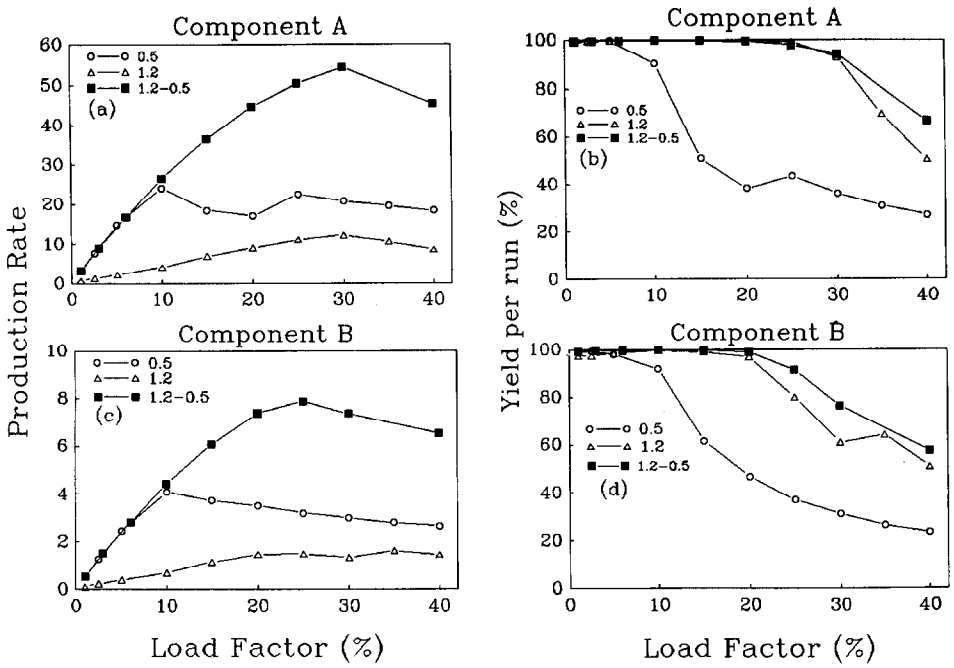

Fig. 10. Production rate and yield for the 6:1 (A:B) binary mixture. S_f = 2.41. Feed concentration: A = 4.2 kg/m^3; B = 0.7 kg/m^3.

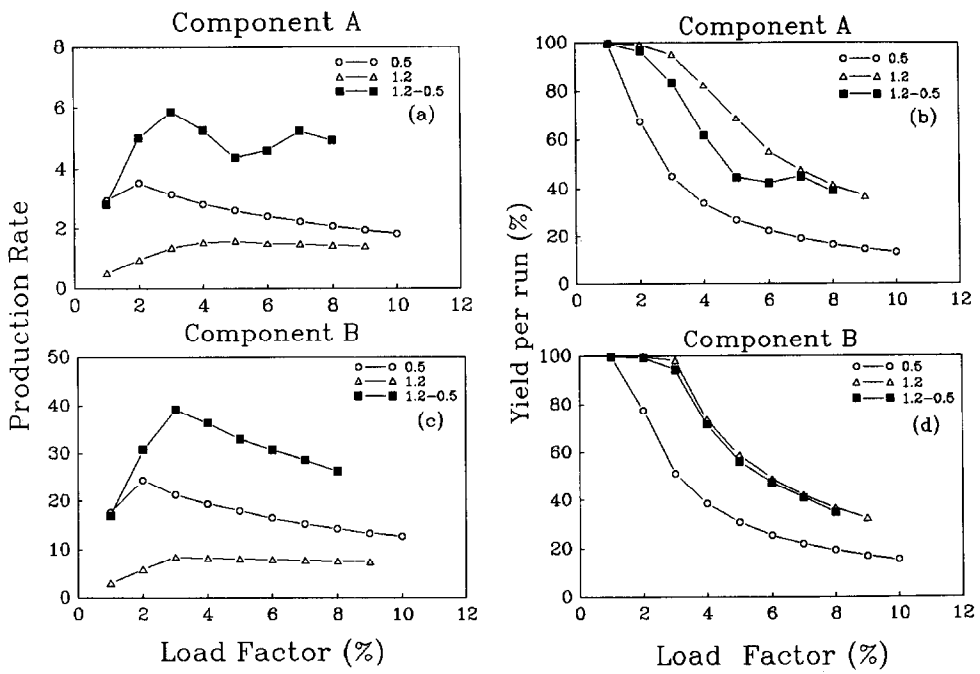

Fig. 11. Production rate and yield for the 1:6 (A:B) binary mixture. S_f = 2.41. Feed concentration: A = 0.7 kg/m^3; B = 4.2 kg/m^3.

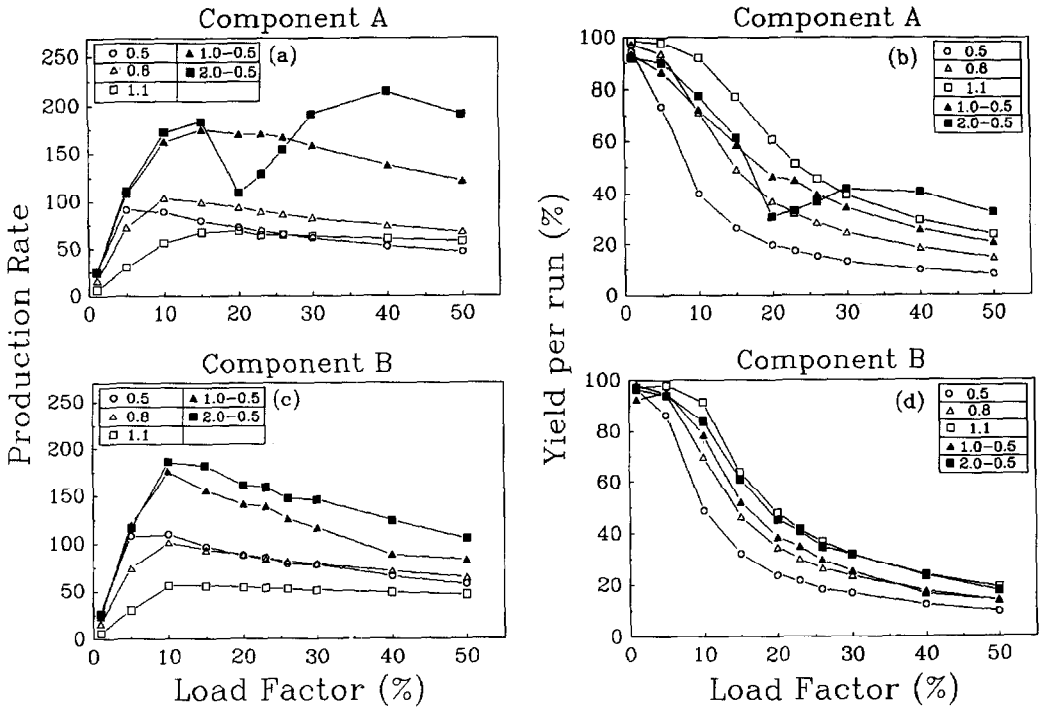

Fig. 12. Production rate and yield calculated at a superficial velocity of $5 \cdot 10^{-5}$ m/s. $S_f = 2.41$; 1:1 binary mixture.

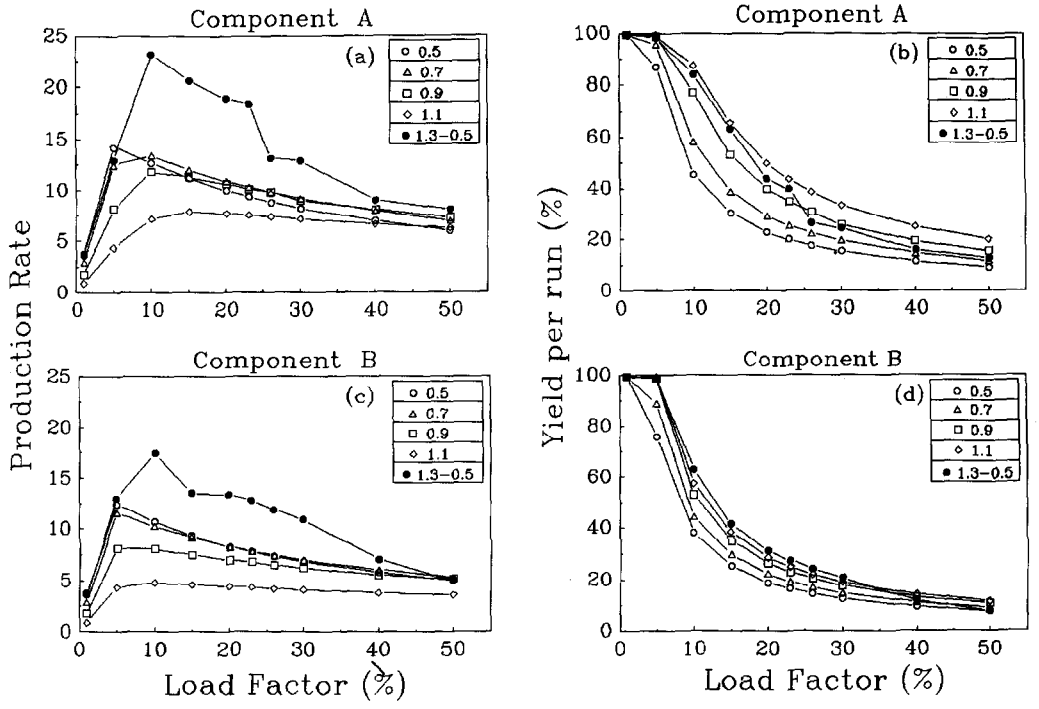

Fig. 13. Production rate and yield for the system having divergent S_f with changing the modulator concentration; 1:1 binary mixture.

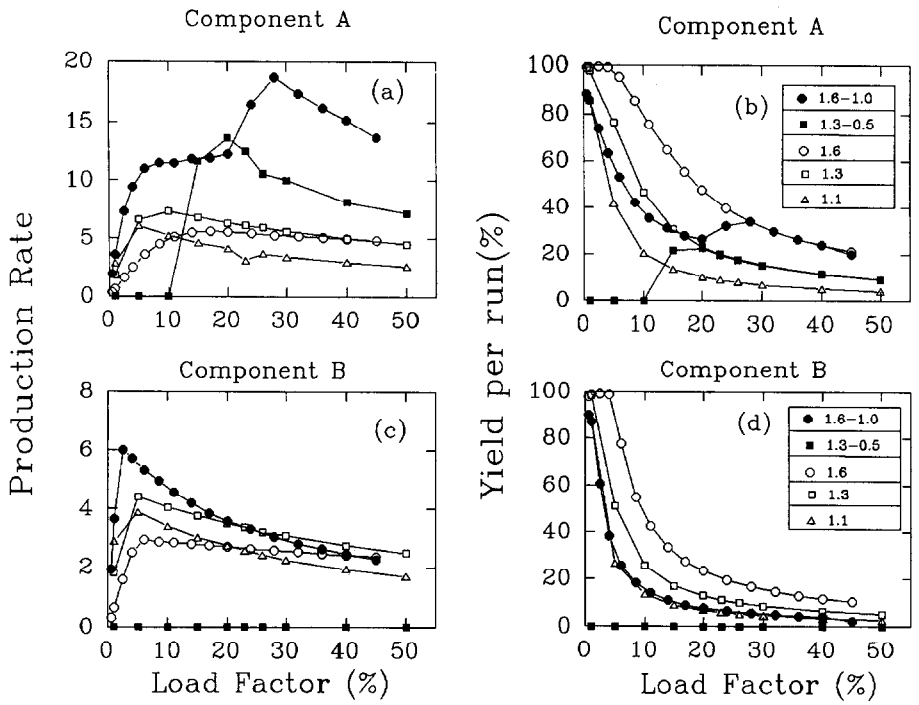

Fig. 14. Production rate and yield for the system having convergent S_f with changing the modulator concentration; 1:1 binary mixture.

concentration is changed from 1.6 to 1.0 M and the corresponding S_f varies from 1.7 to 1.38. Owing to the low S_f of 1.06 in the elution stage for the first case, solute A is not recovered until the column is overloaded to a certain extent. The separation obtained under overloaded conditions results from the front effect. It can be seen that for a low S_f of 1.06 in the elution stage step elution is unfavourable to the production of solute B. However, the improvement in the production rate by step elution is still significant in the second case (S_f = 1.7–1.38). Therefore, when both the initial and terminal S_f are not too low, step elution is superior to isocratic elution whether the separation factor is divergent or convergent with changes in the modulator concentration.

The simulation results reported here are based on the Langmuir competitive isotherm with a saturation capacity independent of modulator concentration, and are also subject to the system without modulator-dependent selectivity reversal. A practical system could follow any type of isotherm, where the separation factor may be dependent on solute and modulator concentrations. As a result, the extension of the conclusion to systems with other isotherms needs to be carefully checked. However, step elution should be still superior to isocratic elution if the following conditions are met by a given practical system: no selectivity inversion due to the changes in solute and /or modulator concentrations is involved; and the separation factor, depending on the solute and modulator concentrations, is not too low during operation.

CONCLUSIONS

A realistic model has been used to simulate step elution in preparative chromatography. In the simulation, a Langmuir competitive isotherm with a saturation capacity independent of modulator concentration has been assumed. The comparison of step elution with isocratic elution, as a function of load factor, shows that the preparative performance of liquid chromatography can be dramatically improved by using step elution for systems having a separation factor that is not too low over the defined range of the modulator concentration. The production rate and enrichment factor for both solutes in

step elution can be higher than those in isocratic elution, and the yield is also slightly improved or is comparable to that in isocratic elution. The maximum production rate achieved in step elution is increased with increase in separation factor. The increases in the production rate and the enrichment factor become significant when the feed solution is dilute. It should be noted that the increase in production rate with step elution would be offset by the column equilibration stage prior to the next run.

The selection of the operational sample size will depend on which solute is the target product and is also dependent on whether a maximum production rate is sought or a compromise between the production rate and the yield is desired. The optimum sample size for maximum production rate is influenced by the separation factor, feed concentration, feed composition and mobile phase flow-rate. With a favourable sample size, the higher the step height the better is the preparative performance of the step elution mode.

SYMBOLS

$b(k)$	Langmuir affinity constant of solute k
C_k^0	Feed concentration of solute k
C_k	Concentration of solute k in macropore fluid
$C_{\mu k}$	Concentration of solute k in stationary phase
$C_{\mu s,k}$	Saturation capacity of solute k
$C_{b,k}$	Concentration of solute k in bulk phase
C_m	Molar concentration of modulator in macropore fluid
C_r	Reference concentration
d_p	Particle diameter
$D_{AB,k}$	Molecular diffusivity of solute k
$D_{p,k}$	Effective diffusivity of solute k in particle
D_{bm}	Molecular diffusivity of modulator
D_{pm}	Effective diffusivity of modulator in particle
D_{ax}	Effective axial dispersion coefficient
k_f	Film mass transfer coefficient
l	Solute l in the mixture.
L	Column height
NC	Total number of solutes in the mixture
R_0	Particle radius
r	Radial coordinate
t	Time
t_{inj}	Sample loading duration time
t_0	Time scale
V_f	Superficial velocity
V_r	Reference superficial velocity
X	Dimensionless radial coordinate
$Y(k)$	Dimensionless concentration of solute k in macropore fluid
$Y_b(k)$	Dimensionless concentration of solute k in bulk fluid phase
Y_b^0	Dimensionless concentration of feed solution
$Y_e(k)$	Dimensionless outlet concentration of solute k
$Y_\mu(k)$	Dimensionless concentration of solute k in stationary phase
Y_m	Dimensionless concentration of modulator
Y_m^0	Dimensionless concentration of modulator in initial buffer
Y_m^e	Dimensionless concentration of modulator in elution buffer
Z	Axial coordinate

Greek letters

α	Coefficient in eqn. 15
β	Coefficient in eqn. 15
γ	Coefficient in eqn. 15
ε_b	Bed void fraction
ε_p	Particle porosity
μ	Viscosity of fluid
ρ	Density of fluid
τ	Dimensionless time
τ_c	Dimensionless cycle time
τ_{inj}	Dimensionless sample loading duration time

ACKNOWLEDGEMENT

M.M.H. acknowledges the support of the Australian NRF in the form of a fellowship.

REFERENCES

1 S. Ghodbane and G. Guiochon, *J. Chromatogr.*, 450 (1988) 27.
2 S. Golshan-Shirazi and G. Guiochon, *J. Chromatogr.*, 523 (1990) 1.
3 S. Ghodbane and G. Guiochon, *J. Chromatogr.*, 440 (1988) 9.
4 S. Ghodbane and G. Guiochon, *J. Chromatogr.*, 444 (1988) 275.
5 A. Katti and G. Guiochon, *Anal. Chem.*, 61 (1989) 982.
6 S. Ghodbane and G. Guiochon, *J. Chromatogr.*, 452 (1988) 209.
7 S. Golshan-Shirazi and G. Guiochon, *Anal. Chem.*, 61 (1989) 1368.

8 G. Cretier, L. Macherel and J. L. Rocca, *J. Chromatogr.*, 590 (1992) 175.
9 L. R. Snyder, J. W. Dolan and G. B. Cox, *J. Chromatogr.*, 484 (1989) 437.
10 L. R. Snyder, J. W. Dolan and G. B. Cox, *J. Chromatogr.*, 540 (1991) 21.
11 L. R. Snyder, G. B. Cox and P. E. Antle, *J. Chromatogr.*, 444 (1988) 303.
12 F. D. Antia and Cs. Horváth, *J. Chromatogr.*, 484 (1989) 1.
13 G. B. Cox and L. R. Snyder, *J. Chromatogr.*, 590 (1992) 17.
14 S. Yamamoto, M. Nomura and Y. Sano, *J. Chromatogr.*, 512 (1990) 89.
15 S. Yamamoto, K. Nakanish and R. Matsuno, *Ion-Exchange Chromatography of Proteins*, Marcel Dekker, New York and Basle, 1988.
16 S. Yamamoto, M. Nomura and Y. Sano, *Chem. Eng. Sci.*, 47 (1992) 185.
17 S. G. Foo and R. G. Rice, *AIChE J.*, 54 (1975) 1149.
18 S. F. Chung and C. Y. Wen, *AIChE J.*, 14 (1968) 857.
19 P. Jandera and G. Guiochon, *J. Chromatogr.*, 588 (1991) 1.
20 W. R. Melander, Z. E. Rassi and Cs. Horváth, *J. Chromatogr.*, 469 (1989) 3.
21 T. Gu, Y.-H. Truei, G.-T. Tsai and G. T. Tsao, *Chem. Eng. Sci.*, 47 (1992) 253.
22 C. F. Carey and B. A. Finlayson, *Chem. Eng. Sci.*, 30 (1975) 587.
23 L. R. Petzold, *A Description of DASSL: a Differential/Algebraic System, Solver,* Technical Report, Sand81-8637, Sandia, CA, 1982.
24 T. Gu, G. T. Tsao, G. Tsao and M. R. Ladisch, *AIChE J.*, 36 (1990) 1156.
25 S. Jacobson, S. Golshan-Shirazi, A. M. Katti, M. Czok, Z. Ma and G. Guiochon, *J. Chromatogr.*, 484 (1989) 103.
26 H. Engelhardt and H. Elgass, *J. Chromatogr.*, 112 (1975) 415.
27 H. Engelhardt and H. Elgass, in Cs. Horváth (Editor), *High-Performance Liquid Chromatography — Advances and Perspectives*, Vol. 2 Academic Press, New York, 1980, pp. 57–111.
28 Y.-B. Yang, K. Harrison and D. Carr, *J. Chromatogr.*, 590 (1992) 35.