ELSEVIER

Journal of Chromatography A, 687 (1994) 13–31

JOURNAL OF CHROMATOGRAPHY A

Recycling in preparative liquid chromatography

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First received 26 November 1993; revised manuscript received 2 August 1994

Abstract

A comparison is made between the performance achieved in preparative chromatography when using elution, the most usual operating mode of this technique, and the different implementations of recycling. This work is based on the experimental results obtained in the separation of the enantiomers of ketoprofen on a cellulose-based stationary phase, using the various chromatographic modes. It uses also the modeling of non-linear chromatography to compare the different operating modes considered. Theoretical and experimental results are presented and compared. In some cases, it is possible to achieve simultaneously both an increase in the production rate and a decrease in the amount of eluent needed.

1. Introduction

High-performance preparative liquid chromatography is becoming an increasingly popular industrial separation process [1]. It uses fine particle stationary phases, typically in the 10–30 μm range, thus ensuring faster mass transfers than in classical adsorption processes. Although high column efficiency is generally achieved, the production rate is limited by the relatively low capacity of the stationary phases, so the preparative chromatographic process remains expensive and requires careful optimization in order to minimize production costs. In particular, it has long been recognized that preparative columns must be operated under strongly overloaded conditions, in a concentration range where adsorption isotherms are no longer linear.

A number of modifications of the conventional elution mode have been suggested to enhance further the performance of the process [2–4]. Some, such as simulated moving bed [4], are complex and require dedicated equipment. Others are simple and can be implemented with minor changes of the equipment used in elution. Bailly and Tondeur [5–7] proposed different implementations of recycling, taking advantage of non-linear effects arising in chromatography at high concentrations. Using the ideal model of chromatography, they studied the application of these different modes to the separation of binary mixtures by ion-exchange chromatography and showed the potential advantages. Extension of this work to other modes of chromatography is straightforward. However, they did not consider

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0021-9673/94/$07.00
SSDI 0021-9673(94)00728-4

the influence of a finite column efficiency, and there are no studies comparing the performance of the various implementations of recycling. It must be pointed out at this stage that these methods are not related to the conventional "peak shaving" technique, developed by analytical chromatographers. In this method the eluent is continuously recycled from the exit back to the column inlet as long as its composition does not meet one of the criteria for fraction collection. In the present case, the eluent containing the material to be recycled is collected, stored until the end of the cycle and injected at the beginning of the new cycle, before the complement of fresh feed or mixed with it. A steady state is achieved in which the amount of material in the column, the chromatogram and the production are all the same for each cycle, whereas in recycling with peak shaving an injection of pure fresh feed is done only every so many cycles, after the amount of material has been reduced to none by shaving at the end of each cycle [3].

We have undertaken a comparative investigation of the eluent consumption, the production rate and the recovery yield at a given purity of conventional elution and of the recycling procedures [5–7]. This study takes into account the finite rate of mass transfer kinetics in the column. It involves numerical calculations and experimental determinations, all made for the same separation problem. As a case in point, we chose the separation of the enantiomers of ketoprofen. To perform this separation we used a chiral stationary phase (CSP) prepared by adsorption of cellulose tris(4-methylbenzoate) on a macroporous silica support [8], and a mixture of *n*-hexane, 2-propanol and acetic acid as the mobile phase. Among CSPs, adsorbed cellulose esters are characterized by a high loading capacity and a fast rate of mass transfer kinetics, and hence a high column efficiency [9].

This separation was chosen because enantiomer purification is a problem of current concern in the pharmaceutical industry. Further, these binary separations permit simple calculations while giving relevant results of practical importance [10].

2. Theory

2.1. Description of the chromatographic modes studied

In the elution mode, the simultaneous achievement of a large recovery yield and a high product purity requires a significant degree of resolution between the two bands at the column outlet. This limits seriously the amount of feed which can be injected in each cycle. If the feed amount is increased beyond this limit to obtain a higher production rate, a mixed zone of growing importance appears in the chromatogram. To achieve both the high recovery and the purity required, this fraction must be recycled. Different implementations are possible.

Besides the classical overloaded elution mode (Fig. 1), we have studied three different recycling procedures, the recycling of an intermediate, mixed zone (Fig. 2), the recycling of the dilute tail of the second band (Fig. 3) and a combination of these two methods (Fig. 4). Recycling must be combined with sample injection. Fresh sample may be added to the recycled fraction at the end of every cycle, or at the end of every second cycle, or never, the next sample being injected only when all the amount injected has been purified. This last approach has been discussed previously [3]. In this work, we assume that some amount of fresh sample is added to the recyclate at the beginning of each new cycle. In this case, operation under cyclic steady state conditions is required in order to achieve the potential performance of the method.

Elution chromatography

Elution (Fig. 1) is still the most usual operating mode in preparative chromatography. The

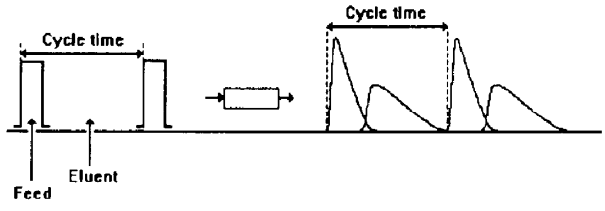


Fig. 1. Schematic representation of elution chromatography.

column is swept by a stream of eluent at constant flow-rate. A given amount of feed is injected periodically at its inlet. The cycle time or period between two successive injections is chosen in order to avoid delay between successive chromatograms. In the separation of a binary mixture, the goal is the production of one component with a minimum degree of purity. Switching valves at appropriate times begins and stops the collection of the useful component. These "cut times" are chosen in order to achieve the required purity.

The performance of the process can be evaluated using one of several criteria: the production rate, the amount of stationary phase immobilized, the amount of solvent needed for the purification of a unit amount of component and the concentration of the collected product. Our purpose is to study operating modes that could successfully replace elution chromatography. In this work, we deal with two operating modes which have already been introduced in the literature [5,7], and with an original combination of them. In all instances, the implementation requires a single column.

Recycling of an intermediate cut

In "recycling with mixing" (Fig. 2A), the non-separated, intermediate zone of the chromatogram is collected during any given cycle, lumped into one fraction and mixed with a given amount of the fresh feed solution. The resulting mixed feed (whose composition differs from that of the actual or fresh feed) is injected into the column as a pulse at the beginning of the next cycle. The cut times are determined in order to satisfy the product purity requirements.

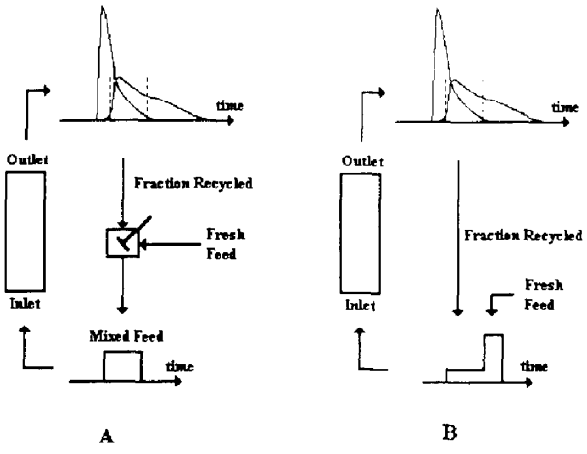


Fig. 2. Schematic representations of (A) "recycling with mixing" and (B) "segmented recycling".

Assuming an infinitely efficient column and a separation factor independent of the concentrations (e.g., with a competitive Langmuir isotherm), Bailly and Tondeur [5] have shown that this procedure could save eluent and give more concentrated products than the elution mode. They derived equations permitting the calculation of the optimum operating parameters of this process.

Because recycled fraction and fresh feed have different compositions, their mixing results in a loss of the separation work performed by the column. "Segmented recycling" (Fig. 2B) is similar to "recycling with mixing", but avoids this loss of separation. The mixed zone of the chromatogram is collected and lumped into one fraction, as in the recycling with mixing mode, but in the next cycle the recycled fraction is pumped into the column just before the new injection of fresh feed is performed [7].

Recycling of the second peak tail

In most instances, equilibrium isotherms are convex upwards, so each band exhibits a steep front, or shock layer, and a diffuse rear boundary [10]. Thus, the peak of the more retained component has a long tail, and the purified fraction containing this component is dilute. The phenomenon may still be aggravated by the consequence of the tag-along effect [10], which spreads the last component over a wide retention range. Rather than collect the highly dilute part of the fraction, it may be more advantageous to recycle it. The corresponding procedure is described in Fig. 3. The injection is made as in "segmented recycling", the recycled fraction being pumped into the column just ahead of the new fresh feed sample. Note that, because the tail is collected, stored and injected ahead of the fresh feed, the tail will not grow longer progressively but will stabilize after a few cycles.

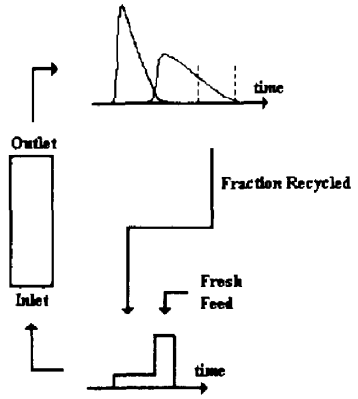


Fig. 3. Schematic representation of "recycling of the second peak tail".

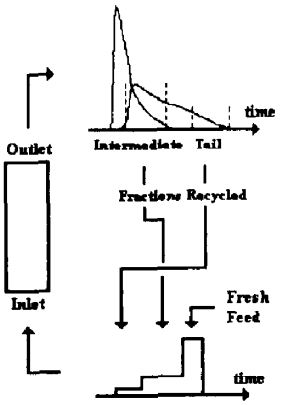


Fig. 4. Schematic representation of "combination of recyclings".

This is what distinguishes this method from the conventional "peak shaving" method [3]. The cut point for the recycling of this tail depends on the optimization criterion.

Combination of recycling modes

Because of the dispersive rear front, the production rate of the more retained component of a mixture is always lower than that of the less retained component in elution, and the collected fractions are less concentrated. The operating modes based on the sole recycling of the intermediate, mixed zone suffer from the same drawback. Hence, it seems attractive to combine the two recycling modes discussed above, as shown in Fig. 4. The injection is made in three steps. The recycled fraction containing the dilute end of the more retained component band is injected first, followed by the mixed, recycled fraction and, finally, a new amount of fresh feed.

2.2. Economic criteria and adjustable variables

Economic criteria were used to optimize the experimental conditions of the chromatographic procedures and compare their performances. The relationships between the cost components of the process and its technical parameters are investigated first.

Economic criteria

Optimizing an industrial process comes down to minimizing its production cost. Following the cost analyses given by Nicoud and Colin [1] and by Felinger and Guiochon [11], the components of the production cost of a chromatographic separation can be arranged into three main groups of contributions, the operating costs, OC, the cost of the crude material lost, CC, and the fixed costs, FC, with

$$PC = CC + OC + FC \quad (1)$$

CC is proportional to the recovery yield, Y_i, of the desired component i:

$$Y_i = \frac{\text{mass of compound of interest recovered}}{\text{mass of compound of interest injected}} \quad (2)$$

To permit a meaningful comparison, the experimental conditions will be chosen so that very close recovery yields be reached with the various operating schemes. In this case, the cost contribution of the loss of crude material has no effect on the comparison of the various procedures.

OC includes mainly the cost of the solvent required, the energy spent and the packing material [1,2,11]. Regeneration of the eluent for its recycling is essential for the economics of the chromatographic process. Its cost, including the cost of the energy required, added to the cost of

fresh solvent, and of waste management, constitutes the essential of the solvent cost and the main part of *OC* in most instances [1]. Although often minor [1], the cost of the packing material must also be taken into account. Labor costs are sometimes included in *OC* [1], which is correct if the duration of a production campaign is inversely proportional to the production rate. Because labor costs are more controlled by safety regulations and possible union contracts than by the actual production rate of a given unit, they are part of the fixed costs for continuous productions [11].

The unit operating costs, or separation cost per unit amount produced, can be reasonably estimated using the following three parameters:

(i) the volume of eluent needed to produce a unit amount of the purified product;

(ii) the specific amount of stationary phase used, or volume of stationary phase immobilized in a column of unit cross-sectional area; and

(iii) the productivity or specific production rate of the desired component, i.e., the mass of this compound produced per unit time and unit column cross-section.

FC represents fixed costs linked to the equipment investments. It includes the capital cost and, at least in the case of short batch production, the labor costs. The additional fixed costs of recycling include only the purchase of a few additional valves and the addition to the control program of the few lines of code needed to operate them, so we can neglect it. As all the operating procedures considered here are performed using virtually the same equipment and the same column, the fixed costs are essentially the same for all the operating modes examined.

Hence the comparison between the cost of the different operation modes will be based on a comparison of the maximum production rates possible and of the volume of eluent required.

Adjustable variables

The performance of the chromatographic separation process, and hence the production cost, depend on a number of parameters that can be adjusted prior to a new campaign. These parameters are:

(i) the concentration of the fresh feed injected, C_F; since in the present case we study the purification of either enantiomers from their racemic mixture, $C_{1F} = C_{2F}$;

(ii) V_F, the volume of fresh feed injected at the beginning of each cycle;

(iii) u_0, the mobile phase velocity;

(iv) L, the column length; because of the availability of dynamic axial compression columns, the column length can be considered as adjustable, at least within a certain range;

(v) for all the recycling modes, the composition and the volume of the recycled fractions are important intermediate parameters. They depend on the previous parameters and on the required values of the yield and purity, but are conveniently treated as variables.

Because the aim of this study is a comparison between the performance of the various operating modes, not their individual optimization, we do not need to optimize separately L and u_0. For each efficiency, the injection conditions (fresh feed and recycling) were optimized. This is done as followed. The composition of the fresh feed and the column efficiency are given, in addition to the purity and recovery yield required for the component of interest. The parameters that must be optimized are the volume of fresh feed injected and the characteristics of the recycled fraction. The optimum values of these parameters can be calculated easily in the case of an infinitely efficient column, using the analytical equations derived by Bailly and Tondeur [5–7] for the ideal model. Starting with this initial guess, numerical solutions of the equilibrium-dispersive model [10] permit the rapid determination of the optimum conditions for any column efficiency. The number of combinations of L and u_0 that permit one to obtain a certain value of N_P with a given column is infinite, but it has been shown that, for a given value of N_P, the production rate does not depend much on the individual choice of L and u_0 [11]. Further, the values of these parameters do not influence the ratios of production rates and of eluent consumptions in elution and in one of the recycling modes. Another advantage of this choice is that, for a given column, the amount of stationary

phase immobilized is the same for all the operating modes, and this criterion disappears from the comparison.

2.3. Modeling of non-linear high-performance liquid chromatography

In high-performance liquid chromatography, the columns have a finite but high efficiency. This fact makes the use of the equilibrium-dispersive model of chromatography particularly attractive [10]. In this model, the differential mass balance equation for one component in a chromatographic column is written as

$$\frac{\partial C_i}{\partial t} + \frac{1-\varepsilon_T}{\varepsilon_T}\frac{\partial q_i}{\partial t} + u_0 \cdot \frac{\partial C_i}{\partial x} = D_{i,ap} \cdot \frac{\partial^2 C_i}{\partial x^2} \qquad (3)$$

where C and q are the concentrations in the mobile and the stationary phases at equilibrium, respectively, ε_T is the total porosity of the bed, and is derived from the retention time, t_0, of a non-retained component whose propagation velocity is u_0, t_0 is also called the column holdup time and $D_{i,ap}$ is the apparent dispersion coefficient.

In the equilibrium-dispersive model, constant equilibrium between the mobile and stationary phases is assumed, and q is related to C by the equilibrium isotherm. The finite column efficiency due to axial dispersion and to the finite rate of the mass transfer kinetics is taken into account by the use of the lumped apparent dispersion coefficient, D_{ap}. We assume D_{ap} to be constant and equal to its value under linear conditions. Thus, D_{ap} is related to the column length, L, and to the number of theoretical plates, N_P, by the equation

$$D_{ap} = \frac{u_0 L}{2N_P} \qquad (4)$$

For a multi-component system, we must write as many Eqs. 3 as there are components. In this case, however, the different components of the mixture compete for access to the adsorption sites on the stationary phase. Thus, the amount q_i of component i adsorbed at equilibrium depends not only on C_i, but also on the concentrations of all the other components in the mobile phase, through the competitive adsorption isotherm:

$$q_i = q_i(C_1, C_2, \ldots) \qquad (5)$$

The determination of the competitive adsorption isotherms of the system components is the keystone of our modeling effort. Different approaches and equations to evaluate competitive isotherms, combined with the use of the equilibrium-dispersive model, have already been used successfully to calculate band profiles [10–15].

Finally, the solution of the system of partial differential equations of the problem requires proper initial and boundary conditions. The initial conditions are identical for all the operating schemes studied: the column is initially empty. As the column efficiency is usually high, the boundary conditions can be simplified by neglecting the dispersion effect [3,10,11], so they are reduced to the injection conditions at the column inlet.

Only numerical solutions of the equilibrium-dispersive model of chromatography can be calculated. The algorithms available have been reviewed recently [10,16]. For most practical applications, the forward–backward finite difference method proposed by Rouchon et al. [16] is the fastest and most efficient algorithm.

3. Experimental

3.1. Equipment

The experiments were performed using an HP1090 liquid chromatograph (Hewlett-Packard, Palo Alto, CA, USA), equipped with a multisolvent delivery system, an automatic sample injector with a 250-μl loop, a diode-array UV detector and a computer data acquisition system. Acquired data were downloaded to one of the computers at the University of Tennessee Computer Center. Also, a Gilson (Middleton, WI, USA) Model 203 fraction collector was used to complement the HP system.

3.2. Materials

Column

A 25 cm × 0.46 cm I.D. Chiralcel OJ column (Daicel, Tokyo, Japan) was used (average particle size 10 μm). This stationary phase was cellulose tris(4-methylbenzoate) adsorbed on macroporous silica [8]. The total column porosity ($\varepsilon_T = 0.674$) was derived from the retention time of the solvent peak. The use of this column was restricted by two independent constraints imposed by the manufacturer, allowing a maximum flow-rate of 1.5 ml/min and a maximum inlet pressure of 50 bar.

Mobile phase and chemicals

In all chromatographic experiments, the mobile phase was *n*-hexane–2-propanol–acetic acid (90:10:0.5, v/v/v). Hexane and 2-propanol were purchased from Burdick and Jackson (Muskegon, MI, USA) and acetic acid from Mallinckrodt (Paris, KY, USA). The *S*-(+)-enantiomer (purity 99%) and a racemic mixture (purity >99%) of ketoprofen were obtained from Rhône-Poulenc (Centre de Recherches de Vitry, Vitry, France). All these compounds were used as received.

3.3. Procedures

All the experiments were performed at 30°C. We determined the adsorption data for the pure *S*-(+)-enantiomer and the competitive adsorption data for different mixtures including the racemic mixture. The column efficiency was also measured at different mobile phase flow-rates. Very small sample amounts (2 μg) were injected. The column efficiency was derived from the width of the peaks at half-height. It was almost the same for the two components.

Determination of adsorption data

The single-component adsorption data were determined using the classical frontal analysis technique [17]. The experiments were performed at a flow-rate of 0.8 ml/min. Data were acquired in two concentration ranges. Twenty data points were measured between 0.2 and 4.3 g/l with detection at 365 nm and ten data points between 0.04 and 0.4 g/l with detection at 295 nm. At these two wavelengths, the detector response was found to be linear in the corresponding concentration range.

Competitive adsorption data were measured using the binary frontal analysis method [18]. In all experiments, the initial concentration of the enantiomers in the column was zero. Hence the concentration of the more retained component in the intermediate plateau was also zero and there was no need to analyze the corresponding eluate. This procedure greatly simplifies the experiments and improves the accuracy of the results. The concentration of the pure less retained component on this intermediate plateau was derived from the calibration graph at 370 nm, at which wavelength the detector response was linear over the whole concentration range studied.

The retention volumes and the corresponding concentrations were inserted into the equation given by Jacobson et al. [18] to determine the amounts adsorbed at equilibrium. The measurements of adsorption data could be made for values of the total concentration up to ca. 5.0 g/l. At higher concentrations, the intermediate plateau, where the less retained component was alone, disappeared.

Determination of elution profiles

Because the HP1090 chromatograph is not equipped with a large enough sample loop, the injections were made by programming the multisolvent delivery system. The individual elution profiles in the mixed band region were determined by collecting fractions of the eluent and reinjecting them on to the same column under analytical conditions. The time of collection for each fraction was chosen according to the flow-rate so that the fraction volume was ca. 80 μl. As the UV spectrum is achiral, the relative concentration of the two enantiomers in a fraction is equal to the peak-area ratio. This permits the rapid determination of the individual concentration profiles.

4. Results and discussion

4.1. Modeling of the separation of the enantiomers of ketoprofen on a cellulose-based stationary phase

We need to find a suitable model for the competitive isotherm data and a proper correlation for the dependence of the column efficiency on the mobile phase flow-rate. These models must then be validated.

Modeling of the competitive equilibrium isotherm

Typically, one of the two enantiomers was not available as a pure compound. Hence it was not possible to determine the competitive isotherm of the system following the classical procedure [12–15,19]. Only the pure *S*-(+)-enantiomer, which is also the more retained, and the racemic mixture were available, allowing the determination of the single-component adsorption data for the *S*-(+)-enantiomer, and the multi-component adsorption data for 1:1 and 1:3 [*R*(−)/*S*(+)] mixtures of *R*-(−)- and *S*-(+)-enantiomers. These experimental data were fitted together to obtain the best possible equilibrium isotherm.

The Scatchard plot of q/C vs. q of the experimental adsorption data for the *S*-(+)-enantiomer is not linear, which suggests that the classical Langmuir model cannot account for these data. Previous studies [12,13,15,19] have shown that on the surface of enantioselective phases two types of sites co-exist, enantioselective and non-selective sites. The former adsorb more strongly, but have a lower saturation capacity than the latter. A two-site adsorption model was successfully adopted, as for other enantiomers on the same stationary phase [19].

Accordingly, we fitted the experimental data to a bi-Langmuir isotherm. The first term represents the contribution of the chiral recognition mechanism and the second term accounts for the non-selective interactions. Previous, independent investigations of the chiral recognition process on cellulose-based CSPs have also shown the existence of two types of retention mechanism [20,21].

In compliance with the assumption that no chiral selectivity is involved in the second term of a bi-Langmuir isotherm, the coefficients A and B (Table 1) must be the same for both enantiomers, which reduces to six the total number of parameters of the two isotherms. We do not know at this stage the nature of the chiral retention mechanism, and thermodynamics cannot identify it. However, by analogy with previous results, we further assume that the column saturation capacities of the two enantiomers on the enantioselective sites are equal. Hence, $a_{(-)}/b_{(-)} = a_{(+)}/b_{(+)}$ (Table 1), which further reduces to five the number of parameters and ensures the thermodynamic consistency of the competitive isotherm model by satisfying the Gibbs–Duhem equation [22]. In spite of all these satisfactory properties, however, the isotherm model must be considered as empirical.

Five different sets of experimental data, one set for the pure *S*-(+)-enantiomer, one set each with the 1:1 mixture and the 1:3 mixture, for both enantiomers were handled together. We used a non-linear regression program based on a simplex algorithm to minimize the following objective function [19]:

Table 1
Isotherm parameters

Type of sites	Isomer	$a_{(*)}$ or A	$b_{(*)}$ or B (l/g)	q_s (g/l)
Selective	*R*-(−)-	4.40	0.202	21.7
Selective	*S*-(+)-	6.79	0.312	21.7
Non-selective	*R*-(−)- and *S*-(+)-	2.89	0.019	152.1

No. of parameters = 5. Model of competitive isotherm:

$$q_{(*)} = \frac{a_{(*)}C_{(*)}}{1 + b_{(-)}C_{(-)} + b_{(+)}C_{(+)}} + \frac{AC_{(*)}}{1 + B[C_{(-)} + C_{(+)}]}$$

where * = − or +. The coefficients $a_{(+)}$, $a_{(-)}$, $b_{(+)}$, and $b_{(-)}$ are the coefficients of the Langmuir adsorption isotherm of the (+) and (−) isomers, respectively, on the chiral selective site; the coefficients A and B are the coefficients of the Langmuir adsorption isotherm of either isomer on the nonselective sites.

$$\delta = \delta_{(-)} + \delta_{(+)} = \sqrt{\frac{1}{N_{(-)}} \sum_{1}^{N_{(-)}} \left(\frac{q_{\exp_i} - q_{\mathrm{cal}_i}}{q_{\exp_i}} \right)^2_{(-)}} + \sqrt{\frac{1}{N_{(+)}} \sum_{1}^{N_{(+)}} \left(\frac{q_{\exp_j} - q_{\mathrm{cal}_j}}{q_{\exp_j}} \right)^2_{(-)}} \quad (6)$$

where $q_{\exp}$ and q_{cal} are the experimental amount adsorbed and the value derived from the isotherm (Table 1), respectively, both functions of $C_{(-)}$ and $C_{(+)}$. The *S*-(+)-enantiomer adsorption data were only involved in $\delta_{(+)}$ and used with $C_{(-)} = 0$. The best values obtained for the isotherm parameters are summarized in Table 1. A comparison of experimental and calculated adsorption data is made in Fig. 5, demonstrating an excellent fit of the model to the experimental data. The average difference between the calculated and experimental adsorption data was less than 1% for both components. The initial slopes of the isotherm were in very good agreement with the retention times derived from chromatograms obtained under linear conditions, when very small amounts were injected.

Efficiency of the column

The column efficiency was measured for different values of the mobile phase flow velocity, between 0.03 and 0.20 cm/s, corresponding to flow-rates between 0.2 and 1.4 ml/min for a 0.46 cm I.D. column. Very small sample amounts (2 μg) were injected. The column efficiency was derived from the width of the peaks at half-height. Fig. 6 shows a plot of the experimental values of the height equivalent to a theoretical plate (HETP) (symbols) versus the flow velocity, u_0. The range of flow-rates studied was limited because of the constraint of a maximum of 1.5 ml/min given by the column manufacturer, independent of the maximum inlet pressure equal to 50 bar [with ΔP (bar) = 8.37 L (cm) u_0 (cm/s) $\leq$ 50 bar].

The column efficiency was almost the same for the two components. The difference observed at the highest velocity is lower than the experimental inaccuracy. In the velocity domain studied, a

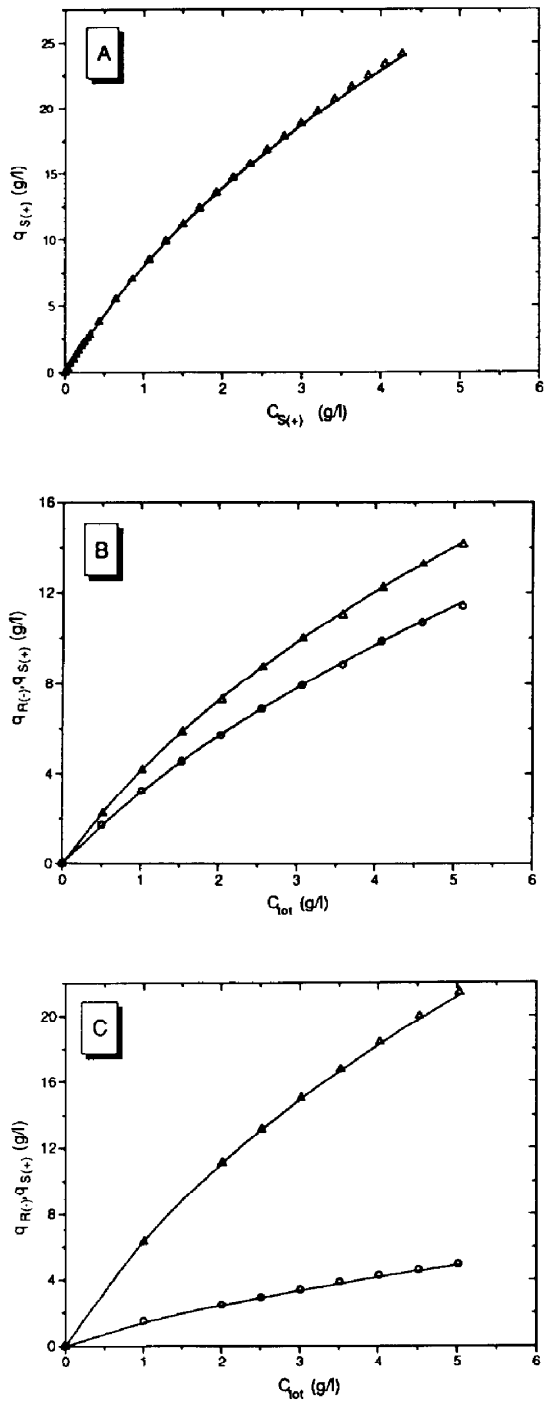


Fig. 5. Experimental adsorption data (symbols) for the ketoprofen on a Chiralcel OJ stationary phase at 30°C. ○ = *R*-(−)-enantiomer; △ = *S*-(+)-enantiomer. (A) *S*-(+)-alone; (B) $C_{(-)} = C_{(+)}$; (C) $C_{(+)} \approx 3.5 C_{(-)}$.

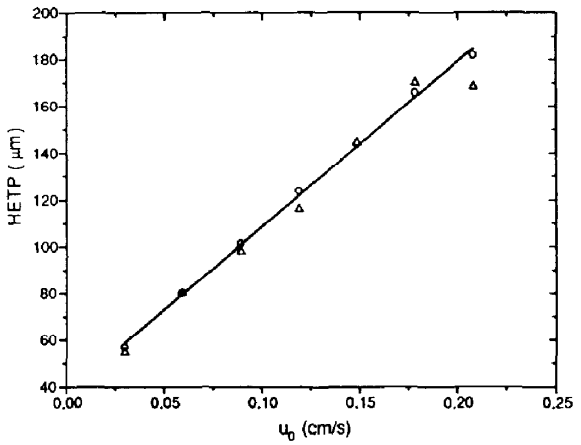


Fig. 6. Experimental [○ = *R*-(−)- and △ = *S*-(+)-enantiomer] and calculated (line) according to Eq. 8 plate heights as a function of the velocity u_0

simple linear relationship could be used to relate the HETP and u_0:

$$\text{HETP}(\mu\text{m}) = 707.4\, u_0\ (\text{cm/s}) + 37.6 \qquad (7)$$

Similar behavior has been previously observed [23]. Horváth and Lin [24] suggested this representation at the relatively (depending on the stationary phase) high mobile phase velocities usually selected in preparative chromatography.

Validation of the model

To check the validity of the band profiles calculations, we compare in Fig. 7 the calculated and experimental individual band profiles obtained in two different cases. The calculated profiles were obtained using the equilibrium-dispersive model [10], the competitive isotherm (Table 1), and the correlation between column plate height and mobile phase velocity (Eq. 7, Fig. 6). The experimental individual band profiles of the two enantiomers were obtained by analysis of collected fractions.

The comparison was done for large samples of two different binary mixtures, having relative compositions 1:1 and 1:3. The total amount injected was about 9 mg for the 1:1 mixture and 7 mg for the 1:3 mixture. These amounts correspond to the high degree of column overload typical of non-linear chromatography. The injection concentration exceeded only slightly the range within which the experimental adsorption data had been determined. Two different flow-rates were used for the two experiments, 0.6 ml/min for the 1:1 mixture, corresponding to an efficiency of 2500 theoretical plates, and 1.2 ml/min for the 1:3 mixture, corresponding to 1500 plates for our column.

As shown in Fig. 7, there is an excellent agreement in both instances between the experimental and the calculated band profiles, demonstrating the validity of the equilibrium-dispersive model, as reported previously [10–16,19]. In both instances, the first component band is slightly shorter than predicted, by approximately 10%. With the 1:3 mixture (Fig. 7B), we observe a small retention time difference of ca. 2% between the calculated and measured profiles. This difference could be explained by a corresponding error on the set flow-rate.

The agreement observed in Fig. 7 between experimental and calculated profiles justifies the use of the equilibrium-dispersive model and the bi-Langmuir isotherm model in all further calculations required by the comparison between recycling procedures.

4.2. Comparison of the various operating modes

We are interested in the production from the racemic mixture of one or other of the two enantiomers, with a purity of 99%. Because of the fundamentally unsymmetrical behavior of the interactions between the two component bands in non-linear chromatography [10], the results of the comparison differ strongly depending on whether the desired component is the less or the more retained component. These two cases are studied successively in the next two sections.

As mentioned in the Theoretical section, the variables involved in the comparison are the injection concentrations and volumes and the column efficiency N_P. For a given value of N_P, we have calculated the optimum injection conditions for the various operating modes and evaluated the performances achieved (prod-

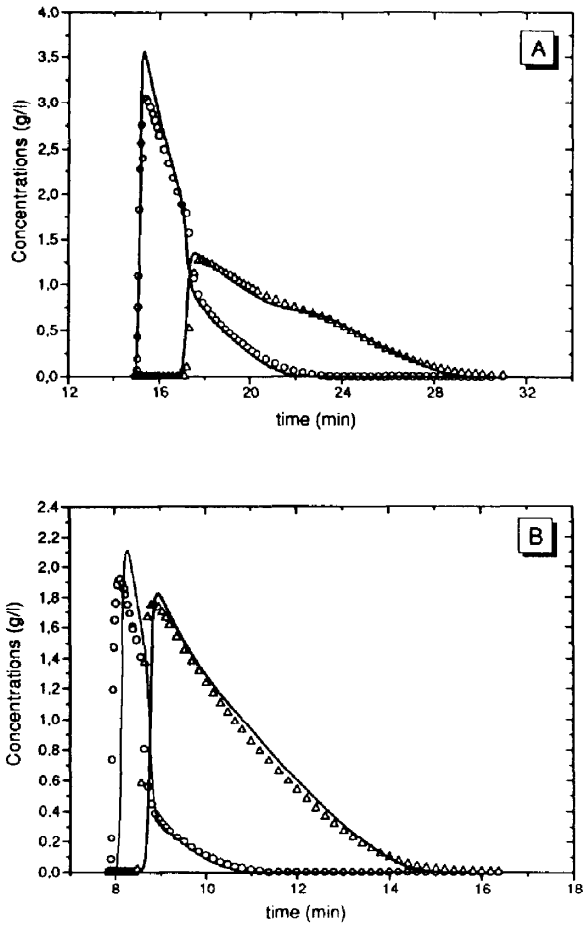


Fig. 7. Comparison between experimental [○ = *R*-(−)- and △ = *S*-(+)-enantiomer] and calculated (lines) individual profiles for an injection of a binary mixture. (A) $C_{1,0} = C_{(+)} = 3.72$ g/l; volume injected, 1.2 ml; flow-rate, 0.6 ml/min; column efficiency, 2500 plates. (B) $C_{1,0} = 1.49$ g/l; $C_{1,0} = 4.74$ g/l; volume injected, 1.2 ml; flow-rate, 1.2 ml/min; column efficiency, 1500 plates.

uctivity, eluent consumption). These calculations were repeated for several values of the number of theoretical plates.

The total concentration of the fresh feed was set equal to 6 g/l ($C_{1F} = C_{2F} = 3$ g/l). Although this injection concentration is not optimum, it was chosen for experimental, practical purposes because it is easy to achieve. Higher injection concentrations are not useful. Numerical calculations showed that no improvements of the economic parameters was obtained when the total injection concentration of the fresh feed exceeded 12 g/l. Moreover, this choice of C_{1F} and C_{2F} was observed to have no influence on the conclusions of the comparison.

First case: production of the less retained component

Figs. 8 and 9 illustrate the performance of the recycling operating modes compared with that of simple elution (for the absolute performance of the elution mode and of combination of recyclings, see below). They show plots of the production rate (Figs. 8A and 9A) and the

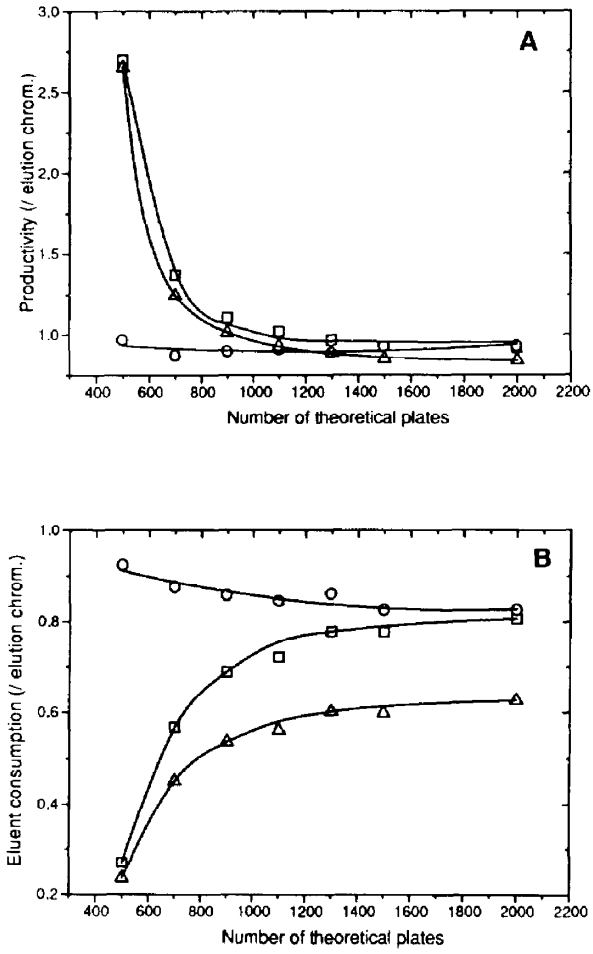
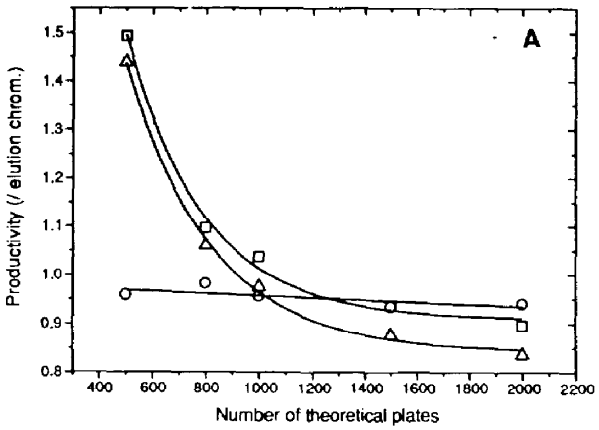


Fig. 8. Comparative performances of the operating modes studied with respect to elution chromatography for various column efficiencies. The less retained component is the product of interest. Minimum recovery yield sought = 99% (purity = 99%). ○ = "Recycling of the second peak tail"; □ = "segmented recycling"; △ = "combinations of recyclings".



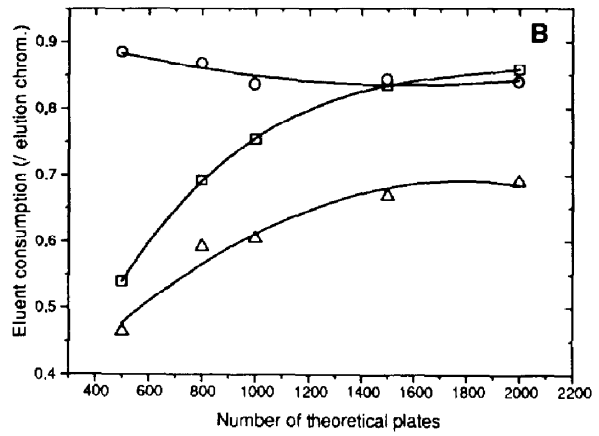


Fig. 9. Same as Fig. 9, but minimum recovery yield sought = 90%.

eluent consumption (Figs. 8B and 9B) versus the column efficiency. Figs. 8 and 9 correspond to two different values of the required recovery yield, 99% and 90%, respectively. The injection conditions selected are optimum for the corresponding recovery yield constraint.

In the elution mode, the volume of fresh feed injected is the only variable there is to optimize for each column efficiency. In practice, this volume is the one for which the recovery yield required is just achieved. For the other operating schemes, the determination of the optimum injection conditions is more complex. A precise analysis is required for all of them. The ideal model is very useful because it gives a good understanding and descriptive tool, and it permits a rapid estimation of approximate values of the optimum conditions, allowing a rapid choice of the proper experimental conditions for band calculations or for actual experiments.

Recycling of the second peak tail. As shown in Figs. 8 and 9, the performance of the mode "recycling of the second peak tail" relative to that of elution is almost independent of the column efficiency. A nearly constant but significant eluent saving, between 13 and 20%, is achieved by recycling the highly dilute fraction, but at the cost of a few percent decrease in production rate. With this recycling mode, the degree of separation reached at the column outlet is of the same order as in elution chromatography, but slightly lower. To obtain the same recovery yield, the amount of fresh feed injected must be slightly smaller. This causes a decrease in the production rate. It is not surprising that recycling the tail of the second band has a minor effect on the production rate and solvent consumption for the first band.

"Segmented recycling" and "recycling with mixing". Different results are obtained in "segmented recycling". At high efficiencies, this mode also offers a compromise between a loss of productivity and a decrease of the eluent consumption. The terms of the compromise are very close to those of "recycling of the tail" of the second peak.

By contrast, at low column efficiency, both an increase in the production rate and a decrease in the eluent consumption can be achieved simultaneously with "segmented recycling" and with "recycling with mixing". This is a very attractive proposition. The importance of the improvement increases with decreasing column efficiency. For example, with an efficiency of 500 theoretical plates and a recovery yield of 90%, the production rate can be increased by 50% and the eluent consumption halved. For a recovery yield of 99%, the gain in solvent consumption is close to 70% while the production rate is multiplied by 2.7, a considerable gain. We note that the rela-

tive retention of the two enantiomers at infinite dilution (Table 1) is 9.68/7.29 = 1.33. With this relative retention and the value of the retention factor (3.1), a resolution of 1.05 is achieved with a 500-plate column.

Fig. 10 explains the relative behavior of the "segmented recycling" and elution modes of operation. It shows plots of the optimum amount of fresh feed injected at the beginning of each cycle versus the column plate number, N_P, relative to the optimum amount which would be injected on an infinitely efficient column, for the two operating modes. Fig. 10A and B correspond to recovery yields of 99% and 90% (always with a purity constraint of 99%), respectively. For an infinitely efficient column, the optimum amount of fresh feed injected for each cycle would be the same with both modes. At high but finite efficiencies, the optimum amounts of fresh feed injected are almost identical. The decrease in production rate observed at high efficiency in "segmented recycling" is due to a longer cycle time. The eluent saving stems from an increase by a factor of 2–3 of the concentration of the less retained component in the eluent. This increase comes from the enhancement at higher concentrations of the non-linear effects involved in preparative chromatography. In this case the results are similar to those predicted by the ideal model.

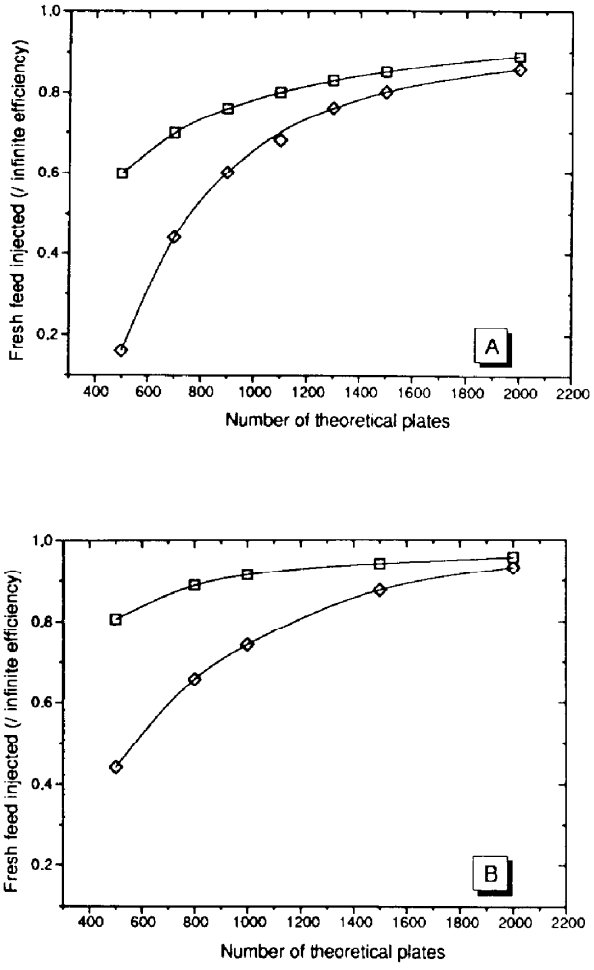


Fig. 10. Amount of fresh feed injected at each cycle for various column efficiencies compared with the amount injected to reach the same purity and recovery yield on a column of infinite efficiency. The less retained component is the product of interest (purity = 99%). Recovery yield = (A) 99% and (B) 90%. ◇ = Elution chromatography; □ = "segmented-recycling".

At low column efficiencies, the optimum amount of feed injected in the "segmented recycling" mode is much higher. The negative influence of the poor column efficiency (i.e., of the kinetic and hydrodynamic effects) is much stronger in the elution mode than in recycling. Elution performance is more strongly affected than recycling performance at small numbers of theoretical plates. This is especially true when the required degree of separation is high, i.e., for a required recovery yield of 99%.

The comparative performance of the "recycling with mixing" mode (Fig. 2A) is not given in Figs. 8 and 9, for the sake of clarity. It is too similar to that of "segmented recycling", albeit slightly inferior.

"*Combination of recyclings*". This combination of operating mode associates positively the performance achieved with the two simple modes. Its main interest appears at high column efficiencies, where the compromise between productivity and eluent consumption that it affords is the most attractive. For instance, with a 2000-plate column and a recovery yield of 99%, there is a 15% decrease in the production rate, but also a 40% saving in the eluent consumption. At low column efficiencies, the results achieved are

close to those given by the mode of "segmented recycling".

Conclusion. It appears that, for the production of the first component, the operating modes of "segmented recycling" and "combination of recyclings" offer better performance than elution, with often a higher production rate and always a lower solvent consumption. These modes are less sensitive to the spreading effects of a finite column efficiency than the conventional elution mode. Accordingly, we can expect their optimum operating conditions to be found at lower values of the column efficiency. At best, both an increase in production rate and a decrease in eluent consumption will be achieved. At worst, significant eluent savings will have to be paid for by a moderate decrease of the production rate. When the eluent consumption is the major contribution to the production cost, recycling could be justified even at the expense of a significant decrease in the production rate.

We compare in Fig. 11 the plots of the absolute values of the production rate or productivity and the eluent consumption obtained in elution and in "combination of recyclings" as a function of the column efficiency. The plots in Fig. 11 were obtained for a recovery yield of 90% and a purity of 99%. The column length, the mobile phase velocity and the amount of feed injected were optimized separately for each value of the column efficiency, as explained above, using Eq. 7 and the condition $\Delta P \leq 50$ bar to derive the mobile phase velocity. These plots demonstrate that the production rate of elution is maximum for an efficiency between 850 and 900 theoretical plates and that the solvent consumption decreases steadily with increasing efficiency, with minimum changes to be expected beyond 2000 plates. These results are in excellent agreement with those derived by Felinger and Guiochon [11]. By contrast, the production rate with "combination of recycling" increases with decreasing efficiency and would exhibit a maximum only below 500 theoretical plates. This is a result of the relative insensitivity of the production rate on the hydrodynamics and kinetic effects, as noted above. As in elution, the solvent consumption in "combination of recycling" decreases with increasing column efficiency but it is much lower, especially at low efficiencies. Fig. 11 permits further direct comparisons between the performance of elution and "combination of recycling". For example, for N_P = 800, a number close to that for which the production rate is maximum in elution, the combination of recyclings offers nearly the same production rate but a 40% saving in eluent consumption. For N_P = 500, this combination permits a production rate 25% larger than elution did at N_P = 800 plates and at the same time a 30% decrease in eluent consumption. This example illustrates the attractive potential of the "combination of recyclings" mode in preparative chromatography.

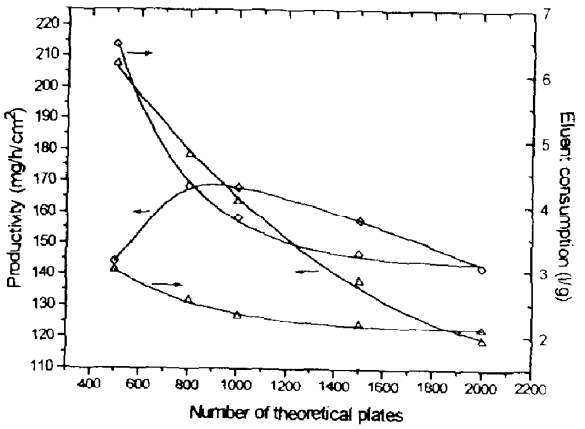


Fig. 11. Dependence of the production rate or productivity ($mg/h \cdot cm^2$) for the less retained isomer and of the eluent consumption (l/g) on the column efficiency (◇) in the conventional elution mode and (△) in "combination of recyclings". Minimum recovery yield = 90%; purity = 99%.

The substitution of conventional elution by a recycling procedure, although admittedly more complex to implement, is especially attractive when, for one reason or another, it is difficult to obtain an efficient column (see Figs. 9 and 10). In such a case, the performance of the chromatographic process is improved considerably. This is the case for certain chiral phases, for example for microcrystalline cellulose triacetate, which is the most widely used phase for the industrial separation of enantiomers by preparative chro-

matography, and with which it is difficult to prepare columns having an efficiency in excess of ca. 1000 plates per metre [13,25]. In this case, the recycling operating modes presented here could be very attractive.

Finally, the selectivity of the system studied here, ca. 1.33, is relatively high. For systems exhibiting a lower selectivity, similar conclusions are expected. Much higher column efficiencies would be required, but the advantage resulting from the enhancement of the displacement effect when the sample size is increased, as allowed by recycling procedures, would permit the same separation to be obtained at efficiencies that are low compared with those which the elution mode would require. Further work is required to reach a definitive conclusion on this point.

Second case: production of the more retained component

If the product of interest is the more retained component, it has been shown that the production rate of a purified product does not increase significantly with increasing sample size beyond the stage where the two bands begin to interfere significantly and the whole amount of second component injected cannot be collected [26]. For an infinitely efficient column, the optimum sample size is that for which the recovery yield begins to drop below 100%. The result is similar for a real column. There is an optimum amount injected, which gives both the maximum production rate and the minimum eluent consumption [27,28]. The corresponding recovery yield of the more retained component depends on the column efficiency. It tends towards 100% with increasing column efficiency.

Fig. 12 compares the performances of "segmented recycling" and "combination of recyclings" with that of the elution mode. The optimum amount injected for elution was determined for each column efficiency. For the other two operating modes, the recovery yield was very close to 99%. Fig. 12 shows that in this case, the simple elution mode is the most favorable approach. "Segmented recycling" and "combination of recyclings" have performances that are nearly independent of the column efficiency. "Segmented recycling" causes a 5–10% decrease in production rate, but also a 20–25% eluent saving. "Combination of recyclings" gives a 10–15% decrease in production rate, but a 35–40% eluent saving. The compromise offered by "combination of recyclings" may be attractive for industrial separations, when the eluent consumption is the major part of the production costs.

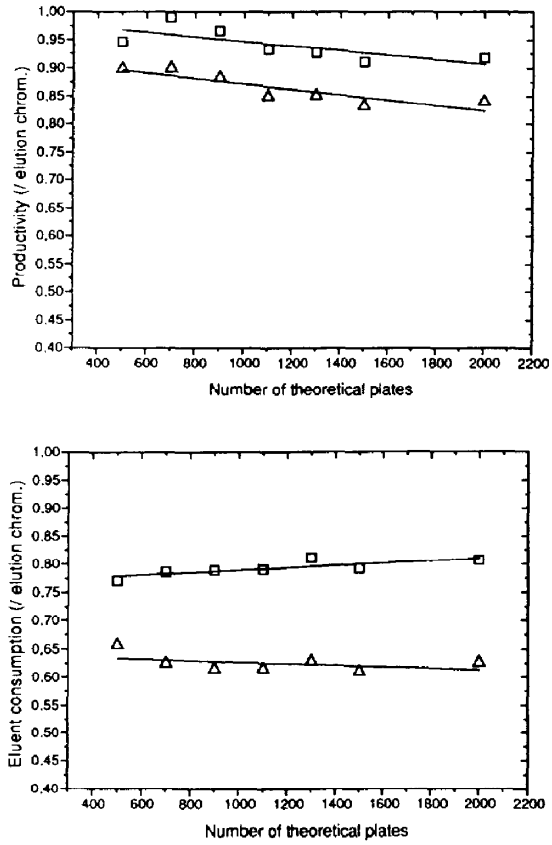


Fig. 12. Comparative performances of the operating modes studied to elution chromatography for various column efficiencies. The more retained component is the product of interest. □ = "Segmented recycling"; △ = "combination of recyclings".

It must be pointed out that a considerable improvement of the separation performance would also be observed at low efficiencies if a high minimum recovery yield was required. The situation would be the same as in Fig. 9 when the less retained component is the product of interest.

4.3. Experimental illustration

Experiments were conducted with the chromatographic system and equipment described above to investigate the conclusions of the theoretical study and the validity of the models used. The column efficiency was 2000 theoretical plates, for a length of 25 cm. The flow-rate was set at 0.8 ml/min, corresponding to a velocity $u = 0.119$ cm/s. Our objective was the production of either enantiomers with a purity and a recovery yield of 99%. As explained above, the total concentration of the fresh feed was chosen equal to 6 g/l, so the injection volumes would be large enough to permit the injection of accurately known volumes using the solvent delivery system.

The various modes of recycling operate under cyclic steady-state conditions. A dynamic equilibrium must be reached before measurements can be carried out. If a production run is started with the injection of pure feed, the chromatogram, the production per cycle at a given purity and the composition of the recycled fraction will drift toward their steady-state values but it may take a significant number of cycles before this dynamic equilibrium is reached. This process can be accelerated by a judicious choice of the start-up conditions, i.e., by mixing the fresh feed with a solution having a composition close to that of the recycled fraction. An educated guess is required, which is easy if previous purifications have been made with the same feed. For a new separation, the easiest method consists in calculating, then preparing the solutions corresponding to the composition of the recycled fractions of the cyclic steady state [5]. This preparation is easily achieved using the solvent delivery system of our chromatograph.

Figs. 13–16 compare the experimental (symbols) and the calculated (lines) chromatograms obtained during the first cycles of the various operating schemes. For these first cycles, synthetic solutions were prepared according to the numerical prediction of the corresponding cyclic steady state with a 2000-plate column. For "recycling with mixing" (Fig. 14), a synthetic solution with the calculated composition of the

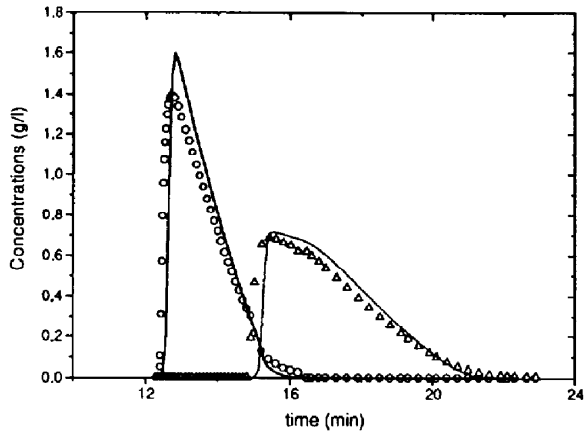


Fig. 13. Comparison between experimental [○ = *R*-(−)- and △ = *S*-(+)-enantiomer] and calculated (lines) concentration profiles for elution chromatography. Flow-rate, 0.8 ml/min (2000 plates); $C_{(-)} = C_{(+)} = 3.0$ g/l; volume injected, 0.62 ml.

mixed feed was injected as a rectangular plug. In the case of "segmented recycling", the first cycle (Fig. 15A) was repeated several times and the mixed zone of the chromatogram was collected according to the cut times given by numerical simulations. The resulting solution was used for a second cycle (Fig. 15B). For "recycling of the second peak tail" (Fig. 16), we made several

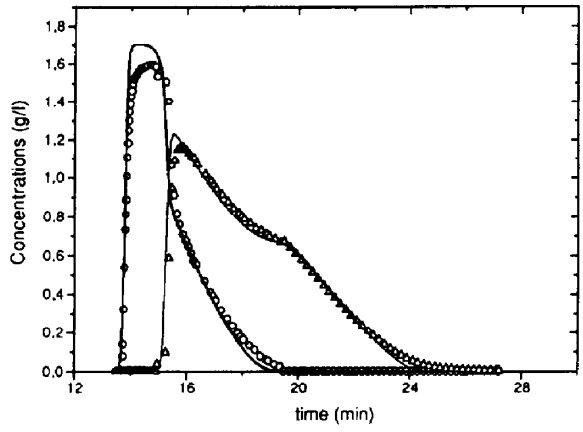


Fig. 14. Comparison between experimental [○ = *R*-(−)- and △ = *S*-(+)-enantiomer] and calculated (lines) concentration profiles of the first cycle of "recycling with mixing". Mixed feed: $C_{(-)} = 0.99$ g/l; $C_{(+)} = 1.38$ g/l; volume injected, 3.06 ml.

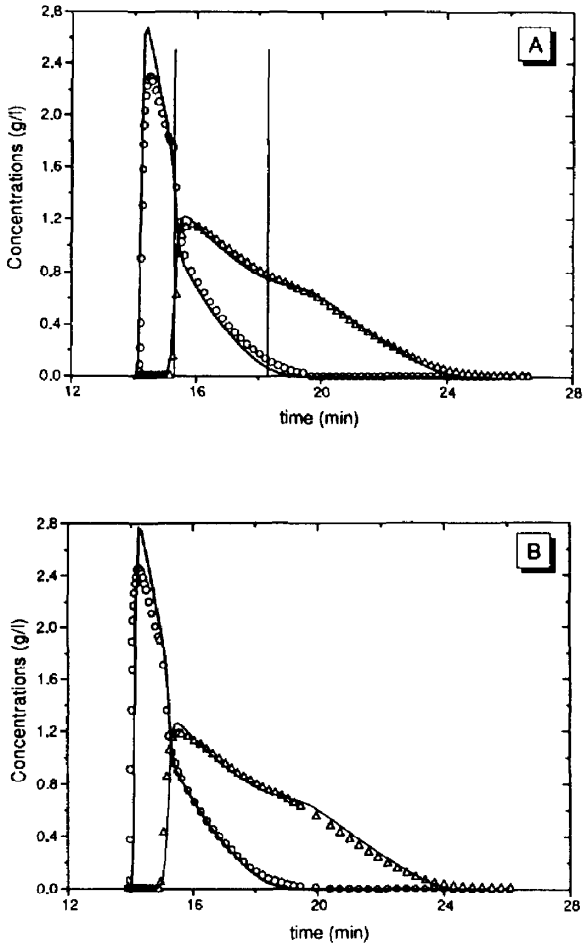


Fig. 15. Comparison between experimental [○ = *R*-(−)- and △ = *S*-(+)-enantiomer] and calculated (lines) concentration profiles of (A) the first and (B) the second cycles of "segmented recycling". Fresh feed: $C_{(-)} = C_{(+)} = 3.02$ g/l; volume injected, 0.62 ml. Recycled fractions: $C_{(-)} = 0.490$ g/l; $C_{(+)} = 0.975$ g/l; volume injected, 2.40 ml (first cycle); and $C_{(-)} = 0.541$ g/l; $C_{(+)} = 1.028$ g/l; volume injected, 2.40 ml (second cycle).

injections of fresh feed and collected the rear part of the elution profiles. This solution was used to generate the volume of recycled fraction of the more retained component which was needed.

A very good agreement is generally observed between the experimental and calculated band profiles shown in these figures, as also seen in Fig. 7. The model used is able to predict with excellent qualitative agreement the results of

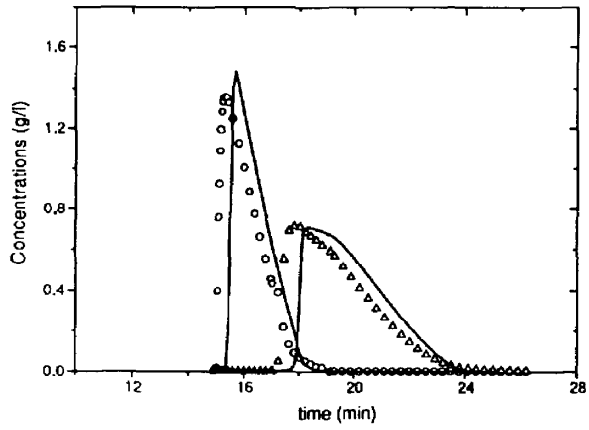


Fig. 16. Comparison between experimental [○ = *R*-(−)- and △ = *S*-(+)-enantiomer] and calculated (lines) concentration profiles of the first cycle of "recycling of the second peak tail". Fresh feed: $C_{(-)} = C_{(+)} = 3.0$ g/l; volume injected, 0.62 ml. Recycled fraction: $C_{(+)} = 78$ mg/l; volume injected, 2.24 ml.

actual series of experiments. The purity and recovery yield of the products were derived from the experimental profiles, as well as the values of the economic criteria. However, the first conclusion from these determinations was that the required values of the purity and the recovery yield were not exactly achieved in the experiments (Table 2). In spite of the excellent agreement between calculated and measured band profiles (Figs. 7 and 13–16), the model does not predict accurately enough the performance of the operating modes. Errors of the same order of magnitude have been reported by Jacobson et al. [15,29].

One of the main reasons for this error is that the model does not predict correctly the tail of the less retained component band in the mixed zone of the chromatogram. Some empirical adjustment (amounts injected, cut times) remains necessary. This error originates from inaccuracies in the competitive isotherm model, which does not predict exactly the magnitude of the displacement effect (e.g., Fig. 14). The errors made in the determination of the competitive isotherm are in part due to the unavailability of the less retained compound, in part owing to the use of a simple model.

Table 2
Purity and recovery yield of the collected fractions

Mode	P_1 (%)	Y_1 (%)	P_2 (%)	Y_2 (%)
Elution	98.1	95.6	96.9	93.3
"Recycling with mixing"	98.1	99.2	97.4	~100
"Segmented recycling"	97.0	97.0	97.0	96.7
"Rectckubg ot the second peak tail"	98.1	95.6	96.9	93.3

The aim was a purity (P) of 99% and a recovery yield (Y) of 99%.

5. Conclusions

Although it is accurate enough to give excellent results in qualitative comparisons between calculated and experimental band profiles, the combination of techniques used for the modeling of competitive equilibrium isotherms and for the calculation of band profiles in non-linear chromatography does not predict exactly the recovery yield or the degree of purity achieved. Nevertheless, there is a semi-quantitative agreement, and important conclusions can be derived from calculated profiles regarding the optimization of experimental parameters.

The theoretical investigation made of several operating modes based on recycling of intermediate fractions shows that they might often be more economically attractive than conventional elution chromatography. Almost always, the eluent consumption is reduced significantly. In a number of cases, the production rate of the first component of a binary mixture is markedly increased. In the case studied, the separation required is easy, the column efficiency needed is low and the use of recycling brings dramatic improvements in both the production rate, which increases, and the solvent consumption, which decreases. The advantage of recycling remains probably as important when the separation carried out is difficult.

The major part of the separation cost of the processes using high-performance preparative liquid chromatography, at least when organic solvents are used, is related to the solvent costs. Accordingly, the operating modes studied here are of great interest because of their potential for significant eluent savings.

Acknowledgements

F.C. thanks Rhône-Poulenc for the financial support of this work. This work was supported in part by Grant CHE-9201663 of the National Science Foundation and by the cooperative agreement between the University of Tennessee and the Oak Ridge National Laboratory. We acknowledge the support of our computational effort by the University of Tennessee Computing Center.

Symbols

a, b	Isotherm coefficients, enantioselective sites (B l/g)
A, B	Isotherm coefficients, non-enantioselective sites (B: l/g)
C	Concentrations in the mobile phase (g/l)
C_{1F}, C_{2F}	Concentrations of the fresh feed (g/l)
D_{ap}	Apparent dispersion coefficient
L	Column length
N_P	Column efficiency
q	Concentrations in the adsorbed phase
t	Time
t_0	Column dead time
u_0	Propagation velocity of a non-adsorbed component
x	Abscissa along the column

z	Reduced abscissa
ε_T	Total porosity of the bed
θ_i	Recovery yield of component i

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