Separation Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

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Online publication date: 29 May 2002

To cite this Article Antos, Dorota and Seidel-Morgenstern, Andreas(2002) 'Continuous step gradient elution for preparative separations', Separation Science and Technology, 37: 7, 1469 — 1487
To link to this Article: DOI: 10.1081/SS-120002732
URL: http://dx.doi.org/10.1081/SS-120002732

SEPARATION SCIENCE AND TECHNOLOGY, 37(7), 1469–1487 (2002)

CONTINUOUS STEP GRADIENT ELUTION FOR PREPARATIVE SEPARATIONS

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ABSTRACT

A continuous step gradient elution process is suggested using several columns fixed in parallel to a multifunctional valve. The positions of the columns are changed in a periodic manner by shifting the valve at discrete times. Thus, the inlet streams into a certain column can be modulated in each time interval. Through one port, the feed is supplied whereas through the next port, eluent with increasing modifier concentration is dosed.

In this work, the principle was studied for a normal-phase high-performance liquid chromatography system using the separation of a binary mixture of two cycloketones as an example. In these experiments, four columns were used. The feed stream was introduced in a relatively weak solvent and then three gradient steps using stronger solvents followed. In order to study the concept quantitatively, a mathematical model describing the column dynamics was developed. In preliminary experiments, the

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most relevant thermodynamic and kinetic parameters of the chromatographic system have been determined. In particular, the dependence of the adsorption isotherms on the mobile phase composition was analyzed. The influence of extra column volumes on the process performance was also considered. Four gradient elution experiments were carried out varying the shape of the step gradients. Besides a modulation of the eluent composition, flow rate gradients have also been considered. In the experiments performed, the separation process turned out to be stable and reproducible. The experimental results were compared with the model predictions. Due to the relatively good agreement found, the model can be applied to design the step gradient process and to evaluate its potential compared to conventional isocratic elution.

Key Words: Preparative chromatography; Continuous overloaded step gradient elution

INTRODUCTION

Gradient elution is used frequently in analytical chromatography to separate complex mixtures. It is based on progressively increasing the elution strength of the mobile phase during the separation process. This allows the separation and concentration of components of widely varying retention behavior in reasonable time. This concept is applied most frequently in ion-exchange processes, in reversed-phase and in normal-phase chromatography (1).

In normal-phase preparative chromatography usually unmodified polar adsorbents are used. In particular, there is a broad spectrum of relatively cheap plain silicas available. They show, e.g., a high ability to separate geometrical isomers (2–4). Under gradient conditions usually, these materials do not suffer from gradual destruction typical for bonded stationary phases (1). Despite the broad application of silicas in analytical gradient elution, only a few systematic studies have been undertaken to analyze preparative separations quantitatively.

Theoretical studies devoted to preparative applications of gradient elution revealed several attractive features compared to conventional isocratic elution. The gradient mode offers the possibility of using higher loading factors and requires less efficient columns. Moreover, the concentration of fractions collected can be significantly higher and the times between repetitive injections can be shorter. Thus, gradient elution can outperform isocratic operation in preparative chromatography (5).

An obvious drawback of gradient elution is the need to handle efficiently more complex mobile phases. For this reason in preparative chromatography gradients using a few discrete (two to five) and stepwise constant mobile phase compositions (step gradients) are preferred to linear or more complex gradient (6). In any case a column regeneration is mandatory prior to a new injection.

Batch wise separation using gradients is in general adequate in the analytical scale. However, in the preparative or production scale there is a tremendous interest in continuous processes as indicated by the recent breakthrough in the simulated moving bed (SMB) process.

In this paper a multicolumn set-up is used to perform a step gradient process. The feed solution is introduced continuously in the unit and product fractions can be collected in a periodic manner. In the experiments, the separation of a binary mixture using silica was studied. The fraction of a polar modifier in the nonpolar solvent was changed in several steps. In order to describe the process quantitatively, the influence of the mobile phase composition on the distribution equilibria was quantified and introduced in a mathematical model.

PRINCIPLE OF EQUISTEP GRADIENT ELUTION

To achieve a continuous introduction of feed, a multicolumn arrangement is suggested in this work. The general principle is illustrated in Fig. 1 for four columns arranged in parallel using a multifunctional valve. These columns can be connected with four pumps that deliver feed and eluent streams with adjusted compositions and flow rates. A step change can be realized by shifting the valve. A constant shifting time is the most simple and attractive regime. Thus, the mode can be designated as "equistep" gradient elution. At each inlet position, i.e., in each step, there is the possibility to choose the eluent composition and the flow rate. One step is reserved obviously for sample injection, where the feed

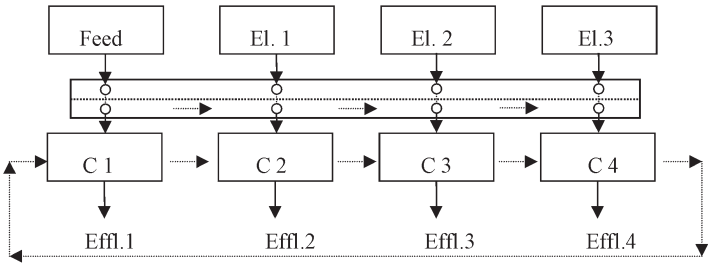

Figure 1. Schematic diagram of a step gradient unit using four columns (C) arranged in parallel (mounted on a multifunctional valve).

concentrations need to be specified. The number of steps can be increased obviously further by adding more columns and pumps. Although, a parallel arrangement of several columns is suggested here, it should be noted that most of the conclusions drawn below are in principle valid for a conventional step gradient elution process using a single column and varying flow rates and mobile phase compositions in equidistant discrete time steps.

Considering the large number of free parameters, the development of an adequate mathematical model appears to be indispensable for an evaluation and an optimized application of the suggested process. Such a model will be presented below.

In order to investigate the concept experimentally a set of four experiments was performed for a binary model system under different process conditions. Varied parameters were the four levels of eluent compositions and flow rates, the shift times, and the feed concentrations.

MATHEMATICAL MODEL

To simulate elution band profiles in efficient columns the equilibrium-disperse model can be used (6).

According to this model, the differential mass balance equation for one column and component i is:

$$\frac{\partial C_i}{\partial t} + F\frac{\partial q_i}{\partial t} + w_K\frac{\partial C_i}{\partial x} = D_a\frac{\partial^2 C_i}{\partial x^2} \quad i = 1, N, \text{ and } m\,(\text{modifier}) \qquad K = \text{I, II, III, IV} \tag{1}$$

where F is the phase ratio depending on the total porosity ε_t, w_K the interstitial mobile phase, D_a the apparent dispersion coefficient, and t and x the time and the axial position in the column, respectively. Further, C and q are the local concentrations in the mobile and stationary phases. The index K designates the current position of the column and the corresponding actual flowrate.

The initial conditions corresponding to a not preloaded column equilibrated with a constant modifier concentration are given by:

$$C_i(t = 0, x) = 0 \quad i = 1, N \tag{2}$$

$$C_m(t = 0, x) = C^0_{mod} \tag{2a}$$

The conventional Danckwerts-type boundary conditions are:

$$u_K C_{fi} - u_K C_i = D_a\frac{\partial C_i}{\partial x}\bigg|_{x=0} \qquad \frac{\partial C_i}{\partial x}\bigg|_{x=L} = 0 \quad i = 1, N, \text{ and } m \tag{3}$$

where C_{fi} is equal to the feed concentration in the different steps. It holds:

$$C_{f\,i} = \begin{cases} C_{fi} & \text{for } t \in [0, t_{shift}]_{K=I} \\ 0 & \text{for } t \in [0, t_{shift}]_{K=II,III,IV} \end{cases} \quad i = 1, N \tag{3a}$$

The modifier concentration can be different in each step:

$$C_{f\,m} = C_{m,K} \text{ for } t \in [0, t_{shift}]_{K=I,II,III,IV} \tag{3b}$$

After each shift K becomes $K + 1$. After four switches (i.e., at $t = 4t_{shift}$) the sequence of boundary conditions (Eqs. (3) and (3a,b)) is repeated.

Assuming permanent equilibrium between the two phases in the above set of equations the concentrations C and loadings q are coupled by the adsorption isotherms. For the sample components the validity of the competitive Langmuir isotherm was assumed:

$$q_i^* = \frac{a_i(C_m)C_i}{1 + \sum_{i=1}^{N} b_i(C_m)C_i} \quad i = 1, N \tag{4}$$

The influence of the modifier on the equilibrium can be included by specifying analytical expressions for the dependences $a = a(C_m)$ and $b = b(C_m)$. On the basis of the Snyder–Soczewinski model of adsorption in normal-phase systems (7) the following two-parameter equation describing the dependence of the isotherm coefficients on the concentration of the stronger polar solvent can be used:

$$a(C_m) = (p_a C_m)^{-m_a} \tag{5a}$$

$$b(C_m) = (p_b C_m)^{-m_b} \tag{5b}$$

The constants, p_a, m_a, p_b, and m_b need to be determined from experimental investigations for the chromatographic system of interest.

Concerning the adsorption of the modifier it can be assumed that it does not depend on the concentrations of the other components. Thus, for the modifier the following single-solute isotherm equation holds:

$$q_m^* = \frac{a_m C_m}{1 + b_m C_m} \tag{6}$$

The set of equations was solved numerically with the orthogonal collocation on finite element method.

EXPERIMENTAL

Chemicals and Columns

Experiments were performed with single solutes and binary mixtures of cyclopentanone and cycloheptanone (Fluka, Buchs, Switzerland).

Four columns packed with silica gel (pore size of 12 nm, particle size of 12 μm, YMC GmbH, Schermbeck, Germany) were used. The columns were 25 cm long and had an internal diameter of 20 mm. In preliminary runs under analytical conditions it was proven that the behavior of the four columns was quite similar.

Mobile Phase

As the mobile phase mixtures of *n*-hexane and ethyl acetate (modifier) were used. These solvents were purchased from Merck (Darmstadt, Germany) and used as received.

It is well known that the reproducibility of normal-phase gradient elution is satisfactory mainly for solvents of low and moderate polarity (4). On the other hand it was often observed that a certain presaturation of the column with the polar modifier is advantageous in terms of reducing band broadening and equilibration time (8). A prefilling of the micropores with the modifier diminishes usually the heterogeneity of the silica surfaces. For this reason and due to the results of preliminary experimental investigations (9) in the gradient elution experiments, the ethyl acetate (modifier) volume fraction was changed in the range between 5 and 30 vol%.

Instrumentation

The unit used for the preparative step gradient experiments was assembled by modification of a SMB unit (Knauer, Berlin, Germany (10)). Four columns were arranged in parallel by appropriately connecting them through a multifunctional valve with four preparative high-performance liquid chromatography (HPLC) pumps type K-1800 (Knauer, Berlin, Germany). Into each of the columns, different streams of feed and eluents with stepwise increased modifier concentration could be delivered. The components to be separated were dissolved in the mobile phase with the lowest volume fraction of modifier (5 vol%). The mixture to be separated was pumped continuously in the unit. By shifting the valve in discrete time intervals, all columns were periodically in one tact in the loading position and subsequently three tacts in the elution positions. After four steps, the sequence was repeated. The effluents of columns were

registered on four UV detectors. The unit was controlled by a personal computer as the user interface. The software allowed to record all detector signals.

An analytical HPLC unit (Hewlett Packard, HP 1100) was used to measure standard chromatographic parameters (capacity factors, column porosities, and efficiencies).

Procedures

Detector Calibration

Calibration factors for the detectors were determined by integration of band profiles of single components recorded (a) for a sample with the maximal concentration used in the experiments and (b) for a sample of a diluted solution. Always, almost the same calibration factors were obtained for both concentration levels confirming the linearity of the calibration curves.

Column Porosity

In order to determine the total porosities each of the columns was connected with the analytical HPLC system. In this unit, extra-column volumes could be neglected. The retention times of *n*-hexane injected in mobile phases with different ethyl acetate/*n*-hexane ratios were measured.

Column Efficiencies

The number of theoretical plates was determined from retention times and half-height peak widths of analytical pulses of cyclopentanone and cycloheptanone. The experiments were performed in the analytical unit using a column equilibrated at different mobile phase compositions and flowrates. The latter were varied in the range between 9 and 20 mL/min.

Extra-Column Volumes

Since in the tubings and the valve of the preparative unit, considerable dead volumes were expected and additional measurements were performed to estimate their size and influence. The retention times and efficiencies were measured again for the column arranged in the preparative unit. The hold-up volumes of the preparative system were determined by comparing retention times of *n*-hexane pulses measured in both analytical and preparative systems.

Isotherm Determination

The measurement of the isotherms of the two cycloketones under isocratic conditions was performed on the preparative unit with one column linked with a Rheodyne sampling valve equipped with a 2.0 cm^3 loop. For the determination of the isotherm parameters of cyclopentanone and cycloheptanone, isocratic elution profiles of single solutes were recorded and analyzed. The modifier contents in the mobile phase chosen for the isocratic experiments were increased from 5 to 30 vol% in 5% steps. The isotherm parameters a and b (Eq. (4)) were determined by fitting elution profiles predicted with Eq. (1) to band profiles recorded experimentally. Based on the dependence of the obtained isotherm parameters on the volume fraction of the modifier the free parameters in Eqs. (5a) and (5b) were determined.

To quantify the adsorption of the modifier (ethyl acetate) a perturbation technique was used (11). The retention times of small ethyl acetate pulses was measured for different column saturation concentrations in the range of interest.

Step Gradient Experiments

Finally, the above-described four-step gradient elution process was investigated experimentally. For this the modifier concentrations and the flow rates for each tact had to be specified as well as the shifting time and the feed concentrations of the components to be separated. A systematic variation of all these parameters was outside the scope of this paper. To test the applicability of the general concept and the multifunctional valve and to validate the presented model, four experiments were performed. The operating parameters of the first three experiments were chosen based on results of a preliminary simulation study using the model explained in the "Continuous Gradient Elution Experiments" section. In order to study consequences of a possible overlap between subsequent cycles in the fourth experiment, the columns were overloaded strongly and the elution strength in the last step was reduced.

RESULTS AND DISCUSSION

Column and Extra-Column Parameters

The total column porosity was found to be approximately $\varepsilon_t = 0.79$. This value was independent of the mobile phase composition.

Values of about 3000 theoretical plates were obtained for the columns in the analytical unit. The numbers did not depend significantly on the component

(cyclopentanone and cyclopentanon), on the mobile phase composition, and within the studied range (9–20 mL/min) on the flow rate.

The results reported above were found to be similar for all four columns applied.

The extra-column volume in the preparative system was equal to 12.5 cm^3. This corresponds to about 20% of the column fluid volume. Because of the high extra-column volume in relation to the column dead volume, the apparent efficiency of the columns in the preparative system was found to be only about half of that in the analytical unit (i.e., approximately 1500 theoretical plates).

Estimation of Isotherm Parameters for Sample Components

Prior to analyzing the isocratic elution experiments in order to determine the adsorption isotherms the measured profiles were shifted along the time axis in accordance with the estimated extra-column contributions. Then the isotherm coefficients were obtained by fitting the numerical solution of Eq. (1) to the observed chromatographic peak shapes (12–14). The estimation was performed using the code COLCHR (15) in which a modified least-square Marquardt method (16) was implemented. Details are given in Ref. (12).

The parameters a and b obtained for different mobile phase compositions are summarized in Table 1. In Fig. 2 for illustration, experimentally determined and predicted elution profiles for the two components are superimposed as single solutes for an ethyl acetate concentration of 15 vol%

The values obtained by nonlinear regression for the constant p_a, m_a, p_b, and m_b (Eqs. (5a) and (b)) are presented in Table 2. Again for illustration, the dependence of the parameter a on C_m is shown in Fig. 3 for the two cycloketones. Obviously a and thus retention is reduced considerably when C_m is increased.

Table 1. The Coefficients of the Langmuir Isotherm, Eq. (4), for Cylcoheptanone and Cyclopentanone

C_m [vol%]	5	10	15	20	25	30
Cycloheptanone						
a [—]	15.99	7.56	4.94	3.40	2.62	2.085
b [1/(vol%)]	0.6	0.267	0.173	0.133	0.091	0.089
Cyclopentanone						
a [—]	21.12	11.00	7.45	5.31	4.06	3.49
b [1/(vol%)]	1.050	0.521	0.380	0.281	0.232	0.206

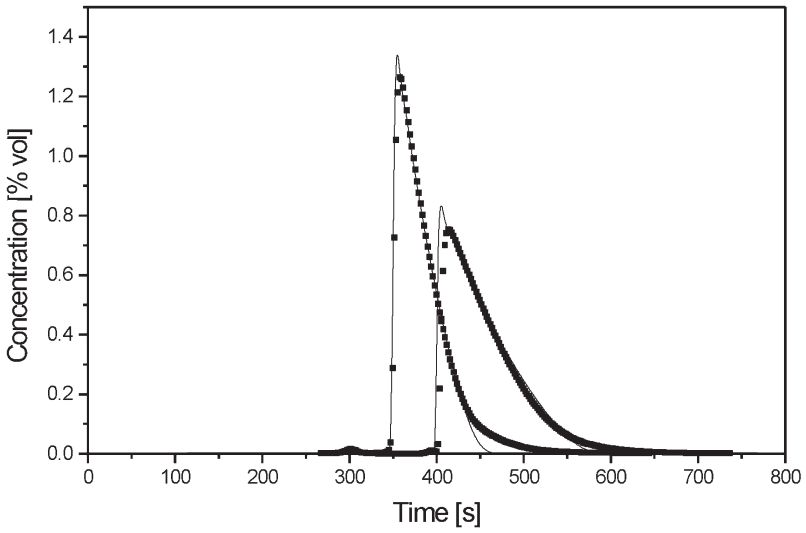

Figure 2. Simulated (lines) and measured (symbols) elution profiles for the single solutes cycloheptanone (first eluting) and cyclopentanone at a constant eluent composition of *n*-hexane:ethyl acetate = 85:15 vol%, $C_{f,1} = C_{f,2} = 10$ vol%, injection time = 6 sec, flow rate = 20 mL/min.

Estimation of Isotherm Parameters for Modifier

In order to simulate the retention behavior of the components to be separated, the local modifier concentration must be known. This value is affected by the retention behavior of the modifier. The modifier ethyl acetate is adsorbed considerably, in particular in the initial concentration range of the gradient (5 vol%).

If the Langmuir isotherm model [Eq. (4)] is applied, for the concentration dependence of analytical retention times holds (11):

$$t_r(C_m) = t_0\left(1 + F\frac{\partial q}{\partial c}\Big|C_m\right) = t_0\left(1 + F\frac{a_m}{(1 + b_m C_m)^2}\right) \tag{7}$$

Thus, measurements of retention times of pulses for different saturation levels provide enough information to determine a_m and b_m by nonlinear regression.

The following isotherm parameters were estimated: $a_m = 0.92$ [—], $b_m = 0.05$ [1/(vol%)]. Comparing the *a*-values for the modifier and the cycloketones (Table 1) it becomes apparent that the modifier is much less adsorbed.

Table 2. Parameter of Eqs. (5a) and (b) for Cycloheptanone and Cyclopentanone (C_m in vol%)

	Cycloheptanone	Cyclopentanone
p_a	0.01613	0.0090
m_a	1.102	0.985
p_b	0.316	0.1903
m_b	1.126	0.951

Continuous Gradient Elution Experiments

Table 3 contains the most relevant conditions characterizing the four experiments (runs 1–4) performed.

The parameters of run 1 were specified after preliminary calculations using the described model and the determined parameters. Since the preparative aspects are essential for the present work, for the feed concentration 1 vol% of each of the

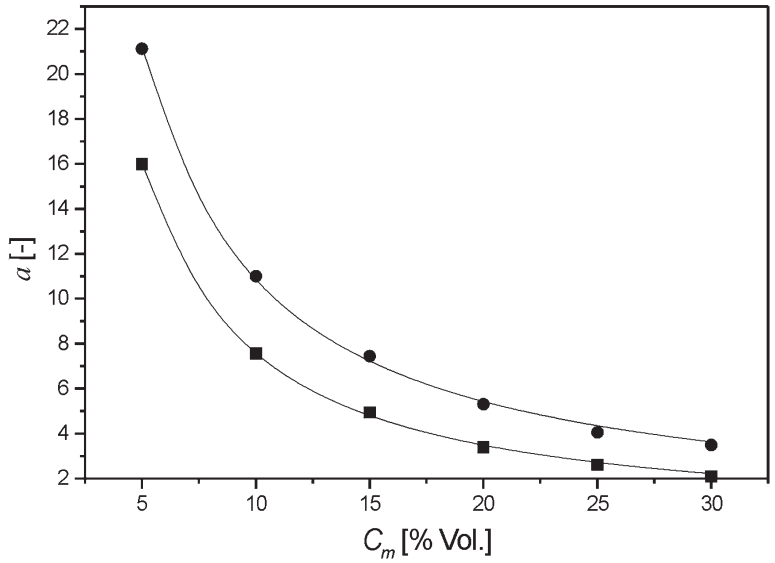

Figure 3. The concentration dependence of the isotherm coefficients a for cyclopentanone (circles) and cycloheptanone (squares). The lines are calculated using Eq. (5a) with the parameters p_a and m_a given in Table 2.

Table 3. Conditions of the Four Performed Step Gradient Elution Experiments

Run	$C_{m,K}$ $K = \text{I, II, III, IV}$ [vol%]	Flow rate $K = \text{I, II, III, IV}$ [mL/min]	t_{shift} [min]	$C_{f,C5} = C_{f,C7}$ [vol%]
1	5, 10, 15, 25	10, 10, 10, 20	2.1	1.0
2	5, 10, 15, 30	10, 10, 10, 20	2.1	1.3
3	5, 10, 15, 30	10, 10, 10, 20	2.1	1.6
4	5, 10, 15, 20	9, 9, 9, 9	2.5	2.0

two cycloketones was chosen. It can be deduced from the isotherm parameters given in Tables 1 and 2 that for this feed concentration there is already a considerable amount of nonlinearity in the process. It is well known that in overloaded gradient elution chromatography initially the lowest possible modifier concentration is favorable for the production rates (17). Thus, for the first step a starting modifier concentration of 5 vol% was set. The corresponding solution contained also the feed components. The modifier content then in the next two steps, was twice increased by 5 vol%. In order to have an efficient column regeneration in the last step the modifier concentration was set to be 25%. The flow rate in the first three steps was 10 mL/min. For more efficient column regeneration the flow rate in the last step was set to be 20 mL/min. An appropriate shift time has to be adjusted in accordance with the migration velocities of the components and the modifier. Significant filling of a column in the first step and sufficient regeneration in the last step are obvious requirements. There should be no overlap and also not too much gap between subsequent cycles. For run 1 a shift time of 2.1 min was chosen promising in simulations a satisfactory operation.

In the experiments essentially the effluent of one arbitrarily chosen column was analyzed. Since all columns are rather similar and work in parallel just a time transformation by multiples of the shift time can be expected to describe the concentration vs. time profiles for the other three columns. In order to investigate the quality of the model the band profiles registered as detector responses were compared with the simulated ones. For this the simulated concentration profiles were converted into theoretical detector signal using the calibration curves.

The results of run 1 are depicted in Fig. 4. The process was observed over four subsequent cycles. The typical overloaded chromatograms for binary mixtures were obtained indicating a separation much more productive than under analytical conditions. The similarity between the cycles is good. Only the bands for the first cycle are a little bit larger. This might be due to insufficient equilibration prior to the run. There is a remarkable agreement between the experimental results and the model predictions. The time delay between subsequent cycles is properly accounted for as well as the shape of the bands. The

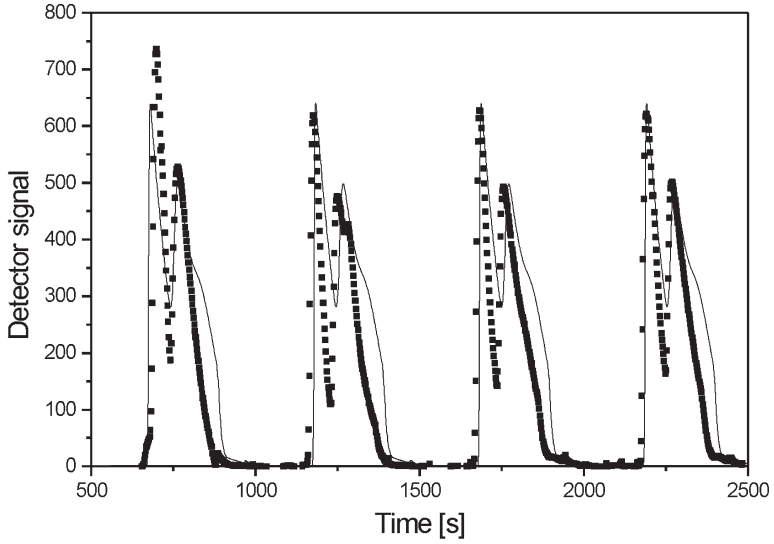

Figure 4. Comparison of the experimental (symbols) and simulated (lines) band profiles for run 1 (conditions as in Table 3).

essential difference is that the theoretical areas under the chromatogram appear to be slightly larger. This might be due to the fact that the extra-column volume in the system was not considered in the calculations. This volume causes additional dilution. The model prediction might be also further improved if the isotherms are described more accurately. It is apparent in Fig. 2 that the Langmuir isotherm does not reproduce the peak tailings correctly, especially for the first eluting component.

Here it should be noted that in run 1 the total amount of eluent used in all four steps is much less than the six-column volumes found in theoretical studies to be a limit for a potential advantage of gradient elution compared to isocratic operation (5).

After this successful run in runs 2 and 3, the feed concentration was further increased (1.3 and 1.6 vol%). To guarantee the completeness of the column regeneration in contrast to run 1 the modifier content in the last step was increased from 25 to 30 vol%. The experimental results for the two runs are shown in Fig. 5. The close agreement between the results of the two runs indicates a good stability and reproducibility of the process. Both components can be collected periodically in certain time windows from these elution profiles. Shifted in time a similar collection is possible at the outlets of the other three columns. Without quantifying productivities run 3 with the higher feed concentrations appear to be

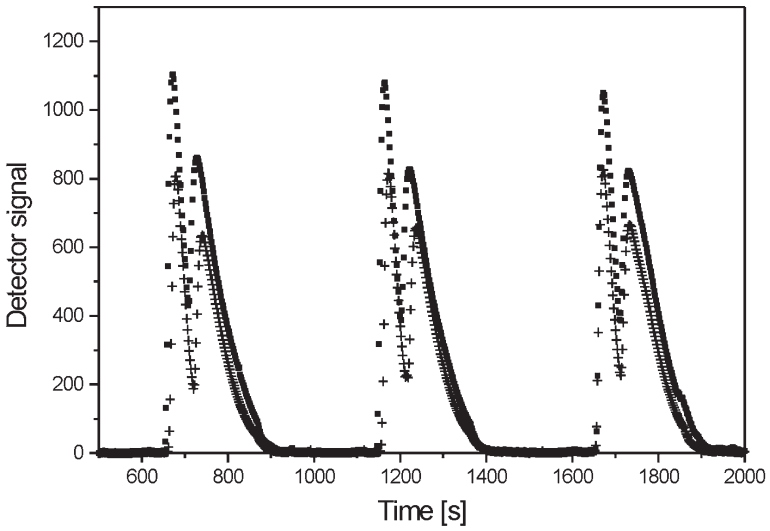

Figure 5. Experimentally determined band profiles for run 2 (crosses, $C_f = 1.3$ vol%) and run 3 (squares, $C_f = 1.6$ vol%). All other conditions as in Table 3.

more attractive. However, in both runs there is still a certain gap between the subsequent cycles. This appears to be not the best possible situation and indicates a considerable potential for optimization.

The comparison between the model predictions and the experimental results for run 2 is shown in Fig. 6. The general agreement is similar to the situation for run 1 (Fig. 4). In this case however the discrepancy between the chromatogram areas is even larger. Again the main reason should be the not considered impact of extra-column volumes. In Fig. 6, in addition results of predicting the process for a constant averaged modifier concentration of 18 vol% are included. All other parameters are kept as in the step gradient simulation. The main difference between the chromatograms is that the isocratic process leads to broader and more diluted bands. Considering a corresponding cyclic process, reduced productivities would result. At this point it should be noted that both theoretical chromatograms presented in Fig. 6 do not represent optimized situations. A more systematic study should take into account the influence of all operating parameters.

Finally another experiment (run 4) was performed in order to exemplify limits of the concept and to stress the need for an accompanying theoretical analysis. The main difference to the previous runs was that the regeneration in step four was weakened. In addition the amount injected was increased by raising the feed concentrations to 2 vol% and the shift time to 2.5 min. The results are

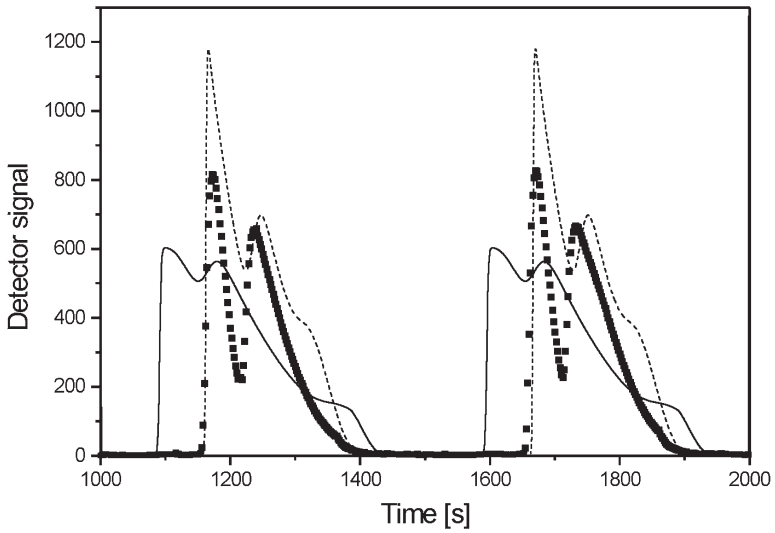

Figure 6. Comparison of the process under step gradient and isocratic conditions. Gradient (dotted): parameters of run 2. Isocratic (solid): constant averaged modifier concentration of $C_m = 18$ vol% (flow rates as for the gradient mode). The symbols are the experimental results of run 2.

shown in Fig. 7. Obviously for these conditions no complete desorption could be accomplished before the next cycle starts and the peaks begin to overlap. This destroys the separation already achieved. A more detailed analysis of this situation is possible using the developed model. In Fig. 8 are shown the theoretical concentration courses of run 4 at the column outlet for the two cycloketones and the modifier. For the second cycle, the positions of the shift times are marked. After the first cycle, there is still a considerable amount of sample in the column. To avoid the resulting overlap between subsequent cycles several measures could be taken to squeeze the bands of the components into the available frame. The obvious (in runs 1–3 applied) way is to increase the elution strength in step four by using larger modifier concentrations and/or flow rates. Of course, a general optimization would also take an alternation of shifting times and feed concentrations into account. In Fig. 8, it can be also noted that the drop of the maximum modifier concentration to its starting value needs some time. Due to the isotherm nonlinearity the corresponding desorption front is dispersed. The time needed to complete this modifier exchange is for the given conditions are 0.95 min. Since after the first step this exchange process should be finished, this time defines a minimal shift time. However, in practice a unit usually will need additional time for reequilibration at the new lower modifier concentration level

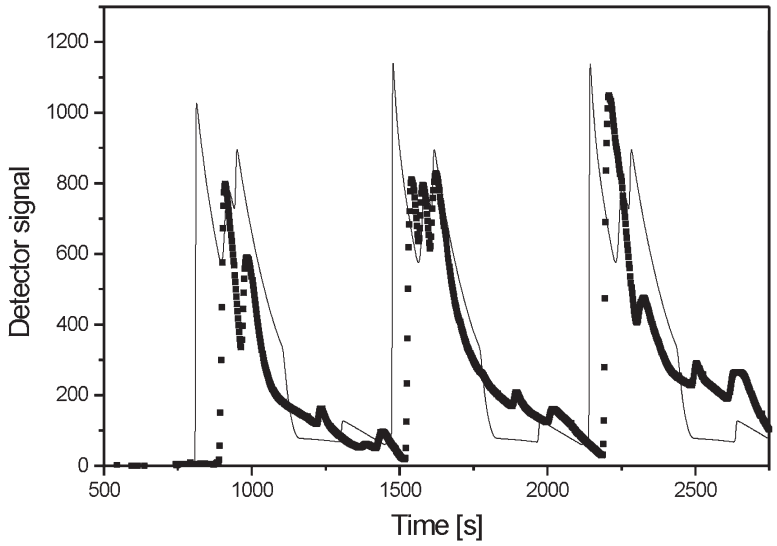

Figure 7. Experimental results of run 4 (symbols) and model predictions (lines). Conditions as in Table 3.

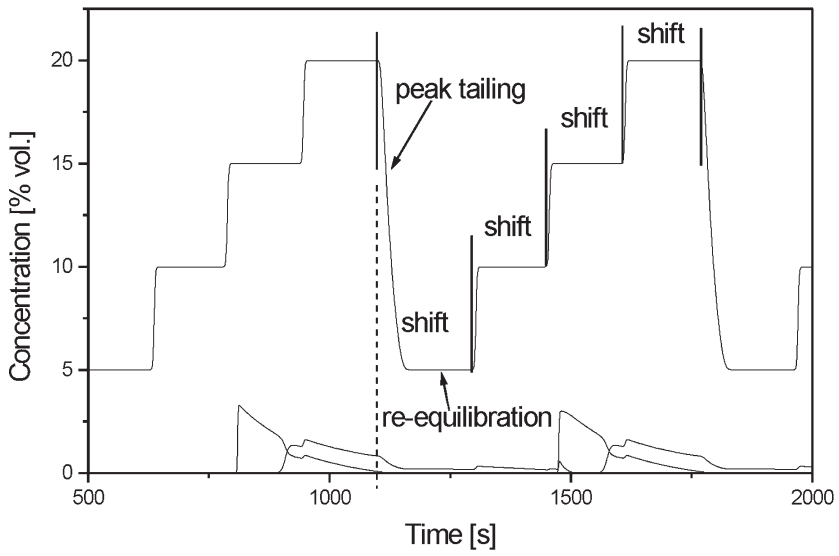

Figure 8. Theoretical analysis of run 4.

(e.g., just to stabilize a detector base line again). The minimum of this additional time can be specified only experimentally.

In Fig. 7 are also shown the superimposed theoretical results for run 4. The model describes qualitatively the uncommon peak shapes and in particular the band distortion due to the sudden decrease of the modifier concentration. However, from the results it can also be concluded that the quantitative representation of the process requires further improvement of the model and the underlying parameters. A corresponding study including a more general optimization of the suggested step gradient process is in progress (18).

CONCLUSIONS

In this work preparative step gradient elution was investigated in a parallel column arrangement. Using a multifunctional valve a continuous dosing of feed was performed. The operating parameters were specified on the basis of preliminary experiments and a developed mathematical model taking into account in particular the periodically changing equilibrium functions. The experiments were carried out under various operating conditions and compared with the model predictions. The separations performed turned out to be stable and reproducible. The concept offers the potential to increase productivities compared to conventional isocratic elution. A relatively good agreement between experiments and predictions was achieved confirming both the model validity and the accuracy of the determined parameters. On the basis of achieved results a more systematic study is required in order to optimize the suggested process more quantitatively. Due to the large number of free parameters, such an optimization can be performed only numerically.

NOMENCLATURE

Symbols

a	coefficient of Langmuir isotherm, Eq. (4)
b	coefficient of Langmuir isotherm, Eq. (4)
C	liquid phase concentrations
C_{mod}	liquid phase concentration of modifier
D	axial dispersion coefficient
F	phase ratio, $F = (1 - \varepsilon_t)/\varepsilon_t$
L	column length
q	concentration in stationary phase
t	time
t_{shift}	shift time, equal to width of a rectangular injection pulse

t_0 hold-up time of the column, $t_0 = L/w$
u superficial mobile velocity
w interstitial mobile phase velocity, $w = u/\varepsilon_t$
x axial positions in the column

Greek Letters
ε_t total porosity

Subscripts
i component index 1,. . .,*NC*
f inlet value
K tact
m modifier

ACKNOWLEDGMENTS

The support of Schering AG (Berlin) and Fonds der Chemischen Industrie is acknowledged gratefully.

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