

Optimization of gradient elution conditions in multicomponent preparative liquid chromatography

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Abstract

Gradient elution is widely applied in analytical chromatography to reduce the separation time and/or to improve the selectivity. Increasingly the potential of modulating the solvent strength during gradient operation is exploited in preparative liquid chromatography. The purpose of this paper is to investigate theoretically the effect of optimizing free parameters available in gradient chromatography (extents and shapes of gradients) on the productivity of isolating a target component in a multicomponent mixture. An equilibrium stage model was used to quantify and compare different modes of operation (isocratic and various variants of gradient elution). By combining experimental design and artificial neural network concepts, optimal conditions were identified for the production of the second eluting component in a ternary mixture. The strong impact of the shape of gradients on process performance is elucidated.

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1. Introduction

In analytical liquid chromatography gradient elution is widely applied to improve the separation of mixtures by varying the solvent strength during the elution process [1–3]. This concept offers the possibility to reduce cycle times, especially if the components in the sample are characterized by a wide range of retention. There are various ways how solvent gradients can be introduced. Most frequently the solvent strength at the column inlet is altered proportional to time (linear gradients). Alternatively, various kinds of step gradients or more sophisticated nonlinear (concave and convex) gradients are applied. The design of suitable gradient shapes is in general a difficult task and optimization is frequently performed empirically.

The possibilities to model mathematically chromatographic processes under overloaded conditions and to optimize the operating parameters have improved considerably in the last years. The suggested models and numerical methods are now widely applied to optimize isocratic preparative chromatography [4,5]. In parallel considerable efforts were undertaken in order to use available mathematical models and tools to analyze and design gradient processes for preparative purposes (e.g. [6–32]). Significant contributions were published by the groups of Guiochon and coworkers [9–16], Jandera et al. [17–20] and Cramer and coworkers [21–24].

Many of the investigations devoted to quantify and optimize gradient operation were focused on studying the effect of linear gradients (e.g. [15,17,22]). Often were considered only the migration of a single component under gradient conditions or the separation of a binary mixture (e.g. [13,29]). Felinger and Guiochon investigated the effect of gradient elution on the production rate and the yield of each component in binary mixtures [12–15]. They also considered

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systematically various possible objective functions (e.g. production rates, recovery yields, solvent consumption [13,14]). Gallant et al. performed systematic studies in order to optimize both step and linear gradients in preparative elution chromatography [21,22]. Jandera optimized also the shape of the gradient using a flexible function [18]. Antos et al. compared the migration of a single solute in linear and non-linear elution chromatography under isocratic and gradient conditions under the influence of mass transfer resistances [29].

Real separation problems are typically characterized by the presence of more than two components in the feed. It is further obvious that the application of nonlinear gradient shapes possesses an additional potential for improving the process performance compared to linear gradient operation. Based on these facts it is the purpose of this paper to perform a theoretical study of gradient elution chromatography optimizing the isolation of the second eluting component out of a ternary mixture. To model the chromatographic process a simple equilibrium stage model is applied. In order to determine optimal operating parameters an artificial network is used. Isocratic operation is compared with linear, stepwise and nonlinear gradient operation. Different relationships between the solvent composition and the corresponding adsorption equilibrium functions of the solutes are used.

2. Theory

2.1. Column model

There are several models available capable to quantify the development of concentration profiles in chromatographic columns [4,5]. Due to the fact that under overloaded conditions the adsorption isotherms are nonlinear, numerical solutions of the underlying model equations are needed. In this paper the Craig model was chosen to simulate elution profiles for multicomponent mixtures. Its simplicity, flexibility and accuracy were the reasons for this choice. It should be noted that the general trends discussed below do not depend on the selection of the column model.

The Craig model [33] is a classical equilibrium stage model suitable to describe the development of concentration profiles in efficient chromatographic columns. The column is hypothetically divided into P (typically a relative larger number) stages of equal size, consisting out of a fraction filled with the stationary phase and a fraction filled with the mobile phase. In a first step, in each stage the components are equilibrated between the two phases in accordance with the adsorption isotherms. Then, in a second step, the liquid phase is withdrawn from the last stage. The liquid fractions in the other stages are transferred in the direction of the mobile phase flow into the next stage. Sample or fresh mobile phase is introduced in the first stage. This process is repeated several times, typically until the whole amount injected has left the

last stage. The mass balance equations of the Craig process can be expressed for a component i , a stage j and an exchange step k as follows:

$$C_{i,j}^{k+1} - C_{i,j-1}^k + \frac{1-\varepsilon}{\varepsilon} (q_{i,j}^{k+1}(C_{1,j}^{k+1}, \dots, C_{N,j}^{k+1}) - q_{i,j}^k(C_{1,j}^k, \dots, C_{N,j}^k)) = 0, \\ i = 1, N; j = 1, P; k = 1, K \quad (1)$$

where $C_{i,j}^{k+1}$ is the concentration of component i in the j stage at step $k+1$, $q_{i,j}^{k+1}$ the concentration of component i in the stationary phase of the j stage in equilibrium with all local mobile phase concentrations $C_{i,j}^{k+1}$ ($i = 1, N$) at step $k+1$, ε the total column porosity, N the number of components and K the number of exchange steps considered.

To describe a rectangular injection profile introduced at the column inlet the following equation can be used:

$$C_{i,0}^k = \begin{cases} C_{i,\text{inj}} & \text{for } k \times \Delta t \leq t_{\text{inj}} \\ 0 & \text{for } k \times \Delta t > t_{\text{inj}} \end{cases}, \quad i = 1, N; k = 1, K \quad (2)$$

In the above $C_{i,\text{inj}}$ is the injected concentration of component i , t_{inj} the injection time and Δt the time step interval for transferring the liquid phase from one cell to the next cell. This interval equals to t_0/P , where t_0 is the dead time of the whole column related to the column length L_{col} , the column area A_{col} and the volumetric flowrate V_F ($t_0 = L_{\text{col}}A_{\text{col}}\varepsilon/V_F$).

The initial conditions corresponding to a not preloaded column are:

$$C_{i,j}^0 = 0, \quad q_{i,j}^0 = 0, \quad i = 1, N; j = 1, P \quad (3)$$

Due to the fact that typically the equilibrium functions $q_i(C_1, \dots, C_N)$ are nonlinear and coupled, the above set of equations has to be solved iteratively. For the Langmuir isotherm model, a simple fixed-point iteration scheme can be used [4]. In general, classical iterative methods capable to solve systems of nonlinear algebraic equations must be applied. Accurate results and fast convergence can be usually achieved using the Newton–Raphson method [34].

2.2. Gradient shapes

Gradients are typically realized by adding gradually at the column inlet a component to the mobile phase that increases the elution strength. This component will be called below *modifier*. A gradient can be described by the following parameters: initial concentration of the modifier ($C_{\text{mod,begin}}$), time for starting the gradient ($t_{g,\text{begin}}$), final concentration of the modifier ($C_{\text{mod,end}}$), time for stopping the gradient ($t_{g,\text{end}}$). Further, the shape of the gradient between $t_{g,\text{begin}}$ and $t_{g,\text{end}}$ needs to be defined. Typical gradients are illustrated in Fig. 1. The shapes of the functions shown were generated using the following flexible function possessing a gradient shape

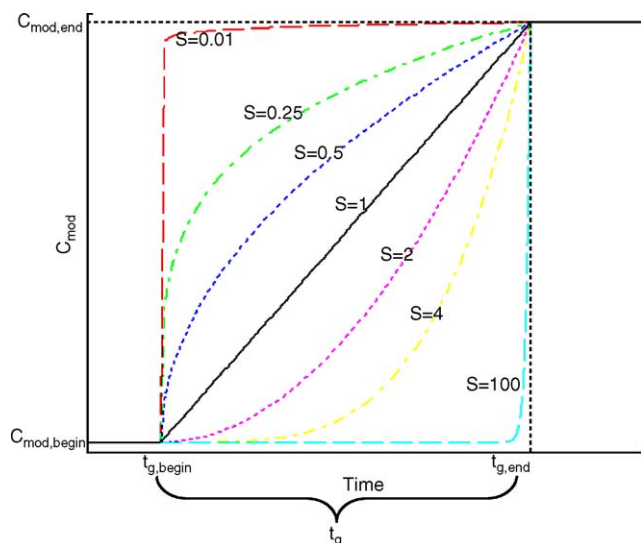


Fig. 1. Notation to define a nonlinear gradient and effect of shape parameter S (Eqs. (4)–(6)).

parameter S :

$$C_{\text{mod}}(t) = C_{\text{mod,begin}} + (C_{\text{mod,end}} - C_{\text{mod,begin}}) \times \left(\frac{t - t_{\text{g,begin}}}{t_{\text{g}}} \right)^S, \quad t_{\text{g,begin}} \leq t \leq t_{\text{g,end}} \quad (4)$$

where t_{g} is the gradient duration which equals to $(t_{\text{g,end}} - t_{\text{g,begin}})$. Useful alternative functions were suggested in [18]. The gradients start often immediately after the end of the injection, i.e. $t_{\text{g,begin}} = t_{\text{inj}}$.

Instead of the gradient duration time, t_{g} , frequently as an alternative an overall gradient slope, G , is used

$$G = \frac{C_{\text{mod,end}} - C_{\text{mod,begin}}}{t_{\text{g}}} \quad (5)$$

Using Eq. (5), Eq. (4) can be expressed also in the following way:

$$C_{\text{mod}}(t) = C_{\text{mod,begin}} + (C_{\text{mod,end}} - C_{\text{mod,begin}})^{1-S} \times (t - t_{\text{g,begin}})^S G^S \quad (6)$$

Eqs. (4) and (6) allow describing several standard shapes for gradients. If the gradient shape factor S is equal to 1, a linear gradient results. In contrast, when the value of S is very small or very big, single step gradient elution is described with the step occurring at $t_{\text{g,begin}}$ or $t_{\text{g,end}}$.

Modern HPLC equipment allows implementing quite accurately nonlinear gradients using several consecutive linear segments with different slopes or a larger number of steps with adjusted step sizes and heights. Of course only a smaller number of linear segments or steps appears to be reasonable for practical application. However, typically two to five optimized steps are already sufficient to reach almost the performance of the nonlinear gradient.

2.3. Equilibrium functions

The most difficult problem in quantifying the gradient effect on the migration speed in chromatographic columns and thus on the shape of elution profiles, is the description of the underlying equilibrium functions. A thorough discussion of two possible approaches was given by Jandera et al. [19]. In general models are favored which consider the modifier explicitly as an additional component of the system. Alternatively the modifier might be included in a simplified manner by affecting only the numerical values of an isotherm model capable to describe the system behavior at a certain isocratic situation. The concept of solving gradient elution optimization problems discussed in this paper is capable to treat both approaches. The mentioned second approach is used below.

In order to quantify the adsorption equilibria required in Eq. (1), $q_i(C_1, \dots, C_N)$, for a certain solvent composition, often the competitive Langmuir isotherm model can be used, i.e.:

$$q_i = \frac{a_i C_i}{1 + \sum_{m=1}^N b_m C_m} = q_{\text{sat},i} \frac{b_i C_i}{1 + \sum_{m=1}^N b_m C_m}, \quad i = 1, N \quad (7)$$

where the a_i are the Henry constants and the b_i are parameters quantifying the isotherm nonlinearity. The $q_{\text{sat},i}$ are the stationary phase saturation capacities of the components, which correspond to a_i/b_i . The ratio between two Henry constants is called separation factor α :

$$\alpha_{i,m} = \frac{a_i}{a_m}, \quad \text{with } a_i > a_m \quad (8)$$

To model the elution profiles under gradient conditions the dependence of the parameters of the adsorption isotherm equation (Eq. (7)), a_i and b_i , on the concentration of the modifier, C_{mod} , must be known. Several models have been suggested that describe relations $a_i(C_{\text{mod}})$ required in analytical chromatography. Hereby the suggested correlations differ for reverse phase and normal phase systems (e.g. [1,2,18,30,34,35]). Often the same correlations are used to describe the additional isotherm parameters required in nonlinear models. In order to demonstrate the optimization concept developed during this study, in the simulations described below as an example the following relations were applied:

$$a_i = (a_{1,i} C_{\text{mod}})^{a_{2,i}}, \quad b_i = (b_{1,i} C_{\text{mod}})^{b_{2,i}}, \quad i = 1, N \quad (9)$$

Consequently, the equilibrium model possesses four free parameters for each component i , i.e. $a_{1,i}$, $a_{2,i}$, $b_{1,i}$ and $b_{2,i}$. Eq. (9) is based on the well-known Snyder-Soczewinski model of normal-phase adsorption chromatography [1,35]. Although in the given references the modifier concentration is expressed in mole fractions, below the more practical volume percents are used [20] leading to the corresponding dimensions of the isotherm model parameters.

2.4. Operating parameters and objective functions

Besides the mentioned parameters of the gradient (Eqs. (4)–(6)), the essential parameters that can be specified in order to optimize a separation in an available column are the volumetric flowrate V_F (or the corresponding dead time t_0) and the injection volume and concentrations, V_{inj} and $C_{i,inj}$. The injected amounts can be conveniently expressed in relation to the saturation capacity of the column as a loading factors $L_{f,i}$ [4]:

$$L_{f,i} = \frac{V_{inj}C_{i,inj}}{(1 - \epsilon)A_{col}L_{col}q_{sat,i}} \times 100\%, \quad i = 1, N \quad (10)$$

Sometimes also a total loading factor, $L_{f,tot}$, is used to characterize the overall injected amount. This factor is defined as:

$$L_{f,tot} = \sum_{i=1}^N L_{f,i} \quad (11)$$

In preparative chromatography the choice of the objective functions depends on the concrete separation problem. Often it is attempted to maximize the rate of producing a certain component i . The production rate, Pr_i , can be defined as the amount collected, m_i , divided by the cycle time, Δt_c , and the column cross-section area:

$$Pr_i = \frac{m_i}{\Delta t_c \epsilon A_{col}}, \quad i = 1, N \quad (12)$$

To calculate for a certain operating point the Pr_i , the corresponding amounts of purified component and the cycle time must be specified. The determination of m_i requires the specification of a desired purity, $Pur_{i,des}$ and a threshold concentration, $C_{threshold}$. A suitable mathematical procedure capable to calculate the Pr_i based on integrating the individual band profiles was recently described [36]. The cycle time, Δt_c , must evaluate the retention time of the injected sample and the time needed for regenerating the column after the end of the gradient. A suitable cycle time can be estimated from the time when the outlet concentration of the most retained component N drops below the threshold concentration, t_N^{end} , and the time needed for the regeneration of the column: t_{reg} .

$$\Delta t_c = t_N^{end} + t_{reg} \quad (13)$$

Another important performance criterion is the recovery yield of a component i , Y_i , defined as the ratio of the amount recovered in the collected fraction over the amount of the same component injected in the sample:

$$Y_i = \frac{m_i}{V_{inj}C_{i,inj}} 100\%, \quad i = 1, N \quad (14)$$

Alternatively, as another useful objective function (OF) the product of the production rate and the yield was introduced [13]:

$$OF_i = Pr_i Y_i, \quad i = 1, N \quad (15)$$

An advantage of this objective function is that the production rate for the optimized experimental conditions is typically only slightly lower compared to the situation when only the production rate is used as the objective function. In contrast, the recovery yield is significantly improved.

Below the product of the production rate and the yield, i.e. OF_i in Eq. (15), was considered as the objective function to be maximized.

2.5. Optimization method

There are several powerful methods and algorithms available which are capable to solve the described or similar optimization problems. Chromatographic processes were already successfully optimized applying the simplex method (e.g. [15]) and genetic algorithms (e.g. [37]).

An alternative optimization concept applied below is based on approximating in a first step the relationships between the free parameters and the objective functions using artificial neural networks (ANN). Subsequently this information can be used efficiently to find the optimum [38]. Initially a suitable architecture and parameters for the ANN must be specified. The size of the input layer is determined by the number of parameters that should be optimized. The output layer contains just one output node representing the value of the objective function. The size of the hidden layer (number of hidden neurons) is a free parameter that should be optimized to obtain best results. The optimum number of hidden neurons is typically decided based on the complexity of the problem, the size of the input and output layers and the number of available training patterns. Since there is not yet a theoretical method available to choose the number of hidden neurons, ANN with different sizes of the hidden layer are usually tested in order to find the best structure. The types of transfer functions used have also an influence on the performance of ANN. Below linear and tansig transfer functions ($f(x) = x$ and $f(x) = 2/(1 + \exp(-x)) - 1$) were used for the hidden and output layers.

To model the response surface accurately and to train the ANN, a set of data covering the whole region of interest must be provided. These training data could be obtained by simulating the chromatographic process for different operating conditions. To reduce the number of calculation and the computation time required, an experimental design method based on orthogonal design [39] was applied to plan the simulations. Such a combination of experimental design and artificial neural networks has been already used successfully in optimizing HPLC and CE conditions [40–43].

The optimization procedure applied can be summarized as follows:

- specify a reasonable range for the parameters to be optimized;
- design conditions for the computer experiments and calculate the corresponding values of the objective function

by simulating the chromatograms using the Craig model (Eqs. (1)–(9));

- (c) train the artificial neural networks using the parameters of the performed computer experiments as the input and the calculated values of the objective function as the output;
- (d) use the ANN to predict values of the objective function in the whole parameter range;
- (e) reduce the optimization region using a subset of the parameters that correspond to the highest objective function values;
- (f) repeat the above procedure until one of the following termination criteria is fulfilled: (1) the optimization range of the parameters is smaller than a specified limit, (2) the difference between the current and the previous maximum objective function values drops below a small specified number.

In this study iterative solutions of the Craig model, Eq. (1), were generated using a self-made C++ program. In addition the neural network toolbox of *Matlab* was used. In the stage of training the feed-forward neural network the Levenberg–Marquardt algorithm was applied [34].

3. Results and discussion

3.1. Typical cases of ternary systems and specified parameters

For the sake of illustration, this study is concerned with the optimal production of the second eluting component present

in a three component mixture. According to the dependencies of the separation factors (Eq. (8)) between the target component and the two neighbors on the concentration of the modifier in the mobile phase, $\alpha_{1,2}(C_{\text{mod}})$ and $\alpha_{2,3}(C_{\text{mod}})$, ternary mixtures can be classified into five main groups:

- Case (1) $\alpha_{1,2}$ and $\alpha_{2,3}$ do not depend on C_{mod} (“constant–constant”).
- Case (2) $\alpha_{1,2}$ and $\alpha_{2,3}$ both decrease with increasing C_{mod} (“convergent–convergent”).
- Case (3) $\alpha_{1,2}$ decreases and $\alpha_{2,3}$ increases with increasing C_{mod} (“convergent–divergent”).
- Case (4) $\alpha_{1,2}$ increases and $\alpha_{2,3}$ decreases with increasing C_{mod} (“divergent–convergent”).
- Case (5) $\alpha_{1,2}$ and $\alpha_{2,3}$ both increase with increasing C_{mod} (“divergent–divergent”).

All five cases were considered in this work. In order to generate empirically different parameter sets for the adsorption isotherm model selected (Eqs. (7) and (9)), two distinct modifier concentrations and two values for the separation factors were fixed corresponding to the five cases. Using $C_{\text{mod}} = 5\%$ and $C_{\text{mod}} = 50\%$ and combinations of 1.2 and 1.5 for $\alpha_{1,2}$ and $\alpha_{2,3}$ as well as the additional assumption that the saturation capacities are the same for all components, the parameters given in Table 1 are obtained. Because of the fact, that the applied assumptions regarding the structure of Eq. (9) and the equality of the saturation capacities are very strong, it cannot be claimed that with these five sets of equilibrium behavior all possible constellations can be successfully described. However, the applicability of the optimization concept developed is not restricted by these assumptions.

Table 1
Adsorption isotherm parameters (Eqs. (7) and (9)) and selected separation factors (Eq. (8))

Cases	Isotherm parameters (Eq. (9)) ^a	First component 1	Intermediate component 2 (target)	Last component 3	Selected separation factors
Case 1: constant–constant	$a_{1,i}$	4.438×10^{-3}	2.857×10^{-3}	1.839×10^{-3}	At $C_{\text{mod}} = 5\%$:
	$a_{2,i}$	−0.9208	−0.9208	−0.9208	$\alpha_{1,2} = 1.5, \alpha_{2,3} = 1.5$
	$b_{1,i}$	1.3999	0.9013	0.5803	At $C_{\text{mod}} = 50\%$:
	$b_{2,i}$	−0.9208	−0.9208	−0.9208	$\alpha_{1,2} = 1.5, \alpha_{2,3} = 1.5$
Case 2: convergent–convergent	$a_{1,i}$	2.836×10^{-3}	2.857×10^{-3}	2.875×10^{-3}	At $C_{\text{mod}} = 5\%$:
	$a_{2,i}$	−0.8239	−0.9208	−1.0177	$\alpha_{1,2} = 1.5, \alpha_{2,3} = 1.5$
	$b_{1,i}$	1.7601	0.9013	0.5243	At $C_{\text{mod}} = 50\%$:
	$b_{2,i}$	−0.8239	−0.9208	−1.0177	$\alpha_{1,2} = 1.2, \alpha_{2,3} = 1.2$
Case 3: convergent–divergent	$a_{1,i}$	2.836×10^{-3}	2.857×10^{-3}	1.389×10^{-3}	At $C_{\text{mod}} = 5\%$:
	$a_{2,i}$	−0.8239	−0.9208	−0.8239	$\alpha_{1,2} = 1.5, \alpha_{2,3} = 1.2$
	$b_{1,i}$	1.7601	0.9013	0.8623	At $C_{\text{mod}} = 50\%$:
	$b_{2,i}$	−0.8239	−0.9208	−0.8239	$\alpha_{1,2} = 1.2, \alpha_{2,3} = 1.5$
Case 4: divergent–convergent	$a_{1,i}$	5.122×10^{-3}	2.857×10^{-3}	2.875×10^{-3}	At $C_{\text{mod}} = 5\%$:
	$a_{2,i}$	−1.0177	−0.9208	−1.0177	$\alpha_{1,2} = 1.2, \alpha_{2,3} = 1.5$
	$b_{1,i}$	0.9342	0.9013	0.5243	At $C_{\text{mod}} = 50\%$:
	$b_{2,i}$	−1.0177	−0.9208	−1.0177	$\alpha_{1,2} = 1.5, \alpha_{2,3} = 1.2$
Case 5: divergent–divergent	$a_{1,i}$	5.122×10^{-3}	2.857×10^{-3}	1.389×10^{-3}	At $C_{\text{mod}} = 5\%$:
	$a_{2,i}$	−1.0177	−0.9208	−0.8239	$\alpha_{1,2} = 1.2, \alpha_{2,3} = 1.2$
	$b_{1,i}$	0.9342	0.9013	0.8623	At $C_{\text{mod}} = 50\%$:
	$b_{2,i}$	−1.0177	−0.9208	−0.8239	$\alpha_{1,2} = 1.5, \alpha_{2,3} = 1.5$

^a Dimensions: $a_{1,i}$ in $\%^{-1}$, $a_{2,i}$ dimensionless, $b_{1,i}$ in $(\%)^{-1-(1/b_{2,i})}$, $b_{2,i}$ dimensionless.

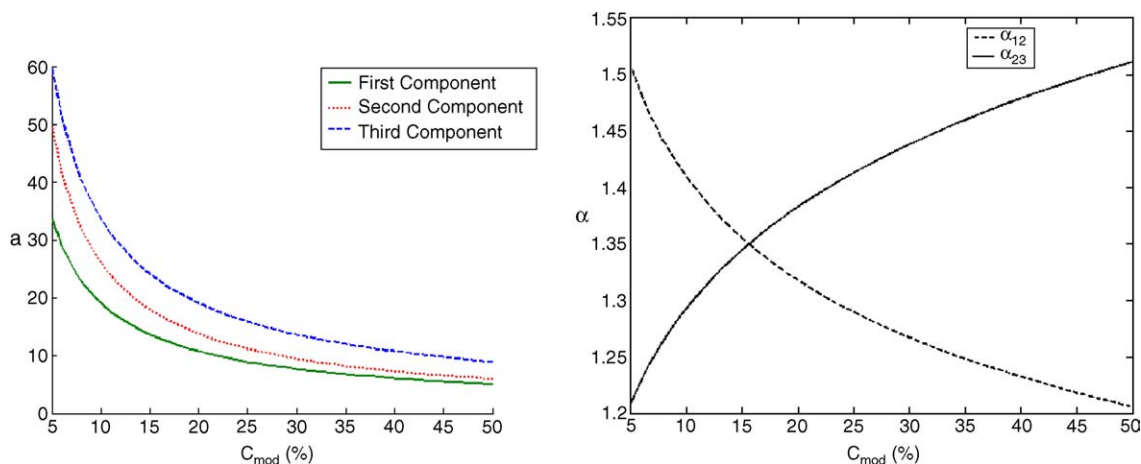


Fig. 2. Dependence of the Henry constants a_i (left) and the separation factors $\alpha_{1,2}$ and $\alpha_{2,3}$ (right) on the concentration of the modifier, C_{mod} , for Case 3 (convergent–divergent) according to the parameters given in Table 1.

For the sake of illustration Fig. 2 shows the resulting variation of the three Henry constants a_i and the corresponding two separation factors as a function of the modifier concentration for Case 3 (“convergent–divergent”).

The study performed was based on geometric (L_{col} , A_{col}) and porosity (ε) parameters typical for laboratory scale HPLC conditions. The stage number P , the flowrate V_F and the injection concentrations, $C_{i,\text{inj}}$, were kept constant. The values for the mentioned parameters and for the threshold concentration, $C_{\text{threshold}}$, and the specified desired purity, $\text{Pur}_{2,\text{des}}$, used during the simulations are summarized in Table 2. The range of modifier concentrations considered during the gradient optimization was restricted between 5% and 50% (which led to separation factors between 1.2 and 1.5). Further, in all calculations performed the start time of the gradient was fixed to be the end of injection, i.e. $t_{g,\text{begin}} = t_{\text{inj}}$.

Since the main goal of this study was to evaluate the influence of the shape and duration of gradients on process performance, during the optimizations the regeneration time t_{reg} was set in Eq. (13) to zero. Thus, the different kind of gradients could be compared directly. A fair comparison with isocratic operation obviously would require specifying for the

Table 2
Parameters characterizing the chromatographic system considered

Column dimensions and efficiency	
L_{col} (cm)	10
A_{col} (cm)	0.6
ε	0.775
P	1000
Flowrate and dead time	
V_F (ml/min)	1.0
t_0 (min)	2.19
Injection concentrations and threshold parameter	
$C_{1,\text{inj}}$ (g/l)	20
$C_{2,\text{inj}}$ (g/l)	20
$C_{3,\text{inj}}$ (g/l)	20
$C_{\text{threshold}}$	$0.01C_{\text{max}}$
$\text{Pur}_{2,\text{des}}$ (%)	99

gradient operation a concrete specific value for the required regeneration time.

3.2. Parametric study for Case 1 (constant–constant)

At first, parametric studies were performed for Case 1. In this case the separation factors $\alpha_{1,2}$ and $\alpha_{2,3}$ were constant (1.5) during the gradient process.

In the calculation at first the gradient shape factor was set to be $S = 1$ (equivalent to a linear gradient) and the final concentration of the modifier was set to be at the upper limit of the interval considered (i.e. $C_{\text{mod, end}} = 50\%$) corresponding to the shortest retention times. For these constraints the effects of the initial modifier concentration, $C_{\text{mod, begin}}$, the gradient slope, G , and the loading factor, $L_{f,\text{tot}}$, on the production rate of the intermediate component, Pr_2 , were studied. Fig. 3

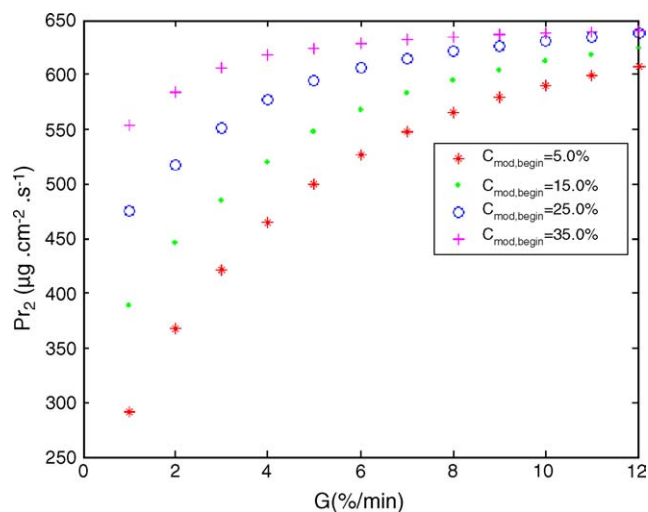


Fig. 3. Effect of initial concentration of the modifier, $C_{\text{mod, begin}}$, and gradient slope, G , on the rate of producing the intermediate component, Pr_2 , for Case 1 (constant–constant) and $S = 1$ (each point corresponds to the specific optimum total loading factor $L_{f,\text{tot}}$).

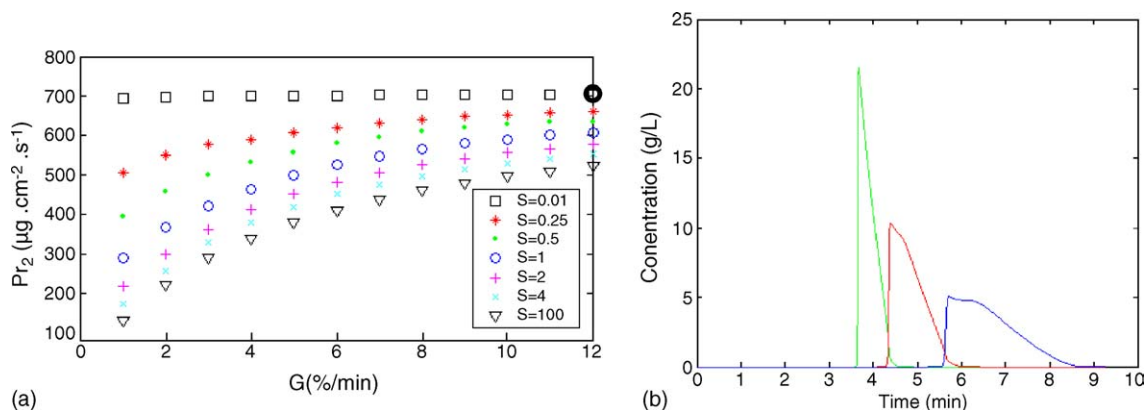


Fig. 4. (a) Effect of gradient slope G and gradient shape factor S on the production rate Pr_2 for Case 1 (constant–constant). $C_{\text{mod,begin}} = 5\%$ (each point corresponds to the specific optimum total loading factor $L_{f,\text{tot}}$). (b) Simulated chromatogram corresponding to the dark black circle in (a). Conditions: $G = 12.0\%$ /min, $S = 0.01$ and $L_{f,\text{tot}} = 19.35\%$.

shows selected results obtained. Each point in this plot corresponds to a different loading factor which specifically allows obtaining the maximum production rate. It can be seen that the production rate increases with increasing gradient slope G . For the conditions considered here gradients steeper than 10% /min do not lead to further improvements. The production rate also increases with increasing $C_{\text{mod,begin}}$. This effect becomes less significant when G is high. Both of the tendencies observed obviously indicate that it is useful to operate the column for the isotherms corresponding to Case 1 under isocratic conditions at $C_{\text{mod}} = C_{\text{mod,max}} = 50\%$.

The effect of the gradient shape factor S on the productivity Pr_2 was studied for a fixed value of $C_{\text{mod,begin}} = 5\%$. Fig. 4a demonstrates that the production rate again increases with increasing G . The rate also increases if S is decreased which leads to faster elution with a stronger mobile phase. When S is very small, the gradient elution corresponds to isocratic elution at a high modifier concentration level. Then the production rate does not depend anymore on G . For illustration, the simulated elution profile corresponding to the optimal elution conditions (black circle in Fig. 4a) is shown in Fig. 4b.

Since in general the effect of a modifier is usually more complex (no constant–constant behavior), subsequently other cases were investigated.

3.3. Parametric study for Cases 2 and 3 (convergent–convergent and convergent–divergent)

Fig. 5 shows the effect of gradient slope and gradient shape for a system belonging to Case 2 (convergent–convergent). Again, the initial modifier concentration was fixed to be $C_{\text{mod,begin}} = 5\%$ and for each situation the optimal loading factor was optimized. The figure reveals that the dependence of the production rate on G and S is different than for Case 1 (Fig. 4a). When the value of S is smaller than 0.5, the change of Pr_2 with variations in the gradient slope G is small. For higher gradient shape factors, the production rate first increases along with the gradient slope due to the reduction

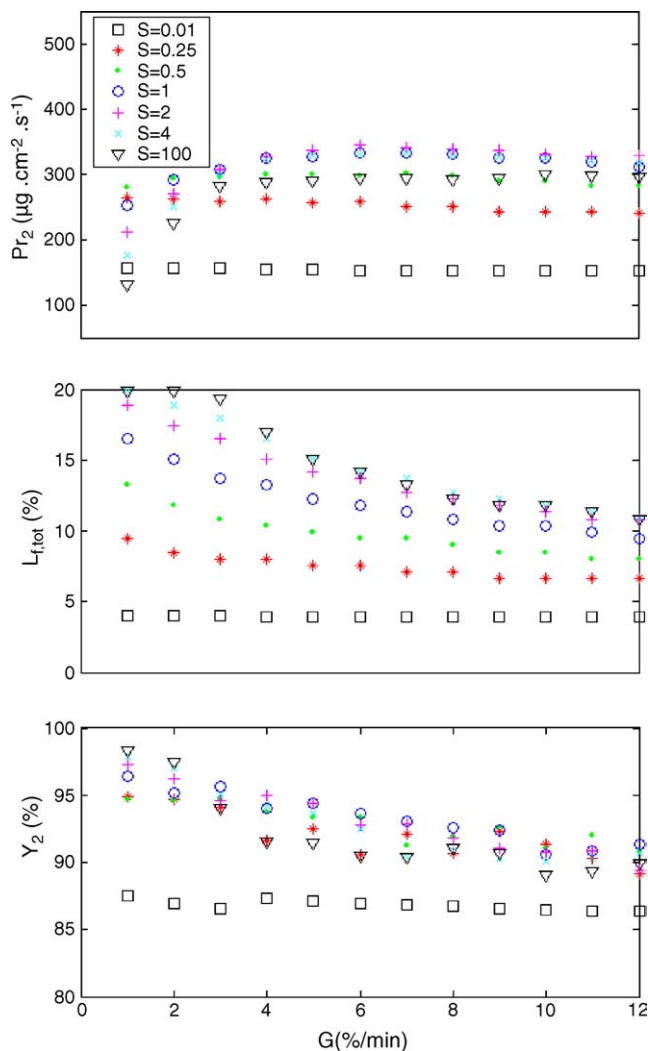


Fig. 5. Effect of gradient slope G and gradient shape factor S on the production rate, the corresponding optimal total loading factor and the recovery yield (Case 2, convergent–convergent).

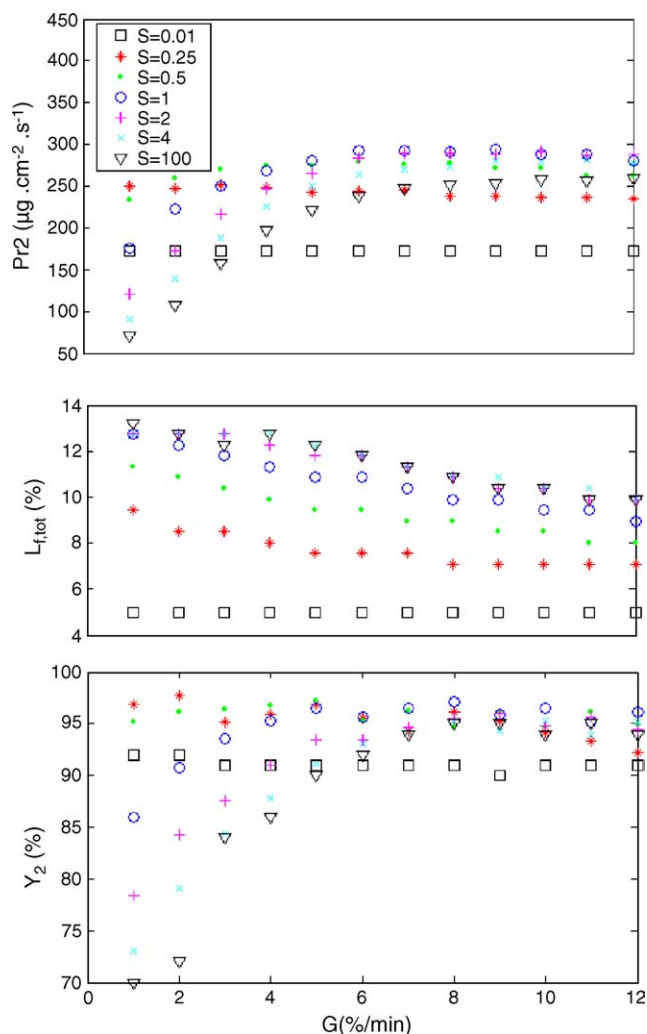


Fig. 6. Effect of gradient slope G and gradient shape factor S on the production rate, the corresponding optimum total loading factor and the recovery yield (Case 3, convergent–divergent).

of separation time. After achieving a maximum, the production decreases because the effect of reducing the separation factors exceeds the effect of reducing the separation time. Another important phenomenon observed is the fact that the optimum production rate increases first and then decreases with an increase of the gradient shape factor. The optimum value of the gradient shape parameter S for the system studied is about 2. In Fig. 5 are also shown the corresponding optimal total loading factors and the recovery yields of the second component. Both the loading factors and the recovery yields decrease gradually when the gradient slope increases.

The results presented on Fig. 6 demonstrate that the effects of gradient slope and shape factor on the production rate of the intermediate component for Case 3 (convergent–divergent) are similar to that for Case 2 (convergent–convergent, Fig. 5). The major difference is that highest production rate can be obtained when the gradient shape factor S is about 1.0 and thus smaller than for Case 2. There appears to exist also an optimum value regarding the gradient slope G .

The results of the parametric studies presented for Cases 1–3 and further results not given here indicate that the effect of gradient elution conditions on the productivity of the target component depend strongly on the specific variation of the adsorption characteristics of the components to be separated with the modifier concentration.

Subsequently systematic optimization runs were performed maximizing the objective function defined in Eq. (15). Besides the nonlinear gradients discussed above, two-step gradients, three-step gradients, linear gradients and isocratic operation were included in the analysis.

3.4. Optimization of isocratic, linear, stepwise and nonlinear gradient elution for Case 3

The operating conditions for Case 3 (convergent–divergent) were optimized for different elution modes.

Before discussing the results, the procedure applied and already briefly described in Section 2.5 is explained in more detail. This is done considering as an example the optimization of the concentration of the modifier, C_{mod} , and the loading factor, $L_{f,\text{tot}}$, in isocratic elution. At first (step a) search intervals were specified for C_{mod} between 5% and 50% and for $L_{f,\text{tot}}$ between 4.7% and 16.5% (based on the results of the calculations described above). Then (step b) 100 experiments with different parameters were designed according to fixed-level orthogonal arrays with 100 runs, 4 factors, 10 levels, and strength 2 [30] and the corresponding values of the considered objective function $\text{OF} = \text{Pr}_2 Y_2$ were calculated after simulating the chromatograms for the corresponding elution conditions. Subsequently (step c) the ANN was trained by taking the selected parameters as input and the corresponding calculated OF-values as output. After training, the ANN was used to model the whole response surface of the objective function over the parameters to be optimized (step d). The results obtained for the isocratic case are illustrated in Fig. 7.

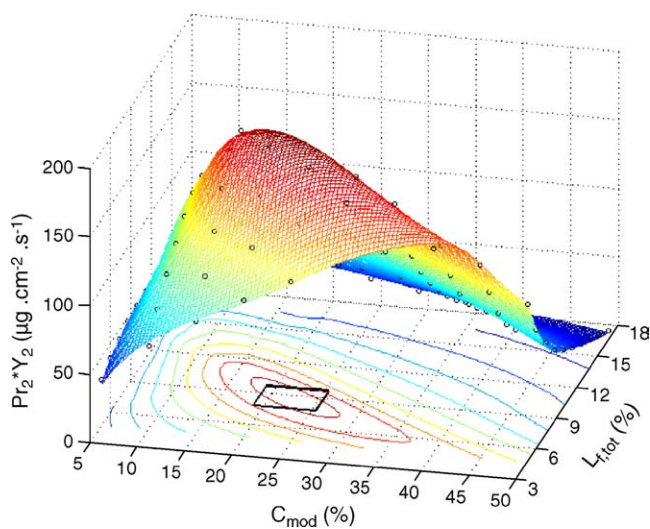


Fig. 7. Dependence of the objective function (Eq. (15)) on the modifier concentration and the total loading factor (Case 3, convergent–divergent).

Table 3
Optimum elution conditions and corresponding performance parameters for the second eluting target component for different elution modes (Case 3)

Elution mode	Number of optimised parameters	Determined optimal values	Pr_2 ($\mu\text{g cm}^{-2} \text{s}^{-1}$)	Y_2 (%)	$Pr_2 Y_2/100\%$ (OF) ($\mu\text{g cm}^{-2} \text{s}^{-1}$)	$C_{2,Ave}$ (g/l)
Isocratic	2	C_{mod} : 21.7%, $L_{f,tot}$: 8.92%	196.2	97.8	191.8	1.52
Two-step gradient	4	C_{mod} (first step): 16.3%, C_{mod} (second step): 49.1%, duration first step: 6.18 min, $L_{f,tot}$: 10.57%	272.3	97.8	266.2	3.96
Three-step gradient	4	C_{mod} (first step): 11.7%, C_{mod} (second step): 24.4%, duration (first step): 2.87 min, duration (second step): 2.87 min, $L_{f,tot}$: 10.62%	283.9	97.4	276.5	3.97
Linear gradient	3	$C_{mod,begin}$: 5.0%, duration of gradient t_g : 5.9 min, $L_{f,tot}$: 9.99%	283.2	95.3	269.8	3.43
Nonlinear gradient	4	$C_{mod,begin}$: 5.4%, duration of gradient t_g : 5.57 min, gradient shape factor S : 1.2, $L_{f,tot}$: 10.34%	294.9	96.0	283.1	3.66

By finding now ranges of parameters that correspond to the highest objective function values, a new reduced region can be specified (step e). In this study, 100×100 sets of parameters were initially scanned and the range of the parameters corresponding to the 100 highest objective function values was used to select the reduced optimization region (shown for the example in Fig. 7 as a smaller rectangle). After the reduced region was determined, the procedure of experimental design, ANN training, modeling the response surface and reducing optimization region was repeated, until a termination criterion was satisfied (step f). In this particular case, values in relation to the accuracy provided by chromatographic instruments were used (0.1% as the minimum difference for the modifier concentration and 0.001 ml ($L_{f,tot} = 0.0472\%$) as the minimum difference for the injection volume).

The obtained optimum operating conditions for isocratic elution and the corresponding performance criteria are listed in Table 3. The corresponding simulated optimal chromatogram is shown in Fig. 8a.

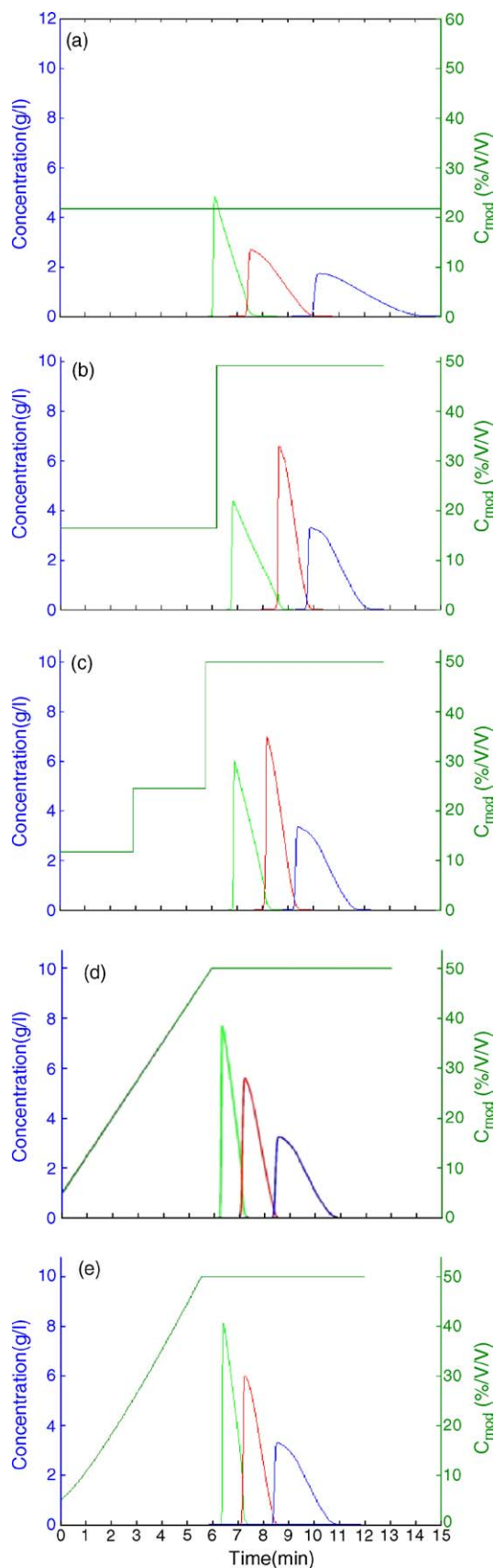
Stepwise gradient elution is often used in preparative chromatography due to the convenience of operation. Hence the procedure described above was subsequently used to optimize the conditions for two possibilities of stepwise gradient elution. At first a two-step step gradient elution was considered. Since the highest concentration of modifier is beneficial to produce concentrated products at reduced separation times, the end concentration of the modifier was fixed to be 50%. Thus, there remain four parameters to be optimized: the initial concentration of modifier, the duration time of first step, the concentration of modifier in the second step and the loading factor. The intervals set for these free parameters were: the concentrations of the modifier were set between 5% and 50%, the duration of the first step was set between 2 and 20 min and the loading factor was set (as for isocratic elution) between 4.7% and 16.5%. The determined optimized conditions are given in Table 3 and the corresponding chromatogram is shown in Fig. 8b. It is illustrated that it is favorable to use at first a mobile phase with a relatively low concentration of

the modifier and then to elute with a significantly stronger mobile phase.

To further improve the separation, three-step gradient elution was also optimized. The free parameters considered were the concentration of the modifier in the first and in the second step. Since the results obtained before indicated that the upper limit is beneficial, the modifier concentration in the final step was fixed to be 50%. Optimized were the durations of the first and second steps (to simplify the analysis, both times were assumed to be the same) and the total loading factor. The initial ranges were the same as in the two-step case. The optimized elution conditions can again be found in Table 3 and the corresponding optimum chromatogram is shown in Fig. 8c. It can be seen that both the production rate and the average concentration of the target component in the collected fraction (C_{ave}) increase compared to isocratic and two-step gradient operation. Obviously the performance of stepwise gradient elution increases along with the number of step numbers. The optimal step height is significantly smaller for the first step than for the second step.

Subsequently the linear gradient was optimized. The following three parameters were determined: the corresponding initial modifier concentration, the gradient slope and the loading factor. The initial search intervals were: the initial modifier concentration was set between 5% and 50%, the gradient slope between 1 and 12%/min, and the loading factor between 4.7% and 16.5%. The results obtained are given in Table 3. The OF-value is between the values for the two- and three-step gradients. The corresponding chromatogram is shown in Fig. 8d.

Finally the nonlinear gradient situation was analyzed using Eqs. (4)–(6). Four parameters were optimized: the initial concentration of the modifier, $C_{mod,begin}$, the gradient slope, G , the gradient shape factor, S , and the total loading factor, $L_{f,tot}$. Initially the concentration of the modifier was set between 5% and 50%, the gradient slope between 1 and 12%/min and the gradient shape factor between 32 and 1/32. The initial search range for the loading factor was between



4.7% and 16.5%. The determined optimum nonlinear gradient elution conditions and the corresponding performances parameters are also included in Table 3. The simulated optimum chromatogram is shown in Fig. 8e. It can be seen that, compared to isocratic elution, not only the production rate increases (from 196.2 to 294.9 $\mu\text{g cm}^{-2} \text{s}^{-1}$), but also the average concentration of the target component in the collected fraction (from 1.52 to 3.66 g/l). The results do not differ very much from the results for the linear gradient, as indicated also by the fact that the optimal gradient shape parameter S is 1.2 and thus close to 1. Obviously for Case 3 and the conditions considered a slightly concave gradient is most beneficial for the production of the intermediate component. Finally the fact should be mentioned, that the optimum situation in all gradient cases shown in Fig. 8b–e leads to an elution of the components after the end of the gradient.

3.5. Optimization of nonlinear gradient elution for Cases 2 and 4

Further optimizations were performed for Cases 2 (convergent–convergent) and 4 (divergent–convergent) using nonlinear gradients based on Eqs. (4)–(6). The results obtained are shown in Fig. 9. It can be seen in Fig. 9a that for Case 2 (convergent–convergent), a significant concave gradient is beneficial. In contrast, for Case 4 (divergent–convergent), a significant convex gradient was found to be favorable (Fig. 9b). The figure captions contain the determined free parameters for the two cases.

In general both shorter separation times and larger separation factors (both α_{12} and α_{23}) are advantageous for the production of the second eluting component. If there is conflict between these objectives the optimal conditions correspond to a compromise. Always a “quick” (convex) gradient ($S < 1$) is beneficial for shortening the separation time. Since it is useful for Case 2, that both separation factors stay longer on a high level, a “slower” (concave) gradient ($S > 1$) is advantageous from this side. In the particular case a slightly concave ($S = 1.72$) represent the optimum. If the gradient would be more concave ($S > 1.72$), the separation time would be longer and the accessible value of the objective function would be lower.

For Case 4, a concave gradient elution is advantageous to increase the second separation factor but disadvantageous regarding the first separation factor. In contrast, a convex gradient elution is advantageous to increase the first separation factor but disadvantageous regarding the second separation factor. For the example considered a slightly convex gradient is optimal because the development of α_{12} is more important

Fig. 8. Simulated chromatograms under various conditions (see Table 3) maximizing the objective function (Eq. (15)) for the second eluting component (Case 3, convergent–divergent): (a) isocratic operation; (b) two-step gradient; (c) three-step gradient; (d) linear gradient and (e) nonlinear gradient.

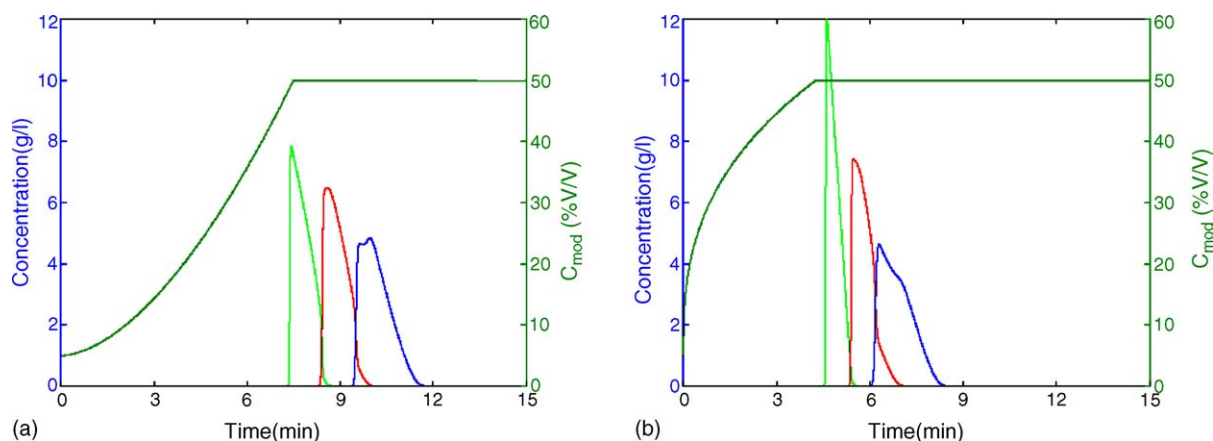


Fig. 9. Optimized nonlinear gradients and corresponding chromatograms for the production of the intermediate component. (a) Case 2 (convergent–convergent): $S = 1.72$, $C_{\text{mod, begin}} = 5.0$, $t_g = 7.5$ min, $\text{Pr}_2 = 344.3 \mu\text{g cm}^{-2} \text{s}^{-1}$, $Y_2 = 93\%$, $\text{Pr}_2 Y_2 / 100\% = 321.3 \mu\text{g cm}^{-2} \text{s}^{-1}$, $L_{f, \text{tot}} = 13.3\%$. (b) Case 4 (divergent–convergent): $S = 0.366$, $C_{\text{mod, begin}} = 5.0$, $t_g = 4.27$ min, $\text{Pr}_2 = 422.3 \mu\text{g cm}^{-2} \text{s}^{-1}$, $Y_2 = 86\%$, $\text{Pr}_2 Y_2 / 100\% = 361.5 \mu\text{g cm}^{-2} \text{s}^{-1}$, $L_{f, \text{tot}} = 12.7\%$.

for the increase of the production of the intermediate component than the development of α_{23} [36].

Results for Case 5 (divergent–divergent) are not presented in detail. It is obvious that for such equilibrium conditions isocratic elution using a mobile phase containing a high concentration of modifier is optimal, as for Case 1 (constant–constant).

In general, it can be concluded that optimum gradient elution conditions are strongly affected by the adsorption characteristics of the components in the sample.

4. Conclusion

The influence of gradient elution parameters on the production of a specific target component using preparative chromatography was studied theoretically. The elution conditions were optimized by combining artificial neural networks and experimental design method in combination with an equilibrium stage model describing the separation process in the column. The concept was found to be useful to compare quantitatively various kinds of gradient techniques (two-step, three-step, linear, nonlinear) with isocratic elution. The increase of production rate due to applying gradients is mainly due to the decrease of the retention and cycle times and the possible increase of the loading factors. The results obtained emphasize that gradient elution possesses the potential to outperform isocratic operation in preparative chromatography. Preparative gradient elution appears to be in particular suitable for the separation of mixtures containing components with strong retention. In addition, to develop a productive process, the time required for column regeneration must be relatively short. It was found that the optimal gradient elution conditions for the production of a certain target component are significantly affected by the dependence of the separation factors between the target component and its neighbours on the mobile phase composition. The optimization concept presented above can be easily applied to analyse

the separation of more complex mixtures possessing the considered or other types of equilibrium relations, provided the latter are available.

5. Nomenclature

a_i	Henry constant for component i , Eq. (7)
$a_{1,i}, a_{2,i}$	parameter of isotherm model, Eq. (9)
A_{col}	cross-section area of the column, Eq. (10)
b_i	parameters of the Langmuir isotherm equation for component i , Eq. (7)
$b_{1,i}, b_{2,i}$	parameter of isotherm model, Eq. (9)
C_{ave}	average concentration in fraction
$C_{i, \text{inj}}$	concentration of component i in the injected mixture, Eq. (2)
$C_{i,j}^k$	concentration of component i in plate j at exchange step k in Craig model, Eq. (1)
C_{max}	maximum concentration in elution profile
C_{mod}	concentration of modifier, Eq. (4)
$C_{\text{mod, begin}}$	initial concentration of modifier
$C_{\text{mod, end}}$	final concentration of modifier
$C_{\text{mod, max}}$	upper limit of modifier concentration during gradient
$C_{\text{threshold}}$	threshold concentration for fractionation
G	gradient slope, Eq. (5)
K	number of exchange steps in the Craig model
L_{col}	length of the column, Eq. (10)
$L_{f,i}$	loading factor of component i , Eq. (10)
$L_{f, \text{tot}}$	total loading factor, Eq. (11)
m_i	amount of component i recovered in the collected fraction, Eq. (12)
N	number of components in the sample, Eq. (1)
OF	objective function
P	number of stages in the column, Eq. (1)
Pr_i	production rate of component i , Eq. (12)
$\text{Pur}_{i, \text{des}}$	desired purity of component i

$q_{i,j}^k$	equilibrium loading of component i in plate j and exchange step k , Eq. (1)
$q_{\text{sat},i}$	saturation capacity of component i in the stationary phase; $q_{\text{sat},i} = a_i/b_i$, Eq. (7)
S	gradient shape parameter, Eq. (4)
t_0	dead time of column, i.e. elution time of a non retained component, $t_0 = L_{\text{col}}A_{\text{col}}\varepsilon/V_{\text{F}}$
t_{g}	duration of gradient ($t_{\text{g, end}} - t_{\text{g, begin}}$), Eq. (4)
$t_{\text{g, begin}}$	start of gradient elution, Eq. (4)
$t_{\text{g, end}}$	end of gradient elution, Eq. (4)
t_{inj}	injection time, Eq. (2)
$t_{\text{N}}^{\text{end}}$	end time of the most retained component, Eq. (13)
Δt	residence time of the mobile phase in a plate, Eq. (2)
Δt_{c}	cycle time, Eq. (13)
V_{F}	volumetric flowrate of the mobile phase
V_{inj}	injection volume, Eq. (10)
Y_i	recovery yield of component i , Eq. (14)

Greek letters

$\alpha_{i,m}$	separation factor between components i and m , Eq. (8)
ε	total column porosity, Eq. (1)

Indices

i	component
j	plate
k	exchange step in Craig model

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