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Analysis of the isolation of a target component using multicomponent isocratic preparative elution chromatography

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Abstract

The separation of a certain target component from a multicomponent mixture using isocratic preparative elution chromatography was studied theoretically. In particular, the important and most complicated case was considered that the target component does not elute in the first or last position. To specify the productivity of collecting this component different options are suggested to identify suitable times for fractionation. Using a conventional Craig model, capable to quantify chromatographic processes, the impact of several essential parameters (e.g. threshold concentration, desired purity, injection volume, separation factor between neighboring components, composition of the mixture) is evaluated for a ternary system based on parametric calculations. The paper provides simple tools to evaluate and optimize the productivity and other objective functions relevant in multicomponent preparative chromatography.

Keywords: Preparative chromatography; Craig model; Production rates

1. Introduction

Preparative chromatography is an important industrially applied separation process for the isolation and purification of pharmaceuticals and other value added products. Since the operating parameters have a strong effect on the productivity with which a certain component can be obtained, much interest has been focused on the investigation and optimization of these parameters (e.g. [1–5]). In preparative chromatography the columns are overloaded and, consequently, the process is characterized by nonlinear and competitive equilibrium functions. Thus, in contrast to analytical chromatography, the migration of one component in the column will effect in a complex manner the migration of the other components. For this reason, many theoretical studies have been focused on analyzing the separation of two components (e.g. [6–8]). However, in general it is necessary to collect a certain component from mixtures containing more than two components. A quantitative investigation of different operating parameters will be helpful to find for such systems suitable operating conditions and to achieve high production rates. Although research has been already carried out to predict and analyze elution profiles for multicomponent mixtures under overloaded conditions (e.g. [9]), there is a need in providing simple and reliable tools capable to quantify the specific productivity for a target component and to analyze systematically the dependence of the course of the chromatographic process on the operating parameters.

This paper attempts to contribute to answer the following questions:

(1) How can appropriate times of fractionation (cut times) be determined for the collection of a certain target component within a multicomponent elution profile taking into account specific purity requirements?
(2) How does the productivity of the separation depend quantitatively on certain parameters describing the chromatographic system?

In order to solve these problems it is useful to apply a simple and reliable model of the chromatographic process.

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2. Theory

There are several models available capable to quantify the development of concentration profiles in chromatographic columns [2]. Due to the fact that under overloaded conditions the adsorption isotherms are nonlinear, numerical solutions of the underlying model equations are needed. The models can be divided into two groups: (a) continuous models (characterized by partial differential equations) such as the equilibrium-dispersive model [2,10], the lumped kinetic model (e.g. [11]), or the general rate model [1,2,9]; and (b) discrete models (characterized by algebraic equations or ordinary differential equations) such as the equilibrium stage model, the Craig model or the "tank in series"-model [12–14]. Combining these models with suitable equilibrium relations, elution profiles can be predicted. It is well known that all the mentioned models deliver almost identical results provided the column efficiency is high [2,10].

In this paper, the Craig model was chosen to simulate elution profiles for multicomponent mixtures. Reasons for this choice were the simplicity and flexibility of this model and the fact that it can be easily extended to describe gradient elution chromatography which is planed for a subsequent work. It should be noted that the general trends discussed below do not depend on the selection of the column model.

2.1. Craig model

The Craig model [13] is a classical tool to describe the development of concentration profiles in chromatographic columns. In the Craig model, the column is divided into P 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 equation of the Craig process can be expressed for a component i, a stage j and an exchange step k as follows:

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

where C is the liquid phase concentration, ε the column porosity, q the concentration in the stationary phase in equilibrium with the local liquid phase concentrations. The difference between two exchange steps, designated by k and $k+1$, corresponds to the characteristic mobile phase residence time in a stage, Δt. It is related to the dead time of the column, t_0, divided by the total number of stages, P:

$$\Delta t = \frac{t_0}{P} \tag{2}$$

with

$$t_0 = \frac{A_{\mathrm{Col}} L_{\mathrm{Col}} \varepsilon}{V_{\mathrm{F}}} = \frac{V_{\mathrm{Col}} \varepsilon}{V_{\mathrm{F}}}. \tag{3}$$

In Eq. (3) A_{Col}, L_{Col}, V_{Col} are the cross section area, the length and the volume of the column. V_{F} is the volumetric flow rate of the mobile phase.

At this point a restriction of the Craig model should be mentioned. Due to the fact that there is applied only one stage number there is no straightforward possibility to take the fact into account that there might be different values optimal for different components. It should be also noted that there exists a relation between the number of stages in the Craig model, P, and the well-known number of theoretical stages (column efficiency), N_{plate} [2].

Considering initially ($k = 0$) not preloaded columns holds:

$$C_{i,j}^{0} = 0 \text{ and } q_{i,j}^{0} = 0 \quad i = 1, N;\ j = 1, P. \tag{4}$$

In elution chromatography typically rectangular injection profiles are imposed at $j = 0$. They can be described as follows:

$$C_{i,0}^{k} = \begin{cases} C_{i,0} & \text{for } k \times \Delta t \le t_{\mathrm{inj}} \\ 0 & \text{for } k \times \Delta t > t_{\mathrm{inj}} \end{cases} \qquad i = 1, N;\ k = 1, K. \tag{5}$$

In Eq. (5), $C_{i,0}$ is the injection concentration and t_{inj} is the injection time, which is the ratio of the injection volume, V_{inj}, and the volumetric flow rate, V_{F}:

$$t_{\mathrm{inj}} = \frac{V_{\mathrm{inj}}}{V_{\mathrm{F}}}. \tag{6}$$

The most important information required to describe a concrete separation process are the adsorption isotherms, i.e. the functions $q(\bar{c})$. Frequently, and also in this work, the following equations of the competitive Langmuir model are used:

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

where a_i (or $q_{\mathrm{sat},i}$) and b_i are the free parameters. The a_i are often called Henry constants and $q_{\mathrm{sat},i}$ stands for the saturation capacity of component i in the stationary phase ($q_{\mathrm{sat},i} = a_i / b_i$).

It should be noted that other adsorption isotherm models capable to describe more complex shapes of adsorption equilibrium functions can be implemented into the Craig model in a similar way.

Table 1
Reference parameters used in calculations

Isotherm parameters	First component	Intermediate component	Last component
a_i[a]	4.79	5.80	6.99
b_i (ml/mg)	0.0266	0.0266	0.0266

System and operating parameters: $L_{Col} = 10\,cm$, $A_{Col} = 0.283\,cm^2$, $\varepsilon = 0.775$, $V_F = 1.0\,ml/min$, $t_0 = 2.19\,min$, $C_{1,0} = C_{2,0} = C_{3,0} = 200\,mg/ml$, $P = 1000$.

[a] $\alpha_{1,2} = \alpha_{2,3} = 1.2$.

The ratio between two Henry constants is called separation factor α:

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

Provided the $C^k_{i,j-1}$, $C^k_{i,j}$, ε, the adsorption isotherms and the relevant initial and boundary conditions are specified, the new equilibrium concentrations, $C^{k+1}_{i,j}$, can be calculated by solving Eq. (1) iteratively along the space (all stages) and time (all considered exchange steps) coordinates. Hereby always all component balances must be solved simultaneously. Suitable iteration schemes have been for example discussed in [14].

A selected set of parameters allowing to generate chromatograms with the Craig model is summarized for a ternary system in Table 1. The numbers given in the table serve in this study as standard parameters. The elution profile simulated for these conditions and an injection volume of $V_{inj} = 10\,\mu l$ is given in Fig. 1. The figure shows the courses of the total and the three individual concentrations. Under these conditions the column is already slightly overloaded. This causes the well-known peak tailing effect.

The injected amount can be also expressed conveniently as a dimensionless loading factor, $L_{f,i}$. This factor, which

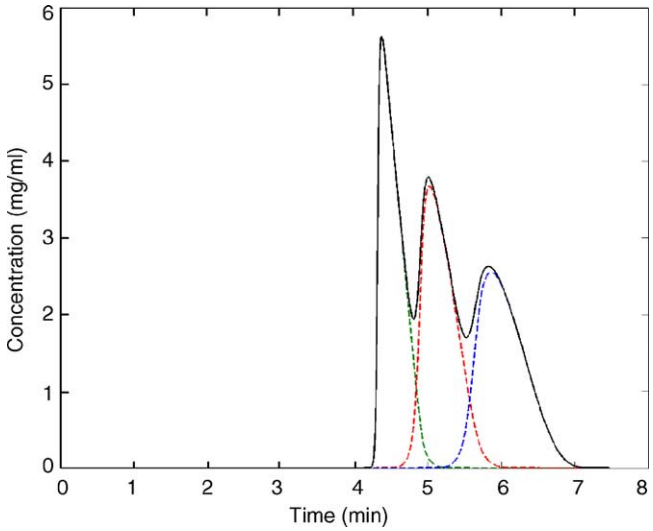

Fig. 1. Reference chromatogram of a three component mixture (individual and total concentrations) simulated with the Craig model (Eq. (1)), $V_{inj} = 10\,\mu l$ ($L_{f,tot} = 4.4$), other parameters as in Table 1.

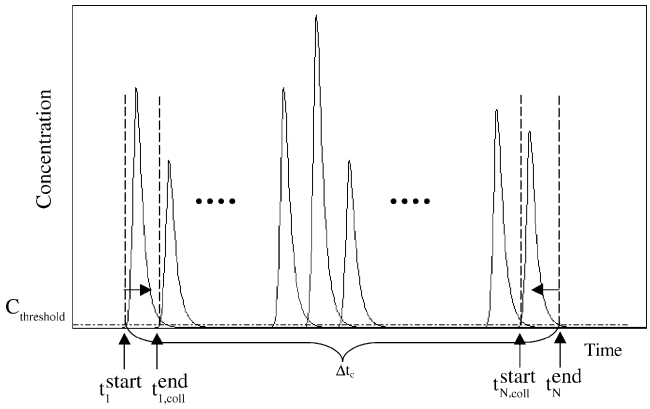

Fig. 2. Schematic presentation of an elution profile for a N-component mixture. Indicated are for illustration the cycle time, the threshold concentration and the times for fractionating the first and the last eluting component.

is the ratio of the amount of component i in the sample to the corresponding specific column saturation capacity, can be expressed as follows:

$$L_{f,i} = \frac{V_{inj} C_{i,0}}{(1-\varepsilon) A_{Col} L_{Col} q_{sat,i}} \times 100. \tag{9}$$

Sometimes also a total loading factor, $L_{f,tot}$, is used which is defined as:

$$L_{f,tot} = \sum_{i=1}^{N} L_{f,i}. \tag{10}$$

The total loading factor corresponding to the chromatogram shown in Fig. 1 is 4.4 indicating a modest degree of overloading.

2.2. Performance criteria

A more general schematic representation of an elution profile corresponding to an injection of a N component mixture is given in Fig. 2. This figure serves to emphasize the fact that the approach presented below can be applied to analyze the isolation of a certain target component from a mixture of an arbitrary number of components.

In order to realize a productive process the injections should be performed as often as possible. The cycle time, Δt_c, is the time between two consecutive injections. It can be specified by the following two characteristic times: (a) t_1^{start}, the time when the concentration of the first eluting component exceeds a given specified threshold concentration, $C_{threshold}$, and (b) t_N^{end}, the time when the concentration of the last eluting component drops below this value. Possibilities to specify $C_{threshold}$ are discussed below (Section 3.1). For Δt_c holds:

$$\Delta t_c = t_N^{end} - t_1^{start}. \tag{11}$$

With the cycle time a production rate of component i, Pr_i, can be defined as the amount recovered from one injection, m_i, over cross-section area and cycle time:

$$\mathrm{Pr}_i = \frac{m_i}{\varepsilon A_{\mathrm{Col}} \Delta t_{\mathrm{c}}}. \tag{12}$$

Hereby the recovered fraction should possess a certain specified (desired) purity with respect to a component i which is defined as:

$$\mathrm{Pur}_{i,\mathrm{des}} = \frac{m_i}{\sum_{m=1}^{N} m_m}. \tag{13}$$

In addition a recovery yield of component i can be defined as the ratio of the amount recovered in the collected fraction over the amount of the same component injected in the sample:

$$Y_i = \frac{m_i}{V_{\mathrm{inj}} C_{i,0}}. \tag{14}$$

To calculate production rates and yields for a certain component i, the cycle time, Δt_{c}, and the amount of purified component collected in a single cycle, m_i, must be known.

2.3. Calculation of cycle times, collection times and amounts of purified component

The determination of the cycle time Δt_{c} (Eq. (11)) requires simply the determination of t_1^{start} and $t_{\mathrm{N}}^{\mathrm{end}}$ using a specified value for $C_{\mathrm{threshold}}$ (Fig. 2).

More complicated is the determination of the collection times, $t_{i,\mathrm{coll}}^{\mathrm{start}}$ and $t_{i,\mathrm{coll}}^{\mathrm{end}}$, and the corresponding amount of purified sample, m_i, for a component i travelling somewhere in the elution train.

The specification of the beginning and the end times for collecting a component i between $t_{i,\mathrm{coll}}^{\mathrm{start}}$ and $t_{i,\mathrm{coll}}^{\mathrm{end}}$ is related to the desired purity of that component in the fraction. This integral purity can be calculated according to:

$$\mathrm{Pur}_{i,\mathrm{int}} = \frac{m_{i,\mathrm{coll}}}{\sum_{m=1}^{N} m_{m,\mathrm{coll}}} = \frac{A_i}{\sum_{m=1}^{N} A_m} \quad i = 1, N, \tag{15}$$

with the corresponding partial peak areas of all components:

$$A_m = \sum_{k=t_{i,\mathrm{coll}}^{\mathrm{start}}/\Delta t}^{t_{i,\mathrm{coll}}^{\mathrm{end}}/\Delta t} C_{m,j=P}^{k} \, \Delta t \quad m = 1, N. \tag{16}$$

Due to the discrete character of the Craig model the time axis is expressed as a function of the number of exchange steps k. For larger time steps (in case of smaller stage numbers) round off error might occur performing these discrete calculations. These round-off errors are negligible if the efficiency is high as it is typically the case in many applications.

Before analyzing the (typical) situation of a target component travelling in the middle of the elution profile at first the trivial cases are considered, that the target component elutes in the first or in the last position.

2.3.1. Calculation of fractionation times for first and last eluting components

It is simple to specify the collection times for the first and last eluting components. For the first eluting component, the purified amount A_1 can be calculated by integrating its concentration between $t_{1,\mathrm{coll}}^{\mathrm{start}} (= t_1^{\mathrm{start}})$ and $t_{1,\mathrm{coll}}^{\mathrm{end}}$ (Fig. 2). The latter can be specified by assuring that the amount of this component collected over the overall amount of the fraction is equal to $\mathrm{Pur}_{1,\mathrm{des}}$. Thus, the time $t_{1,\mathrm{coll}}^{\mathrm{end}}$ should satisfy:

$$\frac{\sum_{k=t_{1,\mathrm{coll}}^{\mathrm{start}}/\Delta t}^{t_{1,\mathrm{coll}}^{\mathrm{end}}/\Delta t} C_{1,P}^{k}}{\sum_{m=1}^{N} \sum_{k=t_{1,\mathrm{coll}}^{\mathrm{start}}/\Delta t}^{t_{1,\mathrm{coll}}^{\mathrm{end}}/\Delta t} C_{m,P}^{k}} = \mathrm{Pur}_{1,\mathrm{int}} = \mathrm{Pur}_{1,\mathrm{des}}. \tag{17}$$

For the last eluting component N, the purified amount A_N can be similarly calculated by integrating between $t_{N,\mathrm{coll}}^{\mathrm{start}}$ and $t_{N,\mathrm{coll}}^{\mathrm{end}} (= t_N^{\mathrm{end}})$ (Fig. 2). The former time is specified by adjusting that the amount of component N collected over the given time interval fulfils the purity requirement $\mathrm{Pur}_{N,\mathrm{des}}$:

$$\frac{\sum_{k=t_{N,\mathrm{coll}}^{\mathrm{start}}/\Delta t}^{t_{N,\mathrm{coll}}^{\mathrm{end}}/\Delta t} C_{N,P}^{k}}{\sum_{m=1}^{N} \sum_{k=t_{N,\mathrm{coll}}^{\mathrm{start}}/\Delta t}^{t_{N,\mathrm{coll}}^{\mathrm{end}}} C_{m,P}^{k}} = \mathrm{Pur}_{N,\mathrm{int}} = \mathrm{Pur}_{N,\mathrm{des}}. \tag{18}$$

2.3.2. Calculation of fractionation times for an intermediate component

It is more complex to specify suitable collection times for a component i eluting at an arbitrary intermediate position ($1 < i < N$). Before identifying such times it is useful to investigate possible courses of the "local" (differential) purity in the whole elution profile. In Fig. 3 is shown for a ternary mixture the course of the local purity of component 2 at the column outlet for the same conditions used to generate

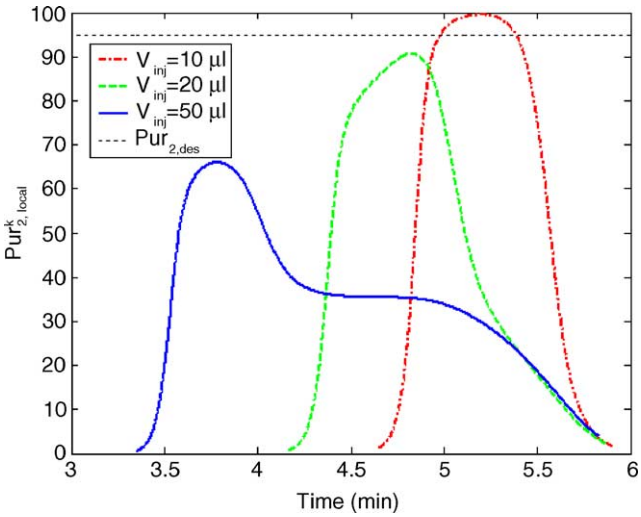

Fig. 3. Typical course of the local purity of the second (intermediate) component in an elution profile for the reference parameters and three different injection volumes. The horizontal line marks a desired purity of $\mathrm{Pur}_{2,\mathrm{des}} = 0.95$ (95%).

Fig. 1 and in addition for two different injection volumes. For the local purity holds:

$$\mathrm{Pur}^{k}_{2,\mathrm{local}} = \frac{C^{k}_{2,P}}{\sum_{m=1}^{N} C^{k}_{m,P}} \quad k = 1, K. \tag{19}$$

Obviously, a target component i can be only collected if there exists a time interval in which the local purity of this component is equal or larger than the desired integral purity ($\mathrm{Pur}^{k}_{i,\mathrm{local}} \geq \mathrm{Pur}_{i,\mathrm{des}}$). If, as assumed in this particular example (Fig. 3), the integral purity of the second component should be larger than 0.95 only with the lowest injection volume considered (10 μl) this goal can be reached in a certain time interval (between 4.93 and 5.33 min). The corresponding integral purity of this fraction would be $\mathrm{Pur}_{2,\mathrm{int}} = 0.983$. Thus, a larger fraction could be collected to achieve exactly the specified purity of 0.95.

Obviously, it is reasonable to identify at first the interval of the elution profile in which the local purity of the target component exceeds the desired purity $\mathrm{Pur}_{i,\mathrm{des}}$, i.e. $[t^{\mathrm{start}}_{i,\mathrm{pur}}, t^{\mathrm{end}}_{i,\mathrm{pur}}]$. Then, as illustrated in Fig. 4, there exist essentially three simple strategies to expand the size of this interval in order to match integral and desired purity. Two strategies consist in expanding the initial interval just in one direction, i.e. in the direction of lower or in the direction of higher retention times. The third strategy is based on expanding the interval simultaneously into both directions. The mathematical description of these three strategies is summarized below.

(1) *Expansion to higher retention times*

In this method, the concentration of component i in the fraction is determined by integrating between $t^{\mathrm{start}}_{i,\mathrm{coll}} = t^{\mathrm{start}}_{i,\mathrm{pur}}$ and a time $t^{\mathrm{end}}_{i,\mathrm{coll}}$. The latter time will be larger then $t^{\mathrm{end}}_{i,\mathrm{pur}}$. It has to be determined in a way that the integral purity of the fraction matches the specified desired value.

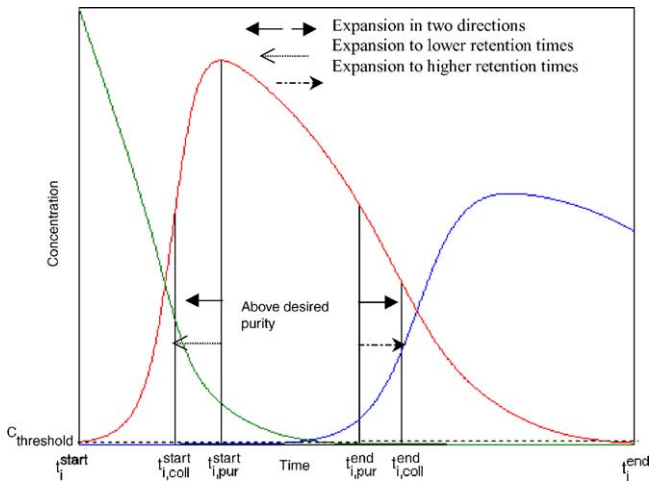

Fig. 4. Chromatogram to illustrate different possible methods to collect an intermediate component with a specified desired integral purity $\mathrm{Pur}_{i,\mathrm{des}}$.

(2) *Expansion to lower retention times*

This method is based on keeping the last time at which the local purity of the target component is larger than the desired purity, $t^{\mathrm{end}}_{i,\mathrm{pur}}$. The method consists in integrating the concentrations of the components in the direction of lower retention times until the integral purity reaches the set value. Thus, a $t^{\mathrm{start}}_{i,\mathrm{coll}}$ can be found which is smaller than $t^{\mathrm{start}}_{i,\mathrm{pur}}$.

(3) *Expansion in two directions*

This more sophisticated expansion is based on enlarging the initial time interval $[t^{\mathrm{start}}_{i,\mathrm{pur}}, t^{\mathrm{end}}_{i,\mathrm{pur}}]$ step by step in one of the two directions. Below only the typical case is considered that the concentrations at the two times $t^{\mathrm{start}}_{i,\mathrm{pur}}$ and $t^{\mathrm{end}}_{i,\mathrm{pur}}$ are above the threshold concentration. The interval is initially characterized by the following two discrete grid points:

$$k^{\mathrm{start}} = \frac{t^{\mathrm{start}}_{i,\mathrm{pur}}}{\Delta t} \quad \text{and} \quad k^{\mathrm{end}} = \frac{t^{\mathrm{end}}_{i,\mathrm{pur}}}{\Delta t}. \tag{20}$$

The specific partial peak areas corresponding to this interval can be obtained by integration as follows:

$$A_{i,\mathrm{pur}} = \sum_{k^{\mathrm{start}}}^{k^{\mathrm{end}}} C^{k}_{i,P}\,\Delta t \quad i = 1, N. \tag{21}$$

In order to decide in which direction the stepwise enlargement of the interval should be performed the following scheme can be used:

$$\begin{aligned} &\text{if } \mathrm{Pur}_i^{k^{\mathrm{start}}-1} = \mathrm{Pur}_i^{k^{\mathrm{end}}+1} \quad \text{then} \\ &\quad A_{i,\mathrm{pur}} = A_{i,\mathrm{pur}} + \max[C_{i,P}^{k_1-1}, C_{i,P}^{k_2+1}]\,\Delta t, \\ &\text{if } C_{i,P}^{k^{\mathrm{start}}-1} \geq C_{i,P}^{k^{\mathrm{end}}+1} :\ k^{\mathrm{start}} = k^{\mathrm{start}} - 1, \\ &\text{if } C_{i,P}^{k^{\mathrm{start}}-1} < C_{i,P}^{k^{\mathrm{end}}+1} :\ k^{\mathrm{end}} = k^{\mathrm{end}} + 1. \end{aligned} \tag{22}$$

$$\begin{aligned} &\text{if } \mathrm{Pur}_i^{k^{\mathrm{start}}-1} > \mathrm{Pur}_i^{k^{\mathrm{end}}+1} \quad \text{then} \\ &\quad A_{i,\mathrm{pur}} = A_{i,\mathrm{pur}} + C_{i,P}^{k^{\mathrm{start}}-1}\,\Delta t \text{ and } k^{\mathrm{start}} = k^{\mathrm{start}} - 1. \end{aligned} \tag{23}$$

$$\begin{aligned} &\text{if } \mathrm{Pur}_i^{k^{\mathrm{start}}-1} < \mathrm{Pur}_i^{k^{\mathrm{end}}+1} \quad \text{then} \\ &\quad A_{i,\mathrm{pur}} = A_{i,\mathrm{pur}} + C_{i,P}^{k^{\mathrm{end}}+1}\,\Delta t \text{ and } k^{\mathrm{end}} = k^{\mathrm{end}} + 1. \end{aligned} \tag{24}$$

This enlarging of the interval can be repeated as long as the ratio of the collected amount of component *i* over the collected amount of the total sample is equal to or larger than $\mathrm{Pur}_{i,\mathrm{des}}$. The termination of this procedure yields the required collection times:

$$t^{\mathrm{start}}_{i,\mathrm{coll}} = k^{\mathrm{start}}\,\Delta t \text{ and } t^{\mathrm{end}}_{i,\mathrm{coll}} = k^{\mathrm{end}}\,\Delta t. \tag{25}$$

It should be mentioned here, that under nonlinear conditions there might exist more than one interval in which the local purity of the target component exceeds the specified purity requirement.

3. Results of parametric calculations

There are several parameters influencing the productivity with which a certain target component can be collected. Some of them will be considered below in a parametric study based on solving Eq. (1). Obviously, the parameters of the adsorption isotherms (in particular the selectivity values $\alpha_{i,m}$, Eq. (8)) and the composition of the feed solution possess a large importance. Before discussing their influence the impact of the concentration threshold, $C_{\text{threshold}}$, the "shaving method" applied (one or two side expansions), the purity requirement specified, $\text{Pur}_{i,\text{des}}$, and the amount injected, V_{inj}, will be discussed. Since in preparative chromatography, in order to maximize the productivity, usually the flow rate is chosen as high as possible (respecting pressure drop limits [2]) the effect of V_{F} is not discussed here.

For the sake of clarity a mixture containing three components is considered in order to illustrate the influence of the parameters mentioned above on the productivity with which each of the three components can be isolated. If there would elute more components before and after the three components considered here, the same methodology can be applied to determine appropriate fractionation times and to determine performance criteria. Obviously, a larger cycle time has to be accepted leading to a reduced productivity. There will be typically also more competition effects caused by the presence of additional components. These effects might be, however, often small compared to the competition caused by the two immediate neighbors of the target component. Currently a study is performed quantifying additional competition effects by more "remote" neighbors.

Mostly the reference parameters listed in Table 1 were used. Solving Eq. (1) with these parameters leads to the reference chromatogram already shown above (Fig. 1).

3.1. Effect of the threshold concentration on the production of component

The choice of the threshold concentration, $C_{\text{threshold}}$, fixes the start and end times for fractionation and thus the cycle time (Fig. 2). In this way it effects the production rates (Eq. (12)).

There exist various possibilities to select the threshold concentration. In order to guarantee that the column is sufficiently regenerated and subsequent cycles are reproducible $C_{\text{threshold}}$ should be kept small. In order to collect concentrated fractions, $C_{\text{threshold}}$ should have a relatively large value.

Obviously, it is most simple to set $C_{\text{threshold}}$ as a fixed value. However, in practical situations, the various concentrations of the different components in the mixture cause difficulties to select a suitable value. Thus, it could be better to set $C_{\text{threshold}}$ as a relative value. One convenient choice is to chose a certain (small) fraction of the usually known concentration of each component in the injected sample. This choice can be made a priori but does not consider the concrete chromatographic conditions (which lead to different degrees of dilution). Regarding this fact an alternative choice is to set $C_{\text{threshold}}$ as a certain small fraction of the maximum concentration at the column outlet. The latter method depends on the knowledge of the corresponding elution profile and could be applied only a posteriori.

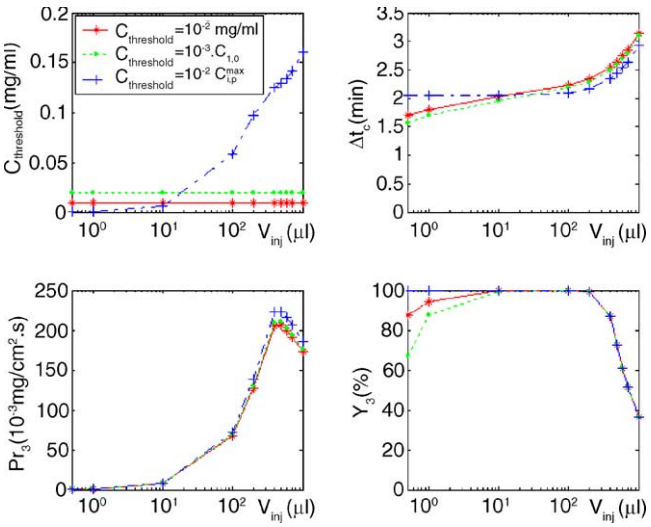

Fig. 5. Effect of three possibilities to specify $C_{\text{threshold}}$ on the production of the third component for $\text{Pur}_{2,\text{des}} = 0.99$. Reference parameters, except $P = 2000$ and $C_{1,0} = C_{2,0} = C_{3,0} = 20$ mg/ml.

To illustrate the effect of the mentioned three different methods of selecting $C_{\text{threshold}}$ in Fig. 5 are shown results of calculating the cycle times, the production rates and the recovery yields with which the last eluting (third) component can be obtained as a function of the injection volume. The threshold concentration is set to be: (a) a fixed value (0.01 mg/ml), (b) a fraction (0.001) of the injection concentration of the target component (i.e. here 0.02 mg/ml), and (c) a fraction of 0.01 of the specific maximum component concentration at the column outlet (which depends on the specific conditions).

In all calculations reported below $C_{\text{threshold}}$ was set as a fraction of the maximum outlet concentration ($C_{\text{threshold}} = 0.01 C_{i,P}^{\max}$), which provided for the particular example studied the highest production rates.

3.2. Effect of specifying fractionation times on the production of an intermediate component

In Section 2.3, three different simple methods have been discussed capable to determine the time interval in which a certain intermediate component should be collected. The effect of applying these three fractionation methods is shown in Fig. 6a where the productivity with which the second component can be obtained is depicted as a function of the injection volume. It can be seen that the productivity for expanding the initial interval $[t_{i,\text{pur}}^{\text{start}}, t_{i,\text{pur}}^{\text{end}}]$ into both directions always leads to a higher productivity compared to the results obtained with the other two expansion methods. These

results could be expected because the former method is more flexible and comprises the two latter methods as special cases. The advantage of the two side expansion method is more pronounced when the production rate is high. When the injection volumes and thus the production rates are small, for the example studied, the results are almost the same for the methods of expansion to lower retention times and expansion to higher retention times. However, when the injection volumes are higher, expansion to lower retention becomes superior. This could be understood in view of the sharpening of the adsorption fronts in case of Langmuirian systems.

In Fig. 6b is given for a certain injection volume (14 μl) a specific part of the corresponding chromatogram showing the elution profile of the second component, the tail of the first component and the front of the third component. The initially determined interval where the local purity of the second component exceeds a specified purity of $Pur_{2,des} = 0.90$ is marked [$t^{start}_{2,pur} = 4.81$ min, $t^{end}_{2,pur} = 5.21$ min]. The corresponding collection times for the three methods are: (a) 4.65 and 5.21 min for expansion to lower retention times; (b) 4.81 and 5.38 min for expansion to higher retention times; and (c) 4.68 and 5.33 min for expansion into both directions (leading to the highest productivity).

Although it is in general simple to apply the expansion into two directions method, it should be noted that the analysis can be more complex if there exists more than one maximum in the $Pur_{i,local}$ versus time curve which is possible under strongly overloaded conditions. A detailed discussion of this aspect is beyond the scope of this paper.

Since the highest productivity can be obtained using the expansion into both directions, only this methods is used below.

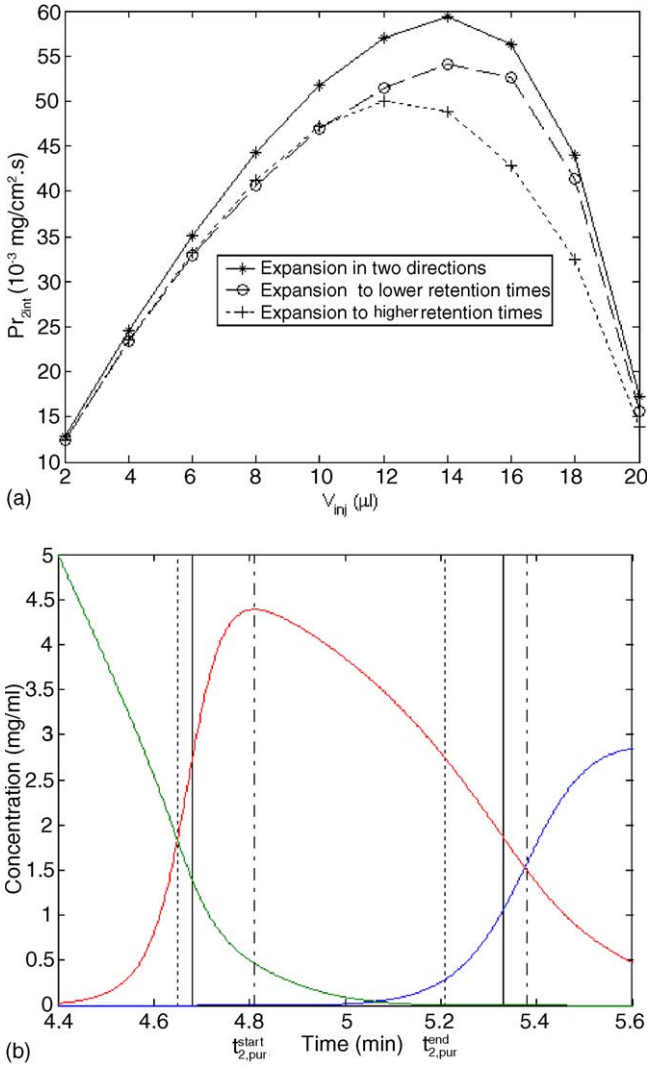

Fig. 6. Influence of method of fractionation: (a) on the production rate of intermediate component ($Pur_{2,des} = 0.90$, reference parameters); (b) on the time interval corresponding to expansion into two directions (solid lines), expansion to lower retention times (dotted line) and expansion to higher retention times (dashed line) for an injection volume of 14 μl.

3.3. Effect of desired purity and injection volume on the production rates

Fig. 7 shows for the reference parameters the dependence of the specific production rate for the three components as a function of the injection volume (or the loading factor) and of the desired product purity. Several conclusions can be drawn from these results.

(i) The production rate is the highest for the first eluting component.
(ii) The specific optimal loading factor is the highest for the first eluting component.
(iii) For each component the production rate can be increased if the desired purity ($Pur_{i,des}$) is decreased.

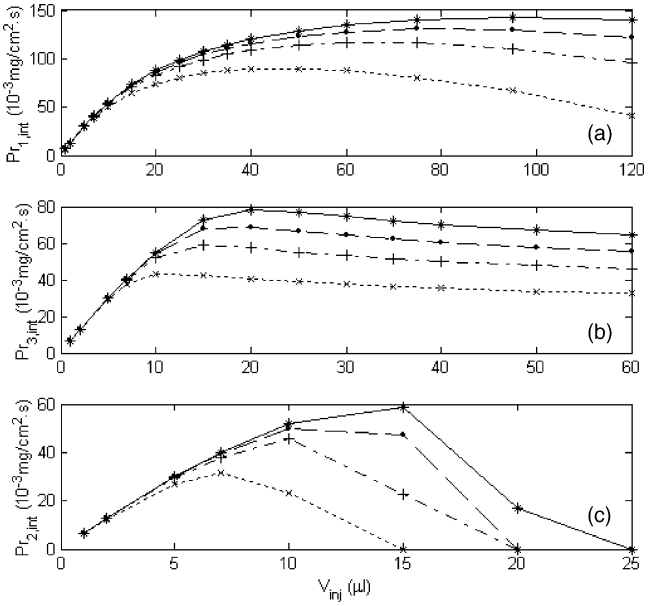

Fig. 7. Effect of desired purity on the production rate of different components. Parameters of reference case: (a) first eluting component; (b) last eluting component; (c) intermediate component. $Pur_{i,des}$: (*) 0.90; (●) 0.93; (+) 0.96; (×) 0.99.

(iv) The specific optimal loading factors are higher for lower purity requirements.
(v) Both the productivity and the optimal loading factor are the lowest for the second (intermediate) component.

It should be noted that these typical trends are valid only for situations similar to the reference case (i.e. 1:1:1 mixture, similar separation factors $a_{1,2}$ and $a_{2,3}$, Langmuirian systems). If the composition of the mixture is different, the optimum loading factor of each component will change. An extensive discussion and analytical solutions regarding the effect of the feed composition are available for binary mixtures and columns with an infinite efficiency [2].

3.4. Effect of injection volume on the recovery yields

Extending the results given in Fig. 7; in Fig. 8 are shown the component production rates and the corresponding recovery yields as a function of the injection volume (or the loading factor) for the reference parameters but a reduced specified purity $\mathrm{Pur}_{i,\mathrm{des}} = 0.85$. The production rates follow obviously the trends visible already in Fig. 7. The recovery yields of all three components decrease when the injection volume is increased (Fig. 8b). This decrease is most pronounced and happens already at lower injection volumes in case of the intermediate component.

3.5. Effect of separation factors on productivity

Obviously, the course of the competitive adsorption isotherms has an essential influence on the separation process. A comprehensive study of the impact of all individual isotherm equation parameters is difficult and outside the scope of this paper. Here, only the effect of the two separation factors $\alpha_{1,2}$ and $\alpha_{2,3}$ was analyzed (varying them between the reference value of 1.2 and 1.8) keeping the reference values for the Henry constant of the intermediate, a_2, and all $q_{\mathrm{sat},i}$ values constant.

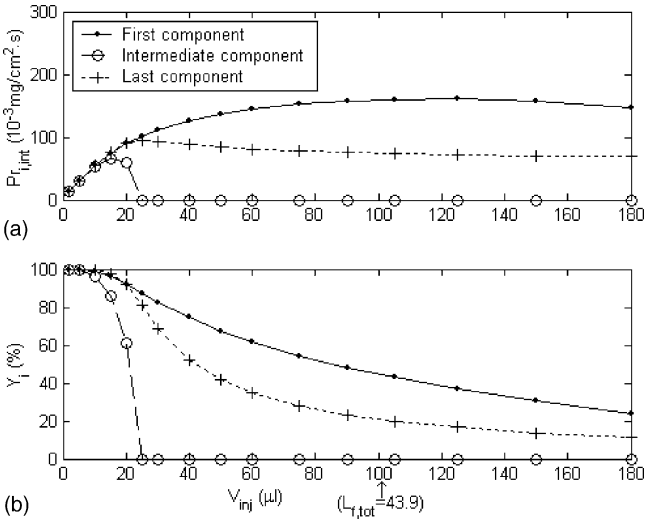

Fig. 8. Effect of injection volume (or loading factor) on: (a) production rate (a) and (b); (b) yield for all three components. Reference parameters. $\mathrm{Pur}_{i,\mathrm{des}} = 0.85$.

Table 2
Influence of the separation factors on the production of the components in a ternary mixture ($C_{1,0}$:$C_{2,0}$:$C_{3,0}$ = 1:1:1, $\mathrm{Pur}_{i,\mathrm{des}} = 0.99$)

$\alpha_{1,2}$	1.2	1.2	1.2	1.5	1.5	1.5	1.8	1.8	1.8
$\alpha_{2,3}$	1.2	1.5	1.8	1.2	1.5	1.8	1.2	1.5	1.8
First eluting component									
$L_{f,tot}$	33.1	33.1	33.1	92.2	92.2	92.2	131.6	144.6	144.6
m_1 (mg)	5.1	5.3	5.4	21.7	23.5	24.2	37.1	40.7	42.4
Y_1 (%)	33.8	35.5	36.3	51.6	56.0	57.7	61.8	61.7	64.2
Intermediate component									
$L_{f,tot}$	3.7	8.1	8.1	15.8	23.8	31.9	23.8	39.6	47.6
m_2 (mg)	1.1	1.8	1.8	3.5	8.8	9.1	4.4	13.8	16.1
Y_2 (%)	76.0	59.8	60.5	58.8	98.1	75.6	48.7	91.9	89.7
Last eluting component									
$L_{f,tot}$	7.3	21.9	32.8	7.3	21.9	32.8	7.3	21.9	32.8
m_3 (mg)	1.7	8.3	14.4	1.9	8.9	15.2	1.9	9.2	15.7
Y_3 (%)	43.5	69.1	79.8	46.7	74.2	84.5	47.8	76.8	87.3

$L_{f,tot}$: optimum total loading factor; m_i: amount of component i collected in a single cycle at optimum loading factor; Y_i: yield of component i at optimum loading factor.

For each pair $\alpha_{1,2}$ and $\alpha_{2,3}$ and each of the three components a specific optimized loading factor was determined which maximized the amount that could be collected with a desired purity of $\mathrm{Pur}_{i,\mathrm{des}} = 0.99$. The obtained optimum loading factors, the corresponding collected amounts and the yields are listed in Table 2. The results show that $\alpha_{1,2}$ strongly affects the production of the first component. The amount collected increases more than six times (from 5.1 to 37.1 mg) when $\alpha_{1,2}$ increases from 1.2 to 1.8 (for keeping $\alpha_{2,3} = 1.2$). The corresponding optimum loading factors and the recovery yields also increase, but more moderately. In contrast, the amount of the first component that could be collected is hardly influenced by $\alpha_{2,3}$ (5.4 mg instead of 5.1 mg for increasing $\alpha_{2,3}$ from 1.2 to 1.8 and keeping $\alpha_{1,2} = 1.2$). Analogously, the productivity of collecting the last component is dominated by $\alpha_{2,3}$ and less influenced by $\alpha_{1,2}$ (Table 2). Under similar conditions it was found again that the rate of producing the first eluting component is significantly higher then the rate of producing the last eluting component.

Concerning the intermediate component both $\alpha_{1,2}$ and $\alpha_{2,3}$ have a significant effect on the production rate. Taking intermediate separation factors as a reference state (e.g. $\alpha_{1,2} = \alpha_{2,3} = 1.5$ leading to $m_2 = 8.8$ mg) it can be seen in Table 2 that a specific change of $\alpha_{1,2}$ has a larger consequence than a specific change of $\alpha_{2,3}$. An increase or decrease of $\alpha_{1,2}$ to 1.8 or 1.2 led to $m_2 = 13.8$ or 1.8 mg, whereas an increase or decrease of $\alpha_{2,3}$ to 1.8 or 1.2 led to $m_2 = 9.1$ or 3.5 mg.

More results revealing the strong and complex influence of the separation factors on the amounts produced and the corresponding recovery yields of the second component are shown in Fig. 9.

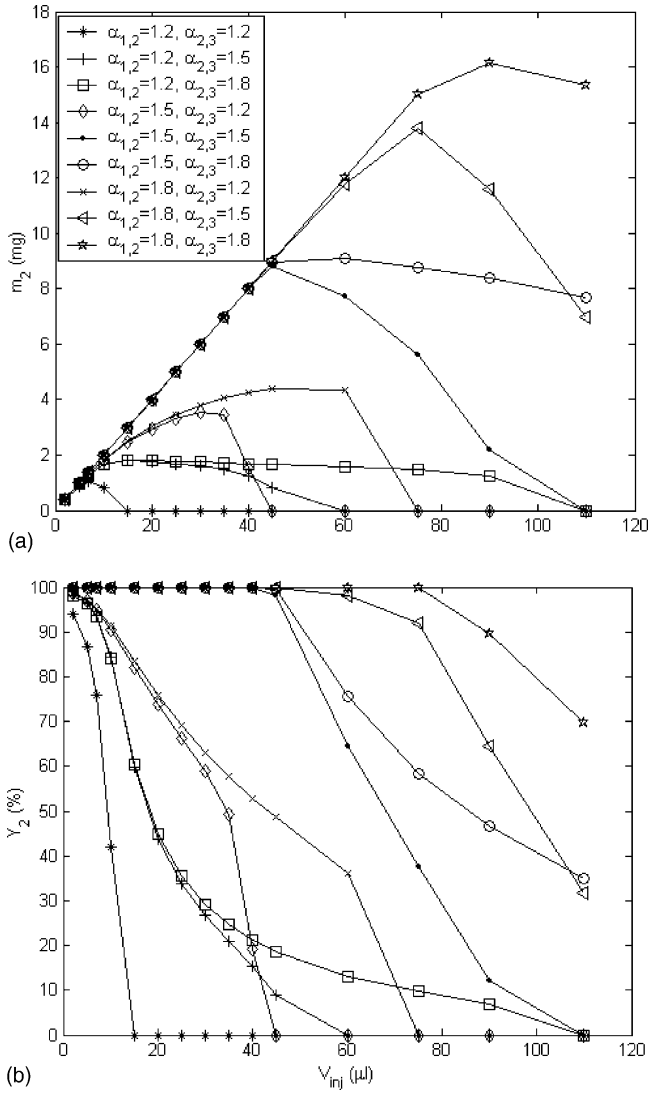

Fig. 9. The effect of separation factors on: (a) the amount that could be collected; (b) yield of the second (intermediate) component. Reference parameters, except isotherm parameters (a_2 was fixed and a_1 and a_3 were set according to the separation factors given, b_1 and b_3 were also change accordingly). $\mathrm{Pur}_{2,\mathrm{des}} = 0.99$.

From the results presented above it can be concluded that the production of one component is mainly influenced by the separation factors with its closest neighbors. The effect of more "remote" peaks is smaller and might be even negligible if the column is not extremely overloaded. This aspect is currently quantified in a separate work in more detail.

3.6. Effect of composition of feed mixture

Fixing the total concentration of all components in the mixture, the influence of the sample composition on the production rates of the intermediate component was investigated for different injection volumes. Fig. 10 shows typical

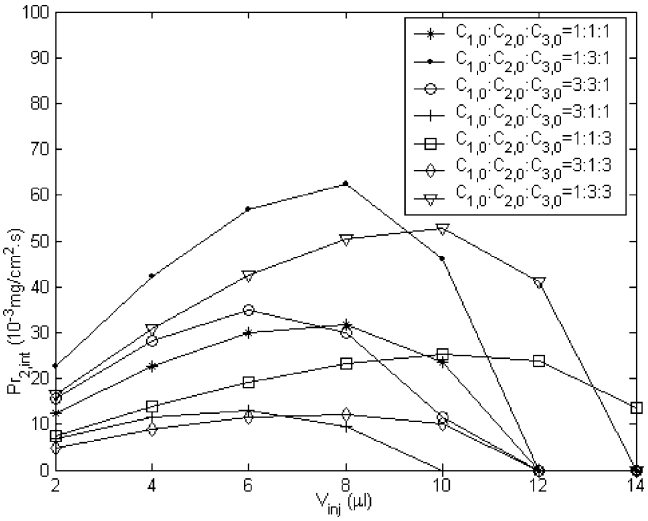

Fig. 10. Effect of relative injection concentrations on the production rate of the intermediate component. Reference parameters, except $C_{1,0} + C_{2,0} + C_{3,0} = 600\,\mathrm{mg/ml}$, $\mathrm{Pur}_{2,\mathrm{des}} = 0.99$.

results. For the case considered relatively high production rates of the intermediate component can be obtained for all of feed compositions studied when the injection volume is between 6 and 10 μl. The highest production rate can be obtained when the target component is enriched in the feed (here for the concentration ratio 1:3:1). In agreement with the above discussion concerning the separation factors $\alpha_{1,2}$ and $\alpha_{2,3}$ the situation 1:1:3 is more favorably for the production of the second component than the situation 3:1:1. The same hold for the comparison 1:3:3 versus 3:3:1. Thus, competition and losses are more increased by an increase of the fraction of the first eluting component in the feed.

4. Conclusions

The effect of several important parameters on the production of different components, especially on the production of an intermediate component, using multicomponent preparative chromatography was discussed. Using the Craig model and a set of reference parameters the separation of a sample containing three components was investigated theoretically.

Simple methods were introduced allowing to specify suitable times to collect a certain target component with a specified purity. The elucidated influences of various parameters on productivity and yields provide useful guidelines for improving the productivity of preparative multi-component chromatography.

The practical application of the methods suggested requires that in the column effluents not only overall but also specific concentrations are measured. Thus, advanced detection and/or fraction collection techniques should be applied to follow the elution profiles of the individual components.

5. Nomenclature

a_i	parameters of Langmuir isotherm for component i, Eq. (7)
A_{Col}	cross section area of the column
A_m	integrated peak area of component m in a certain time period, Eq. (16)
b_i	parameters of Langmuir isotherm for component i, Eq. (7)
$C_{i,0}$	concentration of component i in the injected mixture
$C_{i,j}^k$	concentration of component i in plate j at exchange step k in Craig model
$C_{threshold}$	threshold concentration for fractionation
L_{Col}	length of the column
$L_{f,i}$	loading factor of component i, Eq. (9)
$L_{f,tot}$	total loading factor, Eq. (10)
m_i	amount of component i recovered in the collected fraction
N	number of components in the sample
P	number of stages in the column
Pr_i	production rate of component i, Eq. (12)
$Pur_{i,des}$	desired purity of component i, Eq. (13)
$Pur_{i,int}$	integral purity of component i in a certain fraction, Eq. (15)
$Pur_{i,local}^k$	the course of local purity of component i at the column outlet for exchange step k, Eq. (19)
$q_{i,j}^k$	concentration of component i in the stationary phase in plate j and exchange step k in equilibrium with the corresponding concentrations in the mobile phase
$q_{sat,i}$	saturation capacity of component i in the stationary phase, Eq. (7)
t_0	dead time of the column, elution time of a non-retained component
t_{inj}	injection time
t_1^{start}	time at which the concentrations of the first eluting component exceeds $C_{threshold}$
t_N^{end}	time at which the concentrations of the last eluting component drops below $C_{threshold}$
$t_{i,coll}^{start}$	begin of collecting component i
$t_{i,coll}^{end}$	end of collecting component i
$t_{i,pur}^{start}$	begin of time interval in which the local purity of component i is larger than desired purity
$t_{i,pur}^{end}$	end of time interval in which the local purity of component i is larger than desired purity
V_{Col}	volume of the column
V_F	the volumetric flow rate of the mobile phase
V_{inj}	injection volume
Y_i	recovery yield of component i, Eq. (14)
$\alpha_{i,m}$	separation factor between components i and m, Eq. (8)
ε	column porosity
Δt	residence time of the mobile phase in a plate
Δt_c	duration time of a single cycle

Indices

i	component
j	plate
k	exchange step in Craig model

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