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Novel simulated moving-bed method for reduced solvent consumption☆

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Abstract

Simulated moving-bed (SMB) chromatography is attractive for reducing sorbent and solvent consumption relative to fixed-bed systems. In this contribution, we describe a novel and versatile method for further reducing solvent consumption in the case of reversed-phase chromatography. The method is based on the variation of the distribution coefficients of solutes to be separated upon varying the composition of a multi-component mobile phase. If the solvent strength of the desorbent is set higher than the solvent strength of the feed, the components will have smaller distribution coefficients in the extraction section of the SMB and hence will be more easily eluted. This will result in a lower desorbent flow and possibly also in a shorter desorbent zone, and, ultimately, in more concentrated products. The so-called ''Triangle-method'' by Storti et al. [AIChE J., 39 (1993) 471] to obtain the region of complete separation, is extended for this novel SMB method. Theoretical evaluation of the proposed methodology supports the anticipated solvent reduction relative to fixed-bed RP-HPLC for the cases of the purification of the polyketide antibiotic nystatin and the separation of bovine insulin from porcine insulin.

Keywords: Simulated moving-bed chromatography; Gradient elution; Insulin; Nystatin

1. Introduction

Gradient elution in chromatography was introduced over 40 years ago to permit proper separation of mixtures that are difficult to separate under isocratic conditions. The main difference compared to isocratic elution is that the solvent strength of the mobile phase is increased during the separation, either gradually or step-wise. The main result of using gradient elution is increased resolution of the early eluted components in a mixture and decreased retention time of the latest eluted components, which will result in decreased solvent consumption and less diluted product [1].

Simulated moving-bed (SMB) chromatography, a counter-current continuous process scale separation technology, is gaining more and more popularity in fine chemical and pharmaceutical industries. This is mainly due to its high separating power and low solvent consumption [2]. Even though step-wise and gradient elution have been common practice in fixed-bed chromatography for decades, only one real effort has been made towards applying gradient elution to

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SMB technology, namely in supercritical fluid SMB. Here, step-wise elution is established by applying different pressures in the different sections of the SMB [3].

In this work, we present a novel method for gradient elution in reversed-phase (RP)-SMB chromatography. Its potential is demonstrated using Equilibrium theory and it is elaborated for the simple case of linear isotherms with a common found dependence on solvent consumption. Firstly, we give a detailed description on how to estimate the region for complete separation in the operating parameters space, similar to the methods outlined for isocratic elution in SMB chromatography [4]. Then, we compare several different chromatographic modes, isocratic fixed-bed chromatography, as well as isocratic and gradient SMB chromatography. We will also demonstrate the potential of the method by the theoretical evaluation for two processes: the purification of nystatin from a major contaminant, and the separation of bovine insulin from porcine insulin. The former process, which is not an obvious choice for SMB separation, was chosen because of the low solubility of the product and the associated great need for solvent reduction. The latter process was chosen because its large scale of production makes reduction of solvent consumption urgent. Equilibrium data at different solvent composition were measured for nystatin and available in literature for insulin.

2. Gradient elution in chromatography

Some components in a complex mixture may be unretained or too weakly retained by the stationary phase when the mobile phase has a high elution strength. And conversely, some components may not be eluted at all when the strength of the mobile phase is too low. The solution to this problem is gradient elution, which can be established by increasing the concentration of a strong solvent gradually during the separation. This allows separation of all components in a reasonable process time [1].

Continuous gradient elution is used in analytical high-performance liquid chromatography (HPLC) to improve the resolution of the components in the mixture and to reduce the process time. In preparative chromatography, however, a step-wise gradient is more common, because of the costs associated with the recovery and recycling of the mobile phase components, and because the extraction, enrichment, or purification of a single component rather than of several is the usual goal in preparative chromatography. The combination of high resolution as well as the possibility for considerable solvent reduction are the real driving forces for using gradient elution in production scale chromatography [1].

Separation of proteins and peptides in reversed-phase chromatography is an example where gradient elution can be used. The large size of the protein molecules make their surface area of interaction with the stationary phase large, and their retention factor varies considerably with increasing strength of the mobile phase [1]. Small changes in the solvent concentration of a few tenths of a percent can decrease the retention factor from a very large value to zero. To separate closely related proteins in a reproducible fashion, linear gradient elution is often more reliable than preparation of mobile phase solutions with accurate composition within better than 0.1% [1].

Even though several different gradient profiles can be used, step gradients as well as linear composition gradients are the simplest and most popular, especially in reversed-phase chromatography. In a standard four-section SMB, which is shown schematically in Fig. 1, however, the only option is a step-wise gradient.

Since the SMB is operated in steady-state, a gradient must be used that changes in place instead of time. The variation in solvent composition is obtained by introducing a feed stream with lower solvent strength than compared to the strength of the desorbent. When the function of the different sections is considered in more detail, the reason for applying a gradient becomes clear. In section 1, the most retained component is completely desorbed from the column, which means that we need a strong desorbent. In section 2, the least retained component is desorbed while keeping the most retained component adsorbed to the column material. Ideally the solvent strength should be lowered in section 2 compared to section 1, but since there is no solvent inlet between sections 1 and 2, only the extract outlet, the strength of the solvent is left unchanged.

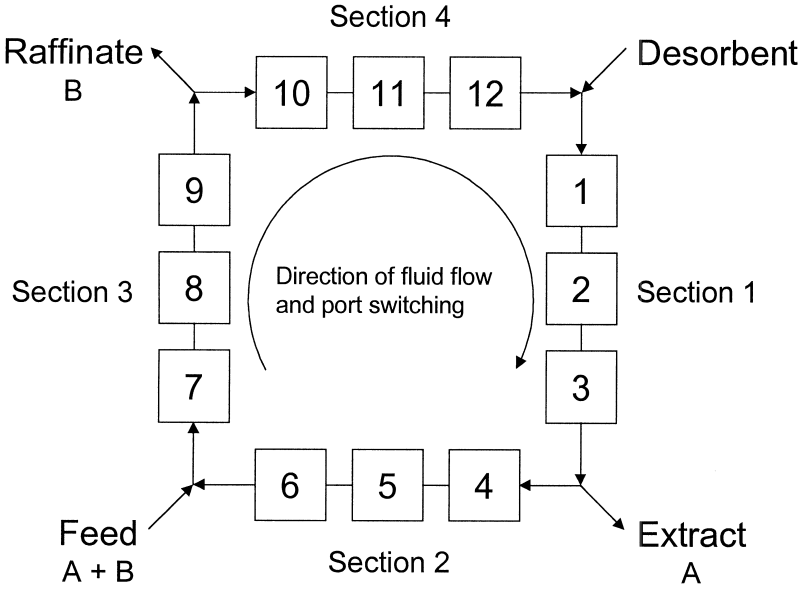

Fig. 1. Schematic drawing of a standard four-section SMB.

In section 3, the most retained component is adsorbed while the least retained component is kept in the liquid phase. This induces the need of an even lower solvent strength and fortunately this can be achieved by lowering the solvent strength of the feed stream, which is introduced between sections 2 and 3. At last, the least retained component is adsorbed in section 4, which ideally demands a solvent with lower strength compared to section 3, but again, there is no inlet between sections 3 and 4, thus making it impossible to alter the strength of the solvent. The conclusion is that a step-wise gradient can be applied to the SMB by introducing a solvent mixture with a lower strength at the feed inlet port compared to the solvent mixture introduced at the desorbent inlet port.

3. Procedure for determining the operating conditions leading to complete separation

3.1. Isocratic mode

Storti et al. [4] have described a method for determining the operation conditions for complete separation based on the Equilibrium theory in SMB technology. This diagram, sometimes called the Morbidelli triangle is used to analyse the optimal conditions, the robustness and the efficiency of the novel SMB process. The methodology is briefly repeated below for the sake of comparison to our new method.

A schematic drawing of an SMB unit is shown in Fig. 1. The unit consists of four sections, 1 to 4, and each section consists usually of two to four columns. Furthermore, five inlet and outlet ports are present for the desorbent (D), extract (E), feed (F), raffinate (R), and recycle stream (the outlet of column 12 in Fig. 1). Five multi-position valves, which are not shown in the drawing, are also present for the simulation of the sorbent flow by switching the in and outlet ports in one direction.

To achieve separation of the feed components, the net flow-rates of the most retained component (A) must be set such that it will be transported to the extract port, while the least retained component (B) most be transported to the raffinate port. If we focus on sections 2 and 3, where the actual separation of the components takes place, a set of constrains is set up on the basis of the separation factors of the components in each of the two sections, where the separation factor is defined as follows [5]:

$$S_{ij} = \frac{F_S}{F_j} \cdot K_i \quad (1)$$

where F_S is the sorbent (solid) flow-rate, F_j is the liquid flow in section j and K_i is the distribution coefficient of component i. Since the solid flow-rate is equal in all sections, it is more practical to use the flow-rate ratio:

$$m_j = \frac{F_j}{F_S} \quad (2)$$

By inserting Eq. (2) in Eq. (1) and using the fact that a separation factor larger than unity means that the component is moving with the sorbent. A separation factor smaller than one means that the component is moving with the solvent, the following set of constraints must be met to achieve complete separation of the two feed components:

$$K_B < m_2 < K_A \text{ and } K_B < m_3 < K_A \quad (3)$$

where K_A is the equilibrium constant for component A, which in this work represents the component with the highest affinity for the sorbent and K_B is the equilibrium constant for the component with lowest affinity for the sorbent. Please note that the subscripts representing the section of the SMB for the equilibrium constants are removed because they are equal in all sections of an isocratic SMB. In order to have a positive feed flow m_3 must be larger than m_2:

$$m_2 < m_3 \quad (4)$$

We can now construct the region of complete separation by projecting the inequalities in the m_2–m_3 plane. This region is shown in Fig. 2 [4].

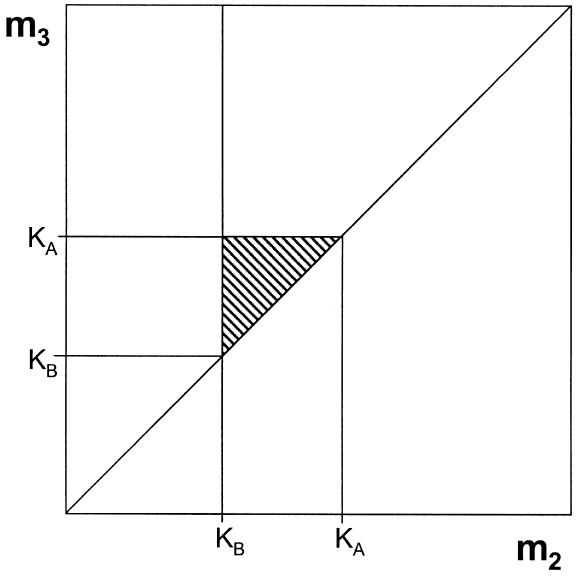

Fig. 2. Schematic diagram (Morbidelli triangle) for an SMB in isocratic mode.

3.2. Gradient mode

A method for determining the region of complete separation can now be extended for operating the SMB in gradient mode. Again, we restrict the analysis to the case of linear isotherms and a binary solvent system. The main difference of this method compared to isocratic operation is that we establish a step-wise gradient between the lower (pre-feed) and the upper section (post-feed) of an SMB unit. This will cause differences in the partition coefficients of each component in lower and upper sections. The most retained component is eluted more easily off the column, which in turn will result in the desired reduced consumption of the solvent and possible enrichment of the product.

The first step is to determine the distribution coefficients for the raffinate and extract products as a function of the solvent composition by performing pulse response experiments. This is a standard technique, described among others by Ruthven [6]. These coefficients are fitted to the following (or a similarly suitable) relation for a binary solvent mixture [7]:

$$\log K_i = \varphi_1 \log K_i^1 + \varphi_2 \log K_i^2 \quad (5)$$

where K_i^k is the distribution coefficient of component i in single pure solvent k and φ_k is the volume fraction of the kth solvent in the solute-free solvent mixture. A step-wise gradient is established by introducing desorbent and feed streams with different organic volume fraction, φ_D and φ_F, which must be set initially. Note that the solvent mixture is not restricted to organic solvent and water and is here only used as an example. A suited value for φ_F should give maximum solubility of the components

in the mixture while keeping the equilibrium constants sufficiently large leading to the required degree of resolution within reasonable column length. The relation between solubility and equilibrium constants is discussed more thoroughly in Ref. [8]. φ_D should be optimised by a mathematical model in a later stage. In this example, the range should be: $\varphi_F < \varphi_D \leq 1$. Then, an expression is found for the organic solvent fraction in section 3 (post-feed, φ_3) by setting up a mass balance over the inlet feed point:

$$\varphi_3 = \frac{m_2\varphi_D + (m_3 - m_2)\varphi_F}{m_3} \quad (6)$$

The distribution coefficients for all species in section 3 (post feed) at this solvent composition restrict the region of complete separation. m_2 is restricted by the K values of the raffinate and extract products for the selected solvent composition of the desorbent stream:

$$K_{B2}(\varphi_D) \leq m_2 \leq K_{A2}(\varphi_D) \quad (7)$$

The maximum and minimum limits of m_3 is restricted by the composition in section 3, φ_3:

$$K_{B3}[\varphi_3(m_2,m_3)] \leq m_3 \leq K_{A3}[\varphi_3(m_2,m_3)] \quad (8)$$

These minimum and maximum values of m_3 can now be calculated implicitly by solving separately the following equations for the unknown m_3:

$$m_3 - K_{A3}[\varphi_3(m_2,m_3)] = 0 \text{ (upper boundary)} \quad (9)$$

$$m_3 - K_{B3}[\varphi_3(m_2,m_3)] = 0 \text{ (lower boundary)} \quad (10)$$

which are single, non-linear functions of m_3, whereas m_2 is set independently. In Eqs. (9) and (10), the K values are given by Eq. (5), φ_3 is given by Eq. (6), and by plotting their solution together with Eq. (4) and Eq. (7) in the m_2–m_3 plane, the region of complete separation is found. This is shown in Fig. 3.

Complete separation of the extract and raffinate product is possible when the values of m_2 and m_3 lie within the hatched areas in Fig. 3. However, to obtain the desired solvent reduction and enrichment of the extract product, m_2 and m_3 must lie within the region above the horizontal line.

Notice that the solution of Eq. (10) is not shown in neither Fig. 5 nor 10 and this is due to the fact that

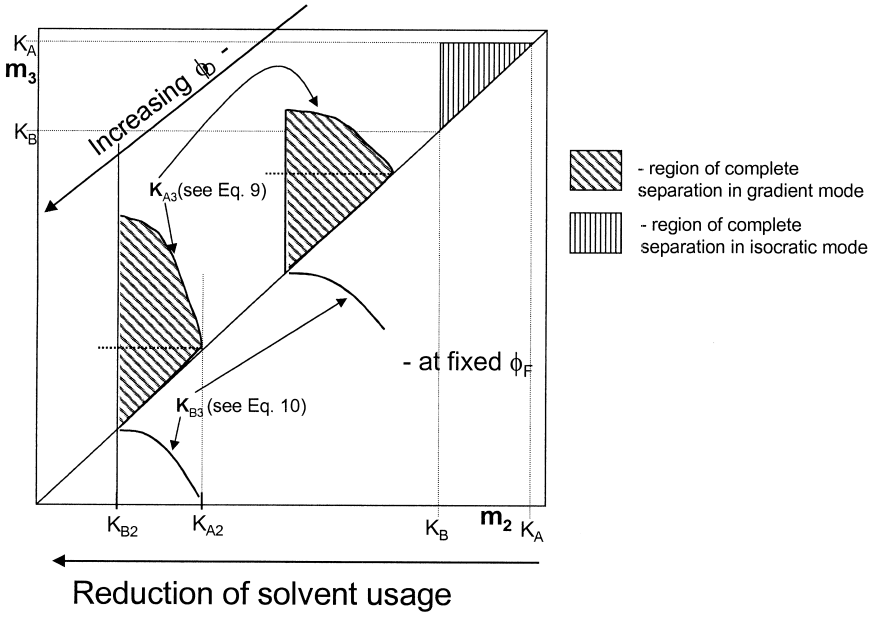

Fig. 3. Schematic diagram (Morbidelli ''triangle'') for an SMB in isocratic and gradient mode. To show how the region of complete separation develops by increasing φ_D, three different regions are shown.

the solution is not influencing the simulations carried out in this work.

For most cases, the solution of Eq. (10) is not of interest because it will lie below the diagonal defined by Eq. (4). However, for a relatively large step in the gradient it will play a role by defining a parabolic region where separation in section 3 will not take place. This region will lie quite close to the intersection between the diagonal and vertical defined by $m_2 = K_{B2}$. A discussion of this issue will be addressed in a later paper.

The methodology can possibly be extended to multi-component solvent mixtures as well as non-linear isotherms, but this has not been carried out, yet.

4. Process performance parameters

To compare the different modes of SMB, certain process parameters must be obtained from each simulation or literature source. In this work we have used purity, recovery, enrichment and desorbent requirement and they are shortly introduced below [9].

The purity of the extract and raffinate product is usually a key parameter, because this is one of the main criteria for the process. Normally, purities higher than 99% or better is often demanded in the fine chemical and pharmaceutical industry. When the most retained product in the system is called component A (the extract product) and the least retained product component B (the raffinate product), the purity is defined as follows:

$$P_E = \frac{100 c_A^E}{c_A^E + c_B^E} \text{ (extract product)} \quad (11)$$

$$P_R = \frac{100 c_B^R}{c_B^R + c_A^R} \text{ (raffinate product)} \quad (12)$$

Complete separation corresponds to the operating regime when both purities are at 100%.

The recovery of the different feed components is defined as follows:

$$R_E = \frac{100 c_A^E F_E}{c_A^F F_F} \text{ (extract product)} \quad (13)$$

$$R_R = \frac{100 c_B^R F_R}{c_B^F F_F} \text{ (raffinate product)} \quad (14)$$

Enrichment tells quite simply if the components to be separated are diluted or concentrated relative to the feed flow during the separation. In our case, large enrichment may be possible and therefore will this variable be important for comparison with isocratic SMB. Enrichment, E, is defined as follows:

$$E_A = \frac{c_A^E}{c_A^F} \quad (15)$$

$$E_B = \frac{c_B^R}{c_B^F} \quad (16)$$

Thus, an enrichment larger than unity means that the product is concentrated relative to the feed and it is diluted if it is less than unity.

Most important for this work is the desorbent requirement, which is a direct indication of solvent consumption. Even though, the remaining solvents are recycled, there is a net consumption of the desorbent during the separation and this must be replaced. This can be done through the make-up stream for compensating the desorbent loss through the product streams. The desorbent requirement, DR, is defined as the mass of desorbent used to recover a unit mass of pure product:

$$DR = \frac{(F_D + F_F)\rho_D}{F_F c_T^F} \quad (17)$$

where ρ_D is the density of the desorbent and c_T^F is the total feed concentration. It has been assumed that the feed mixture is rather diluted so that the feed density is close to the desorbent density.

5. Demonstration of the gradient SMB concept

To compare gradient SMB with isocratic SMB, two relevant cases are discussed below. For the first example, purification of the antibiotic Nystatin, pulse response experiments were carried out in the laboratory, while for the second one, separation of bovine insulin from porcine insulin, the experimental data were found in the literature [10]. They are both simulated with a short-cut model [5], assuming plug

flow for the solid stream and taking axial dispersion and mass transfer limitations for the components into account. Emphasis is laid on the differences in enrichment of the extract product and in consumption of solvent between the isocratic and the gradient operating modes. In addition, all SMB results were compared to fixed-bed isocratic chromatography for the same criteria as mentioned above.

5.1. Case 1: Purification of nystatin from its main contaminant

Nystatin is a prominent member of a relatively large and varied group of structurally related, highly unsaturated antifungal antibiotics produced by various strains of streptomycete species of microorganisms [11]. It was isolated by Hazen and Brown [12] in 1950 in New York State, hence giving the components its name (N.Y.-state-in). During fermentation, several structurally closely related components are also produced, which means that a RP chromatography purification step is necessary to polish the product to meet the demands from the regulatory authorities.

5.1.1. Experimental

There was no literature data available for the nystatin case to fit Eq. (5), so pulse response experiments at different solvent compositions were carried out in the laboratory.

YMC Europe (Schermbeck/Weselerwald, Germany) supplied the column and stationary phase. YMC-pack ODS-AM with a particle size of 30–50 μm with an average pore size of 120 Å was used as the reversed-phase adsorbent in all experiments. The column was pre-packed by the manufacturer and had the following dimensions: 150 mm×10.0 mm I.D.

The mobile phase consisted of methanol of HPLC quality, purchased from Acros (Geel, Belgium) and water from a Millipore water generator, which was filtered (0.22 μm) before use.

Nystatin was a gift from Alpharma (Oslo, Norway) and was dissolved in a 70% aqueous methanol solution to 0.1 g/l.

A Waters Alliance HPLC (Milford, MA, USA), consisting of a 2690 separation module and a 996 photo diode array detector, was used for carrying out the pulse experiments. The column was thermostatted at 25°C throughout the experiments in the attached column oven. The equipment was fully controlled and the data was stored and processed with Waters Millennium[32] software interfaced with an IBM-compatible personal computer.

The pulse experiments were carried out with 60, 70, 80 and 90% aqueous methanol solutions as mobile phase, the pump flow-rate was set to 5 ml/min and 150 μl of the sample was injected for each sample. Chromatograms were recorded at 306 nm and are shown for 60 and 70% aqueous methanol in Fig. 4. The hold-up time (t_0) was determined by taking the retention time of the first visible peak in the chromatogram and was found to be 1.6 min. The nature of this peak is not known, but the retention time for this peak was constant for all different methanol solutions indicating that this component is in fact unretained.

Since many impurities are present in a mixture and SMB is essentially a binary separation step, we choose in this case to separate nystatin from its closest and largest contaminant, which can be seen in the chromatogram in Fig. 4 as the small, but clearly visible peak in front of the nystatin peak, at retention times of 27 and 6 min, respectively.

Two sets of K values were obtained as a function of the methanol volume fraction for the two components in the pulse response experiments. These values were fitted to Eq. (5), the methanol fraction in the desorbent and the feed, φ_D and φ_F, were set at 0.8 and 0.7, respectively, and by solving Eqs. (7) and (8), the region of complete separation was found. In isocratic mode, the solvent strength of the feed, φ_F, was set to 0.8, and the region of complete separation was found. The areas of complete separation are shown in Fig. 5 for both isocratic and gradient mode, where the isocratic region is the triangle enclosed by the two solid (vertical and diagonal) lines and the hatched horizontal line, and the gradient region is enclosed by the two same solid lines and the solid curved line.

We have chosen four different combinations of m_2m_3 to best illustrate the impact of the gradient SMB, one simulation close to the optimal point in gradient mode, one simulation that is more robust, which means that it lays closer to the middle of the region of complete separation in gradient mode, one simulation that lays just outside the region of com-

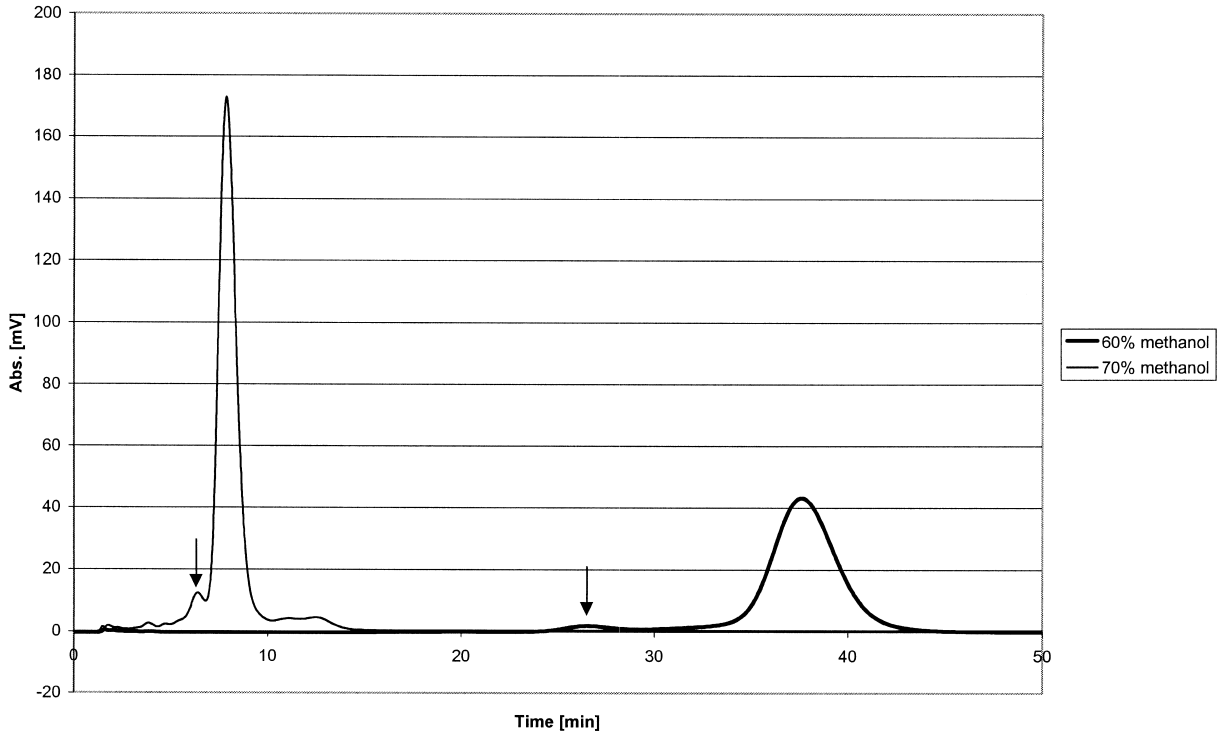

Fig. 4. The chromatogram (at 306 nm) from the pulse experiment for obtaining K values in the nystatin case at 60% (thick line) and 70% aqueous methanol. The large peak at 8 and 38 min is the pure nystatin A, the smaller one in front at 6 and 26 min is the main contaminant, indicated with an arrow.

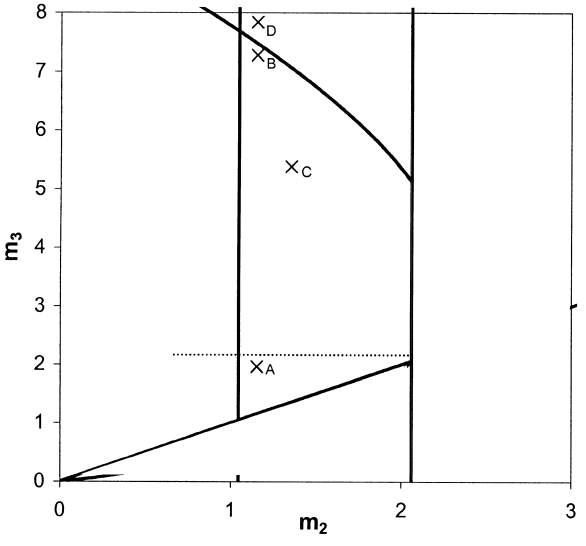

Fig. 5. Operational diagram for the nystatin case, both for isocratic ($\varphi_D = \varphi_F = 0.8$), and for gradient ($\varphi_D = 0.8$, $\varphi_F = 0.7$) mode. The different simulations are labelled and marked with x's.

plete separation in gradient mode and one simulation close to the optimal point in the isocratic region. The different combinations of m_2 and m_3 used in the simulation for purification of nystatin, both for isocratic and gradient mode are shown in Fig. 5, as x's labelled with A, B, C and D. The process parameters used in the simulation are given in Tables 1 and 2 while the results are shown in Table 3. The concentration profiles for each simulation are shown in Figs. 6–9. Please note in Table 2 that the column diameter varies to maintain a constant feed flow for the different runs.

The results indicate clearly that a step-wise gradient will reduce the solvent consumption in RP-SMB chromatography considerably, that is by at least 50%. Furthermore, it is possible to achieve a two-fold enrichment of the extract product and of the raffinate product, compare run A with run B. If a more robust operation point is chosen, such as run C, this robustness must be at the expense of increased

Table 1
Model parameters for the nystatin case

SMB operating conditions		Model parameters	
Feed concentration (g/l)	Nys = 0.9, imp = 0.1	Particle size (μm)	25
Eq. data, isocratic	$K_{nys} = 2.06$, $K_{imp} = 1.05$	Bed porosity (–)	0.65
Eq. data, gradient	$K_{nys}(\varphi) = 10^{(-6.62\varphi)} \cdot 10^{(5.61)}$	Free diffusivity ($m^2\ s^{-1}$)	10^{-9}
	$K_{imp}(\varphi) = 10^{(-7.55\varphi)} \cdot 10^{(6.06)}$	Pore tortuosity (–)	4
Feed (kg/year)	30 000	Particle porosity (–)	0.3
Column length (m)	0.10	φ_D	0.8
Number of columns	12	φ_F (isocratic)	0.8
		φ_F (gradient)	0.7

Table 2
Run specific parameters for the nystatin case

Run	m_1	m_2	m_3	m_4	Column diameter[a] (m)	Switch time (s)
A	2.27	1.15	1.96	0.94	0.55	68.0
B	2.89	1.15	7.28	2.72	0.23	86.6
C	2.89	1.35	5.38	2.31	0.28	86.6
D	2.89	1.15	7.84	2.77	0.22	86.6

[a] This value is variable to be able to keep a constant feed flow for the different runs.

solvent consumption and lower enrichment of the products. For the last run, D, an operation point was chosen just above the optimal point to demonstrate the influence on the declining purity of the products. The purity of the extract product was not influenced, which was expected since the operation point lies well inside the region of complete separation for this product. However, the purity of the raffinate product was decreased indicating that the regions of the operational diagram are quite accurate.

Sauer and Matusch [13] have presented a preparative fixed-bed method for the purification of nystatin. Their results have been compared with the results for both SMB simulations and shown in the last row in Table 3. Even though the 100% purity can be achieved, the results indicate some of the problems related to fixed-bed chromatography, namely large solvent consumption, in this case twice as high compared to isocratic SMB, and strong dilution of the products.

In most SMB separations high purity of both streams occur, normally above 98% for both outlet streams are common. But in this case, raffinate purity around 90% is being reported, see Table 3. The reason is the large difference in the feed concentration of the product and the contaminant, 90% of nystatin compared to 10% of the impurity. Very low amounts of nystatin in the raffinate outlet will have a large impact on the purity, but which in this case is not really a problem since the raffinate product is an

Table 3
Purification of nystatin with isocratic and step-wise elution SMB

Run	P_{nys} (%)	P_{imp} (%)	R_{nys} (%)	R_{imp} (%)	E_{nys}	E_{imp}	DR (kg solvent/kg product)
A (isocratic)	99.9	91.9[a]	99.0	99.8	0.72	0.80	2232
B (gradient)	99.8	87.0	98.3	100	3.47	1.34	870
C (gradient)	100	100	100	100	2.63	1.31	996
D (gradient)	100	56.9	91.6	100	2.80	1.10	1061
FB (isocratic)[b]	100	–	95	–	0.07	–	5700

[a] See remarks in text (Section 5.1) at page 15.
[b] Data are taken from Sauer and Matusch, column size: 250×25 mm [13].

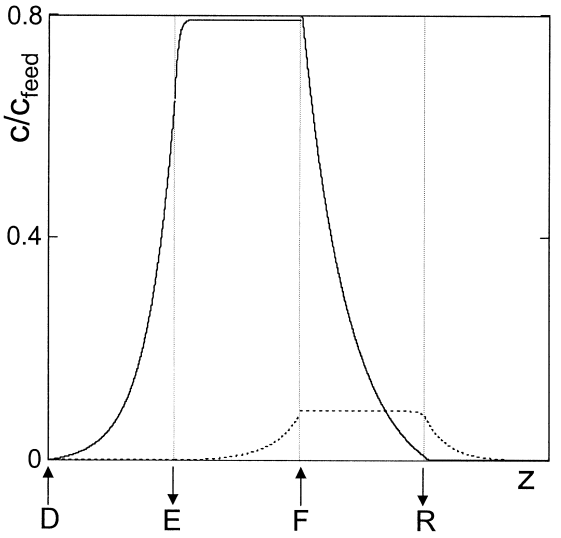

Fig. 6. Concentration profile for the isocratic separation of nystatin (solid line) and its main contaminant (dotted line), run A.

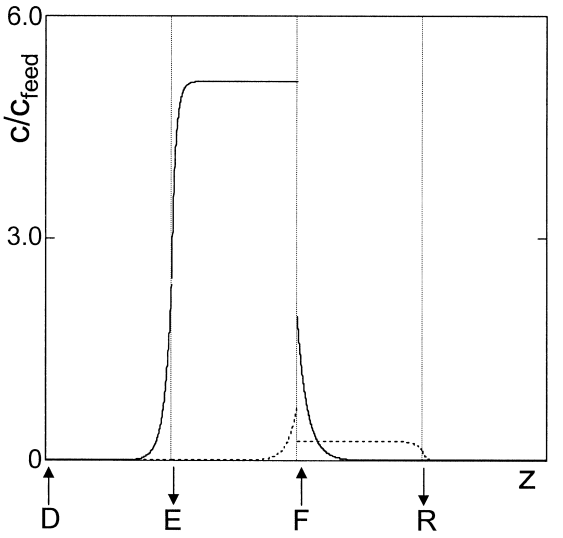

Fig. 8. Concentration profile for the gradient separation of nystatin (solid line) and its main contaminant (dotted line), run C.

impurity and will not be collected as a valuable product.

5.2. Case 2: Separation of bovine insulin and porcine insulin

RP-HPLC has become popular for purification for some proteins for use in humans. One large-scale example is insulin, where RP-HPLC is used in several separation steps for removing contaminating proteins that have very similar structures as the desired product. In Sabharwal and Chase [10], equilibrium data for the separation of bovine insulin from porcine insulin have been given for different

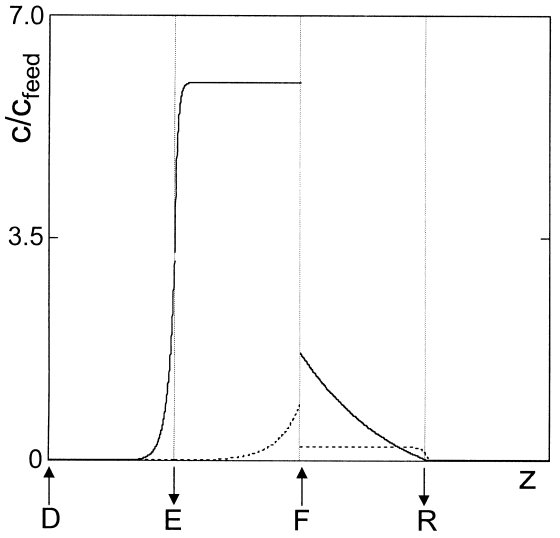

Fig. 7. Concentration profile for the gradient separation of nystatin (solid line) and its main contaminant (dotted line), run B.

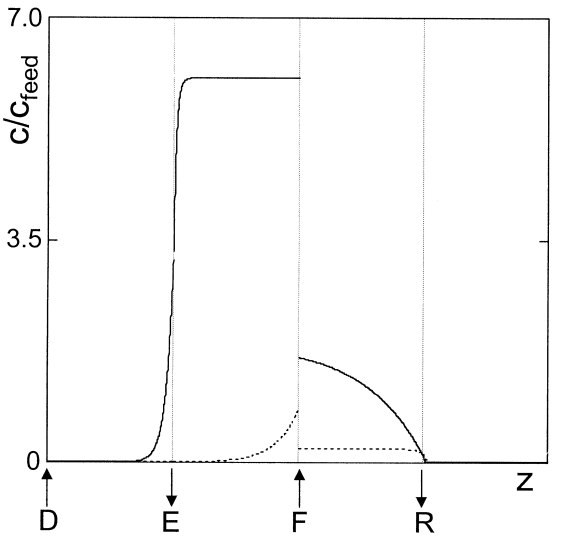

Fig. 9. Concentration profile for the gradient separation of nystatin (solid line) and its main contaminant (dotted line), run D.

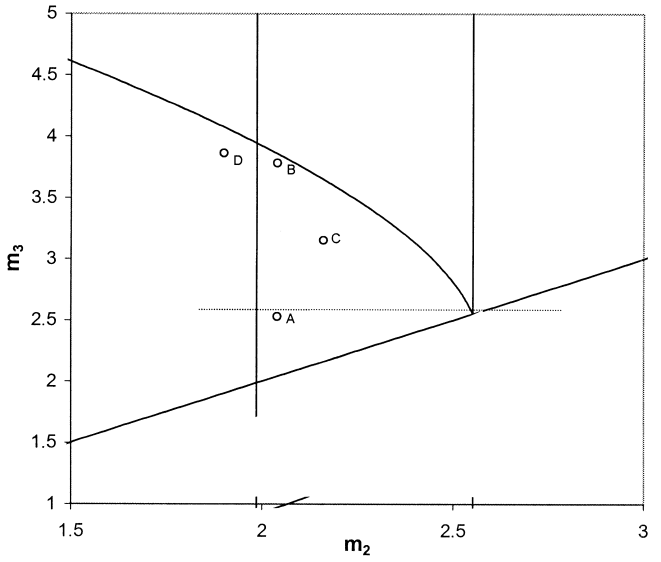

Fig. 10. Operational diagram for the insulin case, isocratic mode ($\varphi_D = \varphi_F = 0.32$) and gradient mode ($\varphi_D = 0.32$, $\varphi_F = 0.30$). The simulations are labelled A, B, C and D and are marked with o's.

concentrations of acetonitrile (ACN) in water, in the range from 30 to 34% ACN. As mentioned above, protein retention is very sensitive to changes in the mobile phase, so the data in this range are comprehensive enough to describe the K values as a function of the organic solvent fraction. Thus, the necessary data are given for estimating the region of complete separation for both isocratic and gradient mode.

The region of complete separation in isocratic mode was estimated with a solvent fraction of 0.30, whereas in gradient mode the solvent fraction of the feed was decreased to 0.28 and the solvent fraction of the desorbent was increased to 0.32.

The same recipe for estimating the region of complete separation was followed here as for the other case. This is shown in Fig. 10 for isocratic and gradient mode, and the different simulations are labelled A, B, C and D.

The process parameters used in the simulation are given in Tables 4 and 5, while the simulation results are presented in Table 6 and concentration profiles are shown in Figs. 11–14. Please note in Table 5 that the column diameter is variable to keep a constant feed flow for the different runs.

Investigation of the purity and recovery of the bovine insulin shows that there is a slight decrease in the purity when applying a gradient. By operating in the robust area, run C, the recovery can be increased while decreasing the solvent consumption with over

Table 4
Model parameters for the insulin case

SMB operating conditions		Model parameters	
Feed concentration (g/l)	Bovine = 1.0, porcine = 0.5	Particle size (μm)	25
Eq. data, isocratic	$K_{bov} = 1.98$, $K_{por} = 2.55$	Bed porosity (–)	0.65
Eq. data, gradient	$K_{bov}(\varphi) = 10^{(-18.57\varphi)} \cdot 10^{(6.24)}$	Free diffusivity ($m^2\ s^{-1}$)	10^{-9}
	$K_{por}(\varphi) = 10^{(-19.04\varphi)} \cdot 10^{(6.50)}$	Pore tortuosity (–)	4
Feed (kg/year)	1000	Particle porosity (–)	0.3
Column length (m)	0.2	φ_D	0.32
Number of columns	12	φ_F (isocratic)	0.32
		φ_F (gradient)	0.30

Table 5
Run specific parameters for the insulin case

Run	m_1	m_2	m_3	m_4	Column diameter[a] (m)	Switch time (s)
A	2.81	2.04	2.53	1.79	0.15	168
B	2.81	2.04	3.78	2.65	0.08	168
C	2.81	2.16	3.15	2.34	0.10	168
D	2.81	1.90	3.86	2.76	0.07	168

[a] This value is variable to be able to keep a constant feed flow for the different runs.

Table 6
Separation of bovine insulin from porcine insulin with isocratic and step-wise elution SMB

Run	P_{bov} (%)	P_{por} (%)	R_{bov} (%)	R_{por} (%)	E_{bov}	E_{por}	DR (kg solvent/kg product)
A (isocratic)	99.4	99.1	98.1	99.7	0.62	0.65	2908
B (gradient)	99.4	99.0	98.0	99.6	2.23	1.53	1020
C (gradient)	100	100	100	100	1.53	1.23	1371
D (gradient)	77.4	99.9	99.8	85.7	2.16	1.53	958

50% and just slightly dilute the product. Note that the result for the solvent consumption for each run is quite high, but this is due to the low feed concentration used, 1 g/l for bovine insulin and 0.5 g/l for the porcine insulin.

Again, the results show that a step-wise gradient will have a large impact on the process performance, namely almost a three-fold reduction of the solvent consumption, two-fold enrichment of the extract product and the raffinate product. Furthermore, a higher solvent consumption and lower enrichment of the products is again the price to pay for a more robust process.

For the last run, D, an operational point was chosen to the left of the region of complete separation. As expected, the purity of the extract product is decreased because the constraints for this component is not met, while the purity for the raffinate product, the desired product, is kept more or less

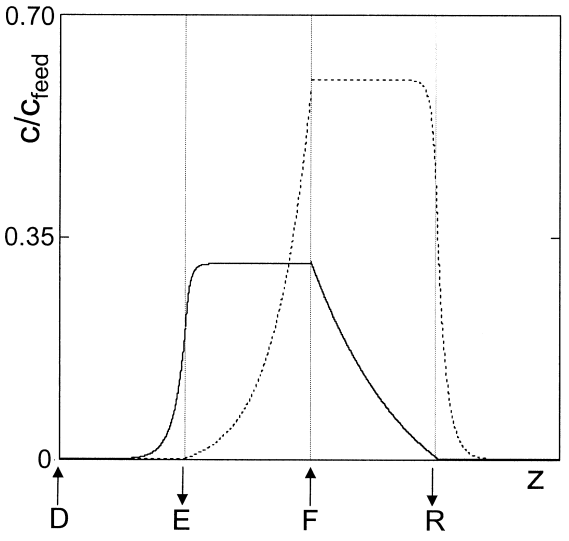

Fig. 11. Concentration profile for the isocratic separation of bovine insulin (dotted line) from porcine insulin (solid line), run A.

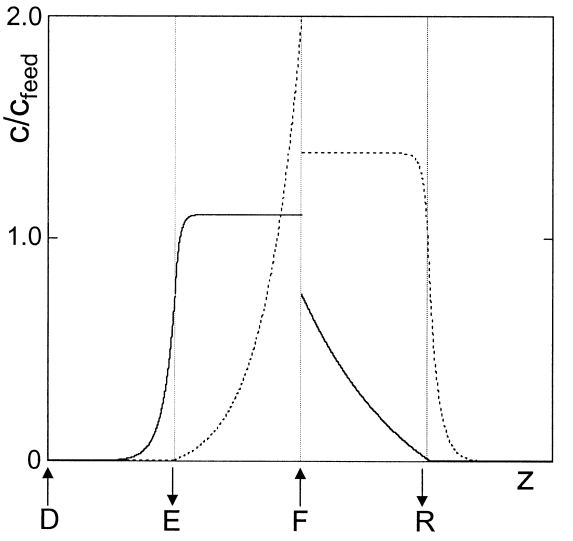

Fig. 12. Concentration profile for the gradient separation of bovine insulin (dotted line) from porcine insulin (solid line), run B.

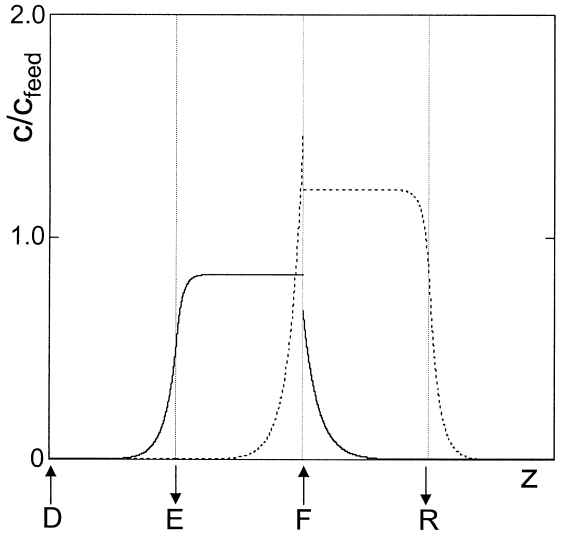

Fig. 13. Concentration profile for the gradient separation of bovine insulin (dotted line) from porcine insulin (solid line), run C.

constant. The recovery for the raffinate product will be less since some of it is lost to the extract outlet.

5.3. General remarks for the two cases

It must be pointed out that the results for the two cases have not yet been verified experimentally on a SMB unit. Experiments have currently been started for the nystatin case and will be presented later. Still, our theoretical results are supported by those of a comparable idea by Mazzotti et al. [3] for supercritical fluid SMB using a pressure gradient over the four SMB zones. The results, based on theoretical modelling and compared with experimental data, show the same trends as presented in this work. In fact, their results seem even more attractive than in gradient SMB because the pressure can be set differently for the four sections, thus having step-wise gradient of three steps, between each section, instead one step between sections 2 and 3 in our case.

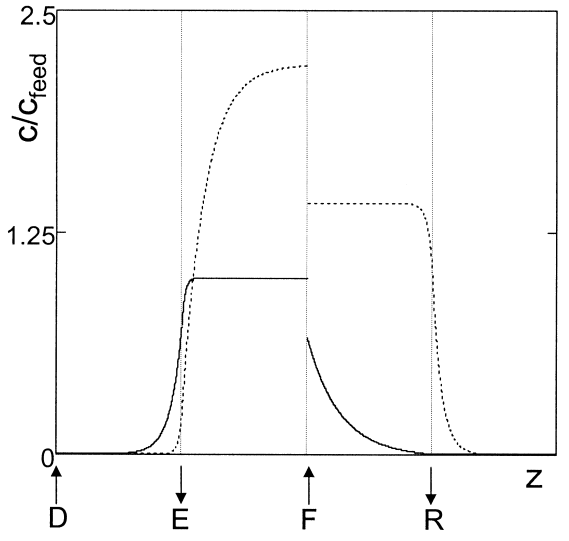

Fig. 14. Concentration profile for the gradient separation of bovine insulin (dotted line) from porcine insulin (solid line), run D.

The solvent strength of the feed and desorbent in gradient mode were not optimised in this work, but only set such that the impact of applying a gradient could be clearly demonstrated. It is obvious that an optimisation of the solvent strengths must be carried out to take full advantage of the SMB in gradient mode. It must also be pointed out that it is not only the solvent consumption that is influenced by a change in the gradient, but also the purity, recovery and enrichment of the products will change. More importantly, the productivity, which indicates how much sorbent material is needed to produce a certain amount of product, will also be influenced by the gradient.

Gradient operation of the SMB introduces a small difficulty in the recycling of the desorbent. Normally, in isocratic mode, the recycle stream can be introduced directly into the beginning of the SMB, together with the make-up stream, but in the case of gradient mode the recycle stream will have a different solvent strength than the desorbent that will be introduced at the beginning. This means that the solvents in the recycle stream must be regenerated to give the desired solvent strength before re-use.

6. Conclusion

It is evident that a step-wise solvent gradient can be applied to a standard four section SMB operating in the reversed-phase mode. The gradient is applied by introducing a feed stream containing a lower solvent strength compared to the desorbent stream. This will result in a shorter elution zone for the two

components to be separated and will lead to lower solvent consumption and less dilution of the products. In the two cases that were presented, purification of the polyketid antibiotic nystatin and separation of bovine insulin from porcine insulin, using a gradient resulted in at least 50% reduction of the solvent and two-fold enrichment of the products. Experimental validation of these theoretical results is part of future work.

Optimisation of the gradient has not been investigated in this work, but it is quite clear that this is important for finding the most efficient process. The optimal combination of the solvent strength for the feed and desorbent should be investigated by minimising the solvent consumption, as well as by taking the purity, recovery, and enrichment of the products and the productivity of the SMB unit into account, since the solvent strength of the two inlet streams will influence all of these parameters. This analysis is also part of future work.

7. Symbols

c	Means fluid phase concentration
DR	Means desorbent requirement, defined by Eq. (17)
E	Means enrichment, defined by Eqs. (15) and (16)
F	Means mass flow-rate
K_i	Means adsorption equilibrium constant of component i in isocratic mode
K_{ij}	Means adsorption equilibrium constant of component i in section j in gradient mode
m_j	Means mass flow-rate ratio in section j, defined by Eq. (2)
P_E	Means purity of the extract product, defined by Eq. (11)
P_R	Means purity of the raffinate product, defined by Eq. (12)
R_E	Means recovery of the extract product, defined by Eq. (13)
R_R	Means recovery of the raffinate product, defined by Eq. (14)
S_{ij}	Means separation factor of component i in section j, defined by Eq. (1)
t_0	Means residence time of non-retained species in a chromatographic column

Greeks

φ_k	Means solvent fraction of solvent k
ρ	Means liquid density

Subscripts and superscripts

A	Means more retained species in the feed
B	Means less retained species in the feed
D	Means desorbent
E	Means extract
F	Means feed
i	Means component index, i=A, B
j	Means section index, $j=1,\ldots,4$
k	Means solvent index, $k=1, 2$
R	Means raffinate
S	Means solid
T	Means total

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