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Optimization of azeotropic protein separations in gradient and isocratic ion-exchange simulated moving bed chromatography

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Abstract

The separation of dilute binary mixtures of proteins by salt aided ion-exchange simulated moving bed (SMB) chromatography is optimized with respect to throughput, desorbent consumption and salt consumption. The optimal flow-rate ratios are analytically determined via an adopted ''triangle theory''. Azeotropic phenomena are included in this procedure. The salt concentrations in the feed and recycled liquid are subsequently determined by numerical optimization. The azeotropic separation of bovine serum albumin and a yeast protein is used to illustrate the procedure. Gradient operation of the SMB is generally preferred over isocratic operation. A feed of azeotropic salt concentration can only be separated in a gradient SMB. Desorbent and salt consumption are always lower in gradient than in isocratic SMB chromatography.

Keywords: Optimization; Simulated moving bed chromatography; Azeotropy; Salt gradients; Proteins; Albumin

1. Introduction

More and more, simulated moving bed (SMB) systems are used for the purification of various components on an industrial scale. Emerging applications are the separations of enantiomers using chiral stationary phases [1,2]. The advantages of SMB chromatography over fixed bed chromatography are the reduced consumption of sorbent and solvent. Furthermore, often a higher purity can be obtained than in conventional fixed bed chromatography. A pure product can already be harvested from an SMB when there is a pure product in only a very small section of the unit, whereas this requires a fair column section in fixed bed separations [3].

Gradients in elution strength may further improve the efficiency of the SMB. By the use of different solvent compositions or physical conditions of the feed and desorbent streams, a situation of a high affinity of the solute for the matrix in the top sections (sections III and IV in Fig. 1) and a low affinity in the bottom sections (sections I and II in Fig. 1) is introduced. The situation of equal affinity in all four sections of the SMB will further be termed ''isocratic operation''. Several papers show that gradient operation may result in an increase of the throughput, i.e., the volume of feed loaded per column volume, and a reduction of the consumption of desorbent per feed volume compared to the isocratic situation. Examples are temperature gradients during separation of sugars [4], pressure gradients in supercritical fluid chromatography [5], and methanol gradients in reversed-phase separation of antibiotics [6]. In the latter (unoptimized) example, the application of the gradient resulted in at least 50% reduction of solvent

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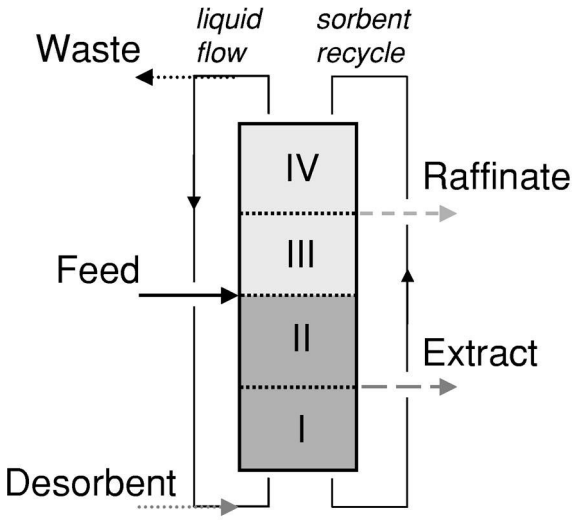

Fig. 1. Schematic representation of gradient in SMB and numbering of sections therein. The desorbent is used for adjustment of volume and modulator concentration of the recycled stream. A dark color indicates a reduced affinity of the protein for the matrix.

consumption and a twofold concentrating of the product compared to the isocratic SMB.

Houwing and co-workers [7,8] investigated the use of salt gradients during separation of dilute mixtures of proteins by ion-exchange chromatography. An important observation was that no complete separation can be obtained at certain combinations of salt concentration in the desorbent and feed. This phenomenon was termed ''azeotropy''. Similar to azeotropic situations such as observed in distillation processes, azeotropy in SMB chromatography is caused by a reversal of selectivity $S_{1,2}$, which is defined as:

$$S_{1,2} = \frac{q_1}{c_1} \cdot \frac{c_2}{q_2}$$

where q is the adsorbed phase concentration and c is the liquid phase concentration. When $S_{1,2}$ exceeds unity, component 1 is the more retained; when $S_{1,2}$ is below unity, component 2 is the more retained. In ion exchange of proteins, the selectivity is a function of the salt concentration. When using different salt concentrations in an SMB, the selectivity may exceed unity in the top sections and meanwhile be below unity in the bottom sections. No complete separation will occur under these conditions. Many examples of selectivity reversals during protein separations can be found in literature. Steric mass action (SMA) isotherms of (dilute mixtures) of proteins in ion exchange frequently lead to a reversal of selectivity as a function of salt concentration. A few examples thereof are the separation of α-chymotrypsinogen and cytochrome *c* [9], of horse and bovine cytochrome *c* [10], and of α-lactalbumin and β-lactoglobulin [11].

In this paper, a procedure for optimization of the separation of dilute, azeotropic mixtures of proteins by ion-exchange chromatography (IEX) in SMB systems is described. The considered optimization functions are: (i) throughput: the volume of feed loaded per sorbent volume; (ii) desorbent consumption: the volume of desorbent required per volume of feed processed; (iii) salt consumption: the amount of salt required per volume of feed processed.

The procedure is based on primary selection of the optimal flow-rate ratios, using an adaptation of ''triangle theory'' [12], which includes azeotropy and is described in Ref. [7]. Secondly, the optimal salt concentrations in the lower sections (the inlet salt concentration) and the feed solution are optimized numerically. Thus, the chance to end up in a local optimum, which is substantial in similar systems with many variables, is reduced. The procedure is illustrated by taking the separation of dilute mixtures of bovine serum albumin (BSA) and a yeast protein (yp) as an example.

2. Theory

2.1. Selection of flow-rate ratios

The selection of the flow-rate ratios m in a gradient SMB follows the ''triangle theory'' [12]; the procedure has been described in [7] and is summarized in Table 1. The constraints on the flow-rate

Table 1
Selection of flow-rate ratios in gradient SMB

		m_1	>	$K_H(c_I)$
$K_H(c_I)$	>	m_2	>	$K_L(c_I)$
$K_H(c_{III})$	>	m_3	>	$K_L(c_{III})$
$K_L(c_{III})$	>	m_4		

ratios are given by the distribution coefficients ($K = q/c$) of the stronger (H) and weaker (L) binding component. These are a function of the salt concentration in the considered section. Furthermore, the addition of the feed dictates that m_3 exceeds m_2, the so called ''positive feed'' criterion.

In Table 1, c_I and c_{III} represent the salt concentrations in sections I and III, respectively. The salt concentration c_I will furthermore be indicated as the ''inlet salt concentration'', as it is the concentration that enters the system upon mixing of the recycle and the desorbent (Fig. 1). The salt concentration in section III follows from the flow-rates, the salt concentration of the feed and the inlet salt concentration via the mass balance over the point of feed introduction. When salt has no interaction with the sorbent, this balance reads:

$$c_{III} = \frac{m_2}{m_3} \cdot (c_I - c_F) + c_F \quad (1)$$

Note that the correct movement of salt also needs to be assured. Houwing et al. [8] showed that salt may adsorb to the sorbent at higher salt concentrations and developed a procedure for correct positioning of the gradient. For reasons of clarity, we have assumed in this paper that salt does not interact with the sorbent.

A mass action isotherm is used to relate the distribution coefficients of the proteins in diluted solutions and the salt concentration [13]:

$$K_i = K_i^0 c_s^{-z_i}$$

where K_i^0 is the (reference) distribution coefficient of component i in a 1 M salt solution, c_s is the salt concentration and z_i is the characteristic charge of component i. This simple mass action model was used for reasons of clarity; however the approach elaborated in this work can be extended to more realistic (and complex) models.

Fig. 2 shows the distribution coefficients of two species as a function of the salt concentration. For illustration purposes, we have used the separation of BSA and a yeast protein on Q-Sepharose FF using NaCl as salt, described in Ref. [7], of which the isotherm parameters are shown in Table 2. This model represents the typical separation problems during the fermentative production of bio-pharmaceuticals, such as recombinant human serum albumin. Only for the sake of readability, the two species to be separated will be named BSA and yeast protein throughout this paper; it should be emphasized that the theory holds for any set of proteins.

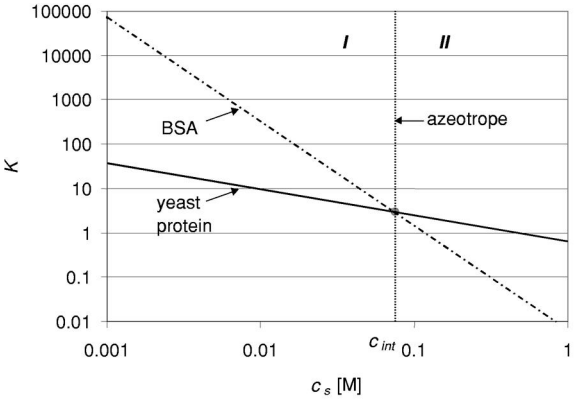

Fig. 2. Intersecting plots of the distribution coefficients of BSA and a yeast protein on Q-Sepharose FF at pH 5.4 in a 10 mM acetate buffer as a function of the salt (NaCl) concentration (from Ref. [7]).

Azeotropic phenomena occur in the system shown in Fig. 2, since the selectivity $S_{BSA,yp}$ exceeds unity at salt concentrations below the concentration of intersection (c_{int}), whereas it is below unity at concentrations exceeding c_{int}. This implies a salt aided SMB can only be operated properly in two regions: region I with salt concentrations below c_{int}, and region II with salt concentrations exceeding c_{int}, such as shown in Fig. 2. Azeotropic phenomena can only be prevented by choosing the salt condition in section III in the same region as the salt concentration in section I. This is established by taking both limits of m_3 into account.

2.2. Optimal flow-rate ratios

Previous work on non-gradient SMB separation of

Table 2
Mass action isotherm parameters of BSA and a yeast protein on Q-Sepharose FF, at pH 5.4 in a 10 mM acetate buffer with NaCl as described in Ref. [7]

	BSA	Yeast protein
K_0	$6.7 \cdot 10^{-3}$	0.64
z	2.35	0.59

components obeying a linear isotherm has shown that throughput is maximal and desorbent consumption is minimal when m_1 and m_2 are chosen near their lower limit, whereas m_3 and m_4 are chosen near their upper limit [14]. The validity of this approach for the output functions considered in this paper has been tested. The results shown in Appendix A indicate that these flow-rate ratios are indeed optimal for optimization of throughput and desorbent consumption. In some cases, optimization of the salt consumption may require a full numerical optimization of both flow-rates and salt concentrations. This paragraph continues with the derivation of explicit relations for the optimal flow-rate ratios in azeotropic SMB separations.

In an azeotropic situation, the choice of m_1 and m_4 is similar to that in non-azeotropic systems. The optimal values, close to the limiting values, are found by multiplication (m_1) or division (m_4) of the limits given in Table 1 by a factor α, which is chosen close to unity.

The optimal values of m_2 and m_3 are most conveniently found in a ''region of complete separation'' [12]. Only inside the triangular region enclosed by the lines defined by the constraints in Table 1, the feed is separated into pure extract and raffinate fractions. Fig. 3 shows the superposition of three such regions of complete separation in an SMB for fractionation of BSA and yeast protein operated in region II. As before, the maximum throughput (m_3-m_2) is obtained when m_2 is at the minimum value, whereas m_3 is at the maximum value. The term ''optimal point'' will further be used to indicate the corresponding point in the m_2–m_3 plane.

The optimal point is on the intersection of the $K_{\mathrm{BSA}}(c_{\mathrm{I}})$ and $K_{\mathrm{yp}}(c_{\mathrm{III}})$ lines, i.e., point X in the isocratic situation. It shifts to increased m_3 upon decreasing the feed salt concentration, or increasing the desorbent concentration. The occurrence of azeotropy at these conditions is avoided by the use of the $K_{\mathrm{BSA}}(c_{\mathrm{III}})$ line. The three aforementioned lines intersect at point Y at a feed salt concentration $c_{\mathrm{F,Y}}$. At that point, the salt concentration in section III equals c_{int}, because the distribution coefficients in section III are equal. The corresponding limiting m_3 equals the distribution coefficient of both components at c_{int}, and is further indicated as $m_{3,\mathrm{int}}$. The feed and inlet salt concentration at this point Y are

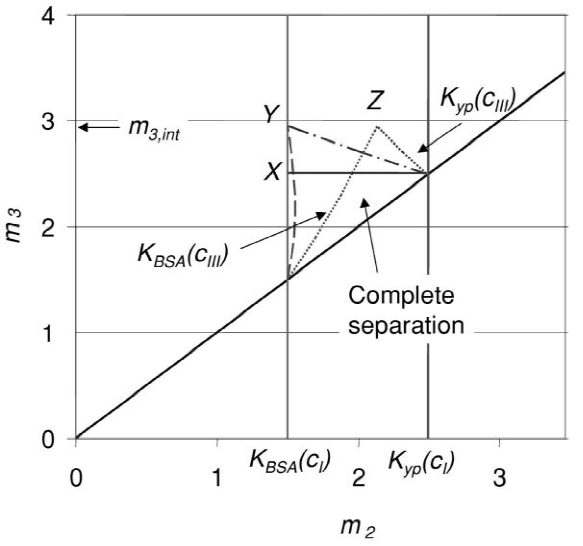

Fig. 3. Superposition of three regions of complete separation during separation of BSA and a yeast protein in region II at $c_{\mathrm{I}}=0.1$ M NaCl. Solid line: isocratic situation ($c_{\mathrm{F}}=0.1$ M NaCl), dash–dotted line: gradient at $c_{\mathrm{F}}=0.042$ M NaCl, dotted line: gradient at $c_{\mathrm{F}}=0.01$ M NaCl. Optimal points X, Y and Z are explained in the main text.

related via the feed mass balance (Eq. (1)), which is rewritten as:

$$c_{\mathrm{F,Y}}\cdot\left(1-\frac{K_{\mathrm{BSA}}}{(c_{\mathrm{I},Y})^{z_{\mathrm{BSA}}}m_{3,\mathrm{int}}}\right)=c_{\mathrm{int}}-\frac{K_{\mathrm{BSA}}c_{\mathrm{I},Y}}{(c_{\mathrm{I},Y})^{z_{\mathrm{BSA}}}m_{3,\mathrm{int}}} \tag{2}$$

At a feed salt concentration below $c_{\mathrm{F,Y}}$, azeotropic phenomena can only be avoided when m_2 exceeds the lower limit $K_{\mathrm{BSA}}(c_{\mathrm{I}})$. Only then, the salt concentration in section III is (larger than) c_{int}. The optimal point is indicated by Z when the point of intersection of the $K_{\mathrm{yp}}(c_{\mathrm{III}})$ and $K_{\mathrm{BSA}}(c_{\mathrm{III}})$ lines is in between $K_{\mathrm{BSA}}(c_{\mathrm{I}})$ and $K_{\mathrm{yp}}(c_{\mathrm{I}})$, by W when it is on the intersection of $K_{\mathrm{yp}}(c_{\mathrm{I}})$, $K_{\mathrm{yp}}(c_{\mathrm{III}})$ and $K_{\mathrm{BSA}}(c_{\mathrm{III}})$, and V when it is on the intersection of $K_{\mathrm{BSA}}(c_{\mathrm{III}})$ and $K_{\mathrm{yp}}(c_{\mathrm{I}})$.

The feed and inlet salt concentration at W are related via:

$$c_{\mathrm{F},W}\cdot\left(1-\frac{K_{\mathrm{yp}}}{(c_{\mathrm{I},W})^{z_{\mathrm{yp}}}m_{3,\mathrm{int}}}\right)=c_{\mathrm{int}}-\frac{K_{\mathrm{yp}}c_{\mathrm{I},W}}{(c_{\mathrm{I},W})^{z_{\mathrm{yp}}}m_{3,\mathrm{int}}}$$

Points V and W are not shown in Fig. 3, because

these do not occur during the separation of BSA and the yeast protein taken as an example.

When the SMB is operated in region I, no azeotropy can occur. The optimal point is invariably at *X*, whereas the value of m_3 at the optimal point increases at decreasing salt concentration of the feed.

Table 3 summarizes the explicit equations on the optimal points that are required in analytical optimization. In Appendix B, we have shown that the gross shape of the region of complete separation and the occurrence of one optimal point, that can be at *V*, *W*, *X*, *Y*, and *Z*, is general and holds for any mass action isotherm. Thus, Table 3 is also generally applicable.

2.3. On the optimization of desorbent and salt consumption

Optimization of isocratic SMB systems only requires optimization of the throughput and the consumption of desorbent. In the gradient SMB, an additional optimization function is the consumption of the gradient forming agent, which is salt in our case. The salt may have large implications on the process economy, possibly via environmental regulations. Obviously, there is an economic optimum between desorbent consumption (i.e., the volume of desorbent used per feed volume, irrespective of the salt concentration) and the salt consumption (i.e., the absolute amount of salt used per feed volume, irrespective of the volume it is dissolved in). At low salt costs, the optimum is at minimum desorbent use. This is favored by a high inlet concentration, which reduces the flow-rate ratio in the lower sections at the expense of an increased salt consumption. At high salt costs, salt consumption should be minimal. This is favored by a lower inlet concentration, which decreases the salt need at the expense of a higher desorbent flow-rate.

The magnitude of the desorbent and salt consumption are strongly influenced by the possibility to recycle of the liquid leaving section IV to section I. In pharmaceutical applications, an ''open loop'' mode is preferred. The liquid leaving section IV is discarded, which prevents the accumulation of undesired contaminants. In other applications, a ''closed loop'' mode may be used. The complete m_4 is then recycled, and only a small volume of desorbent is added. By this recycle, the consumption of desorbent and salt may be reduced substantially.

In the following, we will consider a partially closed loop system. The recycle of m_4 is then maximal, but not necessarily complete. A desorbent (of high salt concentration) is used to adjust the flow-rate and salt concentration of the recycled stream. Two cases can be distinguished.

(i) When both salt concentration and flow-rate need to be increased, i.e., $m_1 > m_4$ and $c_I > c_{III}$, a desorbent stream of magnitude $m_1 - m_4$ can be used. The required desorbent salt concentration c_D is computed from the mass balance over the position of desorbent addition, as is shown in Fig. 1:

$$c_D = \frac{m_1 c_I - m_4 c_{III}}{m_1 - m_4} \tag{3}$$

(ii) When only the salt concentration needs to be adjusted, or when the salt concentration calculated by Eq. (3) is unrealistic, part of the liquid leaving section IV is replenished by a concentrated salt solution, of a chosen maximum concentration. In this paper, an arbitrary value of $c_D = 1\ M$ has been used. The magnitude of the desorbent stream (*des*) is then obtained from the desorbent mass balance:

Table 3
Location of the optimal point

Operation in region	Criteria		Optimal point	m_2	m_3
I	$c_F < c_{int}$		*X*	$K_{yp}(c_I)$	$K_{BSA}(c_{III})$
II	$c_F < c_{int}$	$c_F < c_{F,W}$ or $c_I > c_{I,W}$	*V*	$K_{yp}(c_I)$	$K_{BSA}(c_{III})$
II	$c_F < c_{int}$	$c_F = c_{F,W}$ or $c_I = c_{I,W}$	*W*	$K_{yp}(c_I)$	$m_{3,int}$
II	$c_F < c_{int}$	$c_{F,W} < c_F < c_{F,Y}$ or $c_{I,W} > c_I > c_{I,Y}$	*Z*	From Eq. (1)	$m_{3,int}$
II	$c_F < c_{int}$	$c_F = c_{F,Y}$ or $c_I = c_{I,Y}$	*Y*	$K_{BSA}(c_I)$	$m_{3,int}$
II	$c_F < c_{int}$	$c_F > c_{F,Y}$ or $c_I < c_{I,Y}$	*X*	$K_{BSA}(c_I)$	$K_{yp}(c_{III})$
II	$c_F > c_{int}$		*X*	$K_{BSA}(c_I)$	$K_{yp}(c_{III})$

$$des = m_1 \cdot \frac{c_I - c_{III}}{c_D - c_{III}}$$

The desorbent consumption (*DC*) and salt consumption (*SC*) are calculated from:

$$DC = \frac{des}{m_3 - m_2}$$

$$SC = \frac{c_D des}{m_3 - m_2}$$

3. Methods

The numerical optimization of salt concentrations and flow-rates was done in Matlab version 5.2 (Mathworks, Boston, MA, USA). The inverse of throughput, the desorbent consumption and the salt consumption were minimized using Matlab's ''constr'' optimization function.

4. Results and discussion

Three situations are described in this paragraph: (i) restricted optimization of an SMB operating at a known inlet salt concentration; (ii) optimization of an SMB operating at a known salt concentration of the feed; (iii) overall optimization of both inlet and feed salt concentration.

4.1. SMB chromatography at known inlet salt concentration

4.1.1. Optimization of the throughput by changing the feed salt concentration

The optimal feed salt concentration with respect to throughput *P* in an SMB with known inlet salt concentration can be found analytically. Whenever the optimal point is at *X* or *Y*, the throughput is determined by the difference in affinity of the more retained component in section III and the less retained component in section II. We illustrate this using Fig. 2. For example in region I, m_2 is on the K_{yp} line at concentration c_I, whereas m_3 is on the K_{BSA} line at concentration c_{III}. The throughput is determined by the ''affinity difference'', the vertical distance between the m_2 and m_3 points. This affinity difference is maximal when the difference in salt concentration in section II and III is maximal; hence in region I, the minimal feed salt concentration is optimal.

In principle, the same holds in region II, unless the optimal point is at *Z*, *V* or *W*. Then, m_2 is elevated relative to its lower boundary in order to maintain the correct salt concentration. Throughput is then maximal when the optimal point is at *Y*; any decrease of the feed concentration would lead to an increase of m_2 at constant m_3 and to lower throughput. The corresponding feed concentration is computed using Eq. (2).

The results of these calculations are plotted in Fig. 4. An unexpected finding is that salt should be added to the feed solution in region II in order to improve throughput. Even, more salt is added than is strictly necessary to overcome the azeotrope. This is counterintuitive, since the throughput of fixed bed separations always increases upon reduction of the salt concentration of the feed.

Fig. 4 also indicates that the optimal feed salt concentration increases with increasing inlet salt concentration. This can mathematically be proofed by taking the derivative of $c_{F,Y}$ (Eq. (2)) to c_I. This derivative is always positive whenever $z_{BSA} > 1$, which explains the observed. However, whenever $z_{BSA} < 1$, the derivative is negative and the optimal feed salt concentration decreases with increasing inlet concentration. This situation is not likely, since BSA is the stronger binding component.

The throughput is high in region I when the

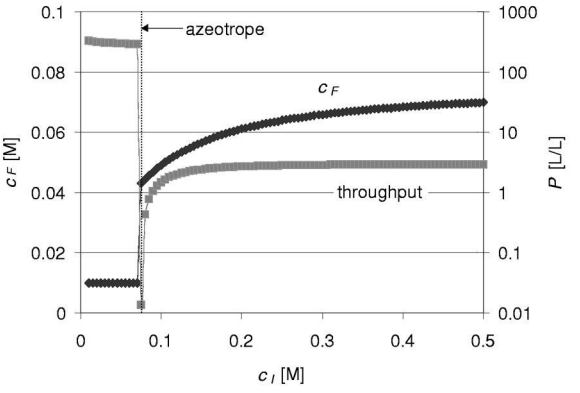

Fig. 4. Optimal feed salt concentration and throughput as a function of the inlet salt concentration.

minimum salt concentration is low (a minimal concentration $c_{F,min}$ of 0.01 *M* NaCl has been used in Fig. 4). As will be shown shortly, the minimum salt concentration strongly influences the magnitude of the throughput. The maximum throughput is obtained in the isocratic situation. The convergence of the isotherms suggests that m_3 profits more than m_2 of a decrease in c_I, which would imply operation at low c_I favors a high throughput. However, by a complete mathematical derivation of the derivative of throughput to c_I, it is found that there can be other optima in c_I in region I, independent of z_y, when $z_{BSA}>1$. The occurrence of such optima strongly depends on the values of *K* and *z*. The derivative in the isocratic situation is always of negative sign, which implies the isocratic situation is always a local optimum.

The throughput at the optimal feed concentration in region II is a weakly increasing function of the inlet concentration, since m_3 is fixed at $m_{3,int}$ and m_2 decreases slowly with increasing c_I. This is due to the small effect of charge on the distribution coefficients at high salt concentrations. The throughput in region II is much lower than in region I, since the maximal m_3 is bound by $m_{3,int}$.

4.2. SMB chromatography at known feed salt concentration

This section deals with a practical question: ''what is the most optimal inlet salt concentration starting from a feed of salt concentration c_F?'' The isocratic operation is evaluated as an alternative.

4.2.1. Optimization of the throughput by changing the inlet salt concentration

In an isocratic SMB operated in region I, the throughput *P* is high and decreases with increasing salt concentration (Fig. 5), because of the convergent lines of the distribution coefficients as a function of the salt concentration (cf. Fig. 2). This means the affinity difference is maximal at low salt concentration and decreases with increasing c_s. Throughput is zero when both c_I and c_F equal c_{int}, because all distribution coefficients are then equal. When the salt concentration is further increased (i.e., operation in region II), the throughput initially increases, because the affinity difference increases with c_s as a result of

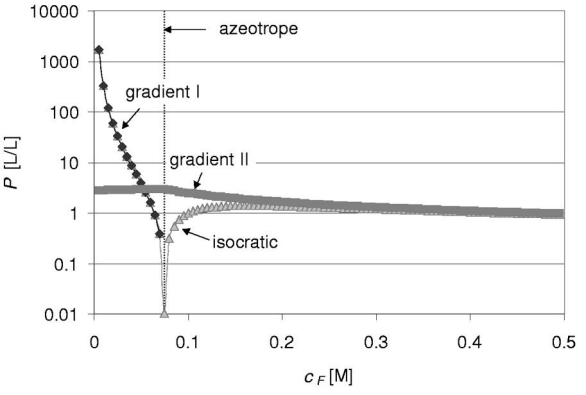

Fig. 5. Throughput in isocratic and optimized gradient SMB at a maximum inlet concentration of 1 *M* NaCl.

the divergent lines of the distribution coefficients. After a certain optimum, the throughput decreases, because the lines of the distribution coefficients converge when plotted at a linear scale. Note that the lines still diverge at a logarithmic c_s scale.

The optimum throughput of the gradient SMB operated in region I is obtained in the isocratic situation ($c_I=c_F$) as has already been explained in Fig. 4. The gradient SMB operated in region II has its maximal affinity difference at the maximum allowed inlet salt concentration. The divergent isotherms suggest that m_2 is more decreased than m_3 at increasing inlet salt concentration, which implies a high inlet salt concentration favors throughput. However, a detailed mathematical analysis of the effect of c_I on throughput in terms of the derivative $\partial(m_3-m_2)/\partial c_I$ shows that this will not always be the case. At feed salt concentrations below c_{int}, the system is operated at *Y* or *Z*. In that case, m_3 and c_3 are constant and the derivative is always positive, which means a high c_I is favorable for the throughput. The same result is obtained when the feed salt concentration exceeds c_{int} and $z_y<1$. However, whenever $z_y>1$ and $c_F>c_{int}$, other optima may exist.

Obviously, there is a limit to the maximum concentration in practical systems. In further calculations, the maximal c_I has been set at an arbitrary value of $c_{max}=1$ *M* NaCl. The throughput in region II is not very sensitive to the chosen value; at a maximum concentration of 0.5 *M* NaCl the throughput is maximally 3% decreased compared to a 1 *M* inlet salt concentration.

When operating in a gradient in region II at increasing feed salt concentration, the throughput first increases, passes an optimum and then decreases. The explanation lies in the location of the optimal point. At low salt concentration, the optimal point is at *Z*. Upon an increase of the feed salt concentration, this point *Z* moves towards the $K_{BSA}(c_I)$ boundary. Hence, m_2 is decreased at constant m_3, so throughput increases. At a feed salt concentration of 0.073 *M* NaCl, i.e., the $c_{F,Y}$ corresponding to the 1 *M* inlet salt concentration, the system is operated at point *Y*; at this point throughput is maximal. At further increasing feed salt concentration, the optimal point is at *X*, at a decreasing m_3 and a constant m_2, which results in a decrease of the throughput with increasing feed salt concentration.

It can be concluded that the isocratic SMB operation leads to a (local) maximum in throughput during separation of a feed of low salt concentration. In the considered example of BSA and the yeast protein, the performance of the isocratic SMB is one- to three-orders of magnitude better than the gradient SMB operated in region II. At low feed concentrations, the throughput in region II is always lower than in region I, since the maximum m_3 is limited. An important finding is that separation of a feed of salt concentration c_{int} is only possible when using a gradient in region II. The gradient introduces the affinity difference required for separation, which is absent in the isocratic situation. At high feed salt concentrations, the gradient is not very powerful. The introduced affinity difference is low and so the performances of the gradient and isocratic SMBs will be similar.

4.2.2. Optimization of the desorbent consumption by changing the inlet salt concentration

In the isocratic SMB, the desorbent consumption DC is close to unity at most salt concentrations, because the desorbent flow-rate ratio m_1-m_4 almost equals the feed flow-rate ratio m_3-m_2. An asymptote in desorbent consumption is found at (feed) salt concentration c_{int} (cf. Fig. 6). At this salt concentration, throughput is zero (see Fig. 5), so the volume of desorbent used per feed volume is infinite.

When the gradient SMB is operated at low feed salt concentration in region I, the optimal inlet concentration is close to c_{int} (Fig. 6). This ''high'' inlet concentration favors a low m_1. Also, the recycle of m_4 is most efficient, and hence a low flow-rate of a desorbent of 1 *M* NaCl is sufficient. At a feed salt concentration of 0.03 *M* NaCl, m_1 starts to exceed m_4 and hence the recycled m_4 no longer suffices to supply the necessary flow-rate. An increased flow-rate of desorbent of decreased salt concentration is then required. This explains why the desorbent consumption abruptly increases at the feed salt concentration of 0.03 *M* NaCl.

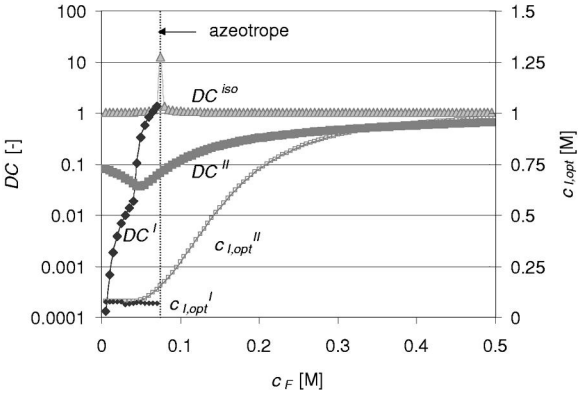

Fig. 6. Desorbent consumption and optimal inlet concentration in isocratic and optimized gradient SMB at a maximum inlet concentration of 1 *M* NaCl.

Also when the gradient SMB is operated in region II, a complete recycle of m_4 in combination with a desorbent of maximal salt concentration (1 *M* NaCl) is optimal. This maximal desorbent concentration is maintained at all feed salt concentrations; never, a more diluted desorbent needs to be used. Thus, the recycle of salt is used to the maximum.

At very low feed salt concentration, the system is operated at optimal point *Z*. By substitution of the known c_3, $m_{3,int}$ and $c_D=1$ *M* NaCl in Eq. (3), an optimal inlet salt concentration of 0.0794 *M* NaCl is found. At increasing feed salt concentration, operation is still at *Z*, so the optimal inlet salt concentration and m_1 do not change. Throughput increases, since point *Z* shifts to lower m_2 at increasing feed salt concentration. Thus, the desorbent consumption decreases. At a feed salt concentration of 0.045 *M*, the system is operated at optimal point *Y*. From that feed concentration on, an increase in inlet concentration leads to: (i) an increased throughput

(as explained at ''throughput''), which decreases the desorbent consumption; (ii) an increased m_1, which increases the desorbent consumption.

This trade-off results in a gradual increase of the optimal inlet salt concentration. Only by optimization, it can be found that the optimum is at an inlet concentration of 0.05 *M*.

It can be concluded that the desorbent consumption in closed loop mode is always lower in gradient operation than in isocratic operation. In gradient operation, m_1 is reduced as a result of the low affinity in section I. Furthermore, most of m_1 can be obtained from the recycle of m_4, since in a gradient it is more likely that the flow-rate ratio m_4 exceeds m_1. The combination of these two factors results in a very low desorbent flow-rate ratio of a desorbent of the maximal salt concentration. This finding seems general and independent of the values of *K* and *z*. In the specific case of the separation of BSA and the yeast protein, a gradient in region I may lead to a 8000-fold decrease of the desorbent consumption at very low c_F; a gradient in region II may lead to a 30-fold decrease of the desorbent consumption at $c_F = 0.05$ *M* NaCl (all in comparison to the isocratic situation).

4.3. Optimization of the salt consumption by changing the inlet salt concentration

The optimization of the salt consumption in a gradient SMB is not as straightforward as the optimization of throughput or desorbent consumption, since it may require rigorous numerical optimization (see Appendix A). However, in all optimizations carried out, the optimal flow-rate ratios in fact equaled the analytical solution. This could not be explained.

In an isocratic SMB, the salt consumption SC is proportional to the feed salt concentration c_F, as is shown in Fig. 7. The proportionality occurs, because the desorbent salt concentration equals c_F and the desorbent consumption is independent of c_F (cf. Fig. 6). The salt consumption has an asymptote at c_{int}, because the desorbent consumption has an asymptote at that concentration.

In a gradient operated in region I at low feed salt concentrations, the salt consumption is minimal when the inlet salt concentration is near c_{int}. At this

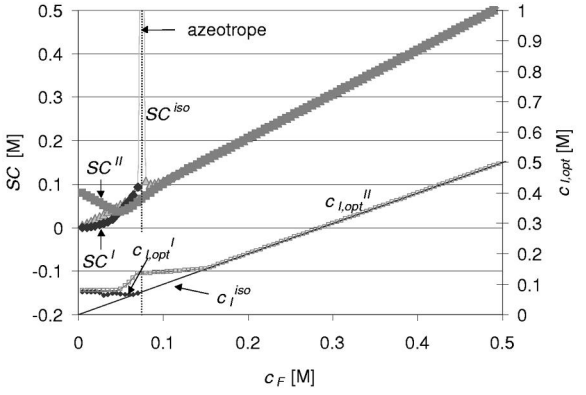

Fig. 7. Salt consumption and optimized inlet concentration in isocratic and optimized gradient SMB at a maximum inlet concentration of 1 *M* NaCl.

inlet concentration, m_1 is small and m_4 exceeds m_1, which allows a maximal recycle of m_4. The salt concentration of the recycled liquid can then be increased by the addition of a small volume of desorbent of high concentration. The salt consumption increases upon increasing c_F, because the affinity difference and throughput are reduced as the gradient approaches the isocratic situation (cf. Fig. 5). The high inlet salt concentration near c_I remains favorable. Also, m_4 is reduced, which leads to a situation where an increased desorbent flow of decreased c_D is required to adjust both flow-rate and salt concentration at $c_F = 0.04$ *M* NaCl. When the feed salt concentration approaches c_{int}, the system can only be operated isocratically.

When the SMB is operated in region II at low feed salt concentration, the conditions of optimal salt consumption coincide with the conditions of optimal desorbent consumption. Again, the maximization of the recycle and the addition of a very small volume of a concentrated desorbent is possible at these low feed salt concentrations. As the throughput increases with increasing c_F and constant c_I and m_1 is also constant, the salt consumption decreases with increasing c_F. Again, as c_F exceeds 0.045 *M* NaCl, an increased inlet salt concentration can be used, since this increases throughput, whereas the recycle is still maximal and little salt needs to be added. The optimal salt concentration of 0.05 *M* NaCl can only be found by optimization.

As c_F further increases, m_4 strongly decreases,

because m_4 is related to the salt concentration in section III via z_{BSA}; a large z_{BSA} introduces a strong dependence. This reduces the percentage of m_1 that can be obtained from the recycle and hence a larger desorbent flow-rate is required. Starting at a certain feed salt concentration of 0.07 *M* NaCl, which cannot be deduced analytically, the optimal inlet salt concentration is ''fixed''; it cannot decrease, because that would further increase m_1, but it cannot increase either, since this would increase c_3, reduce m_4 and reduce the possibility for salt recycle. From this concentration on, the optimal conditions for desorbent and salt consumption are no longer the same. This clearly demonstrates the trade-off mentioned in the Theory section: a smaller m_1 at higher inlet salt concentration may impose a larger salt need and hence be less beneficial than a (somewhat) larger m_1 at lower inlet salt concentration.

At high feed salt concentration, the isocratic situation is optimal to reduce the salt usage. Throughput then hardly profits from the gradient, as shown in Fig. 5. Gradient operation would only result in an increased demand for salt in the desorbent, because the recycle is very small.

It can be concluded that the salt consumption in closed loop mode can be lower in gradient operation than in isocratic operation. The reuse of the liquid leaving section IV to supply a flow-rate and a starting salt concentration for the liquid in section I is very important. Thus, the addition of a very small amount of salt is possible. This finding seems general and does not depend on the values of *K* and *z*. In the specific separation of BSA and the yeast protein separation used as an example, the gradient in region I results in a reduction of the salt consumption of up to a factor 40 compared to the isocratic situation. The gradient SMB operated in region II at feed concentrations near c_{int} is better than isocratic operation, because the isocratic separation has an asymptote at that concentration. However, when the feed salt concentration is about twice c_{int}, isocratic operation is most favorable for reduction of salt consumption.

4.4. Overall comparison of isocratic and gradient SMB chromatography

Thus far, we have only regarded one optimization function at the time. However, the optimization of one function may lead to a poor result with respect to the other functions. In Table 4, for all optimized functions, the other output functions are listed as well. The table clearly indicates the dilemma. For example, a high inlet salt concentration is beneficial to maximize the throughput in region II, but this also means the desorbent and salt concentration are far beyond the optimal values.

Table 4
Optimized and calculated process parameters

Optimized function	c_F (*M*)	c_I (*M*)	*P* (l/l)	*DC* (–)	*SC* (mol/l)
P (region I)	0.005	0.005	1700	1.020	0.005
P (region II)	0.073	1.000	2.953	0.222	0.222
DC (region I)	0.005	0.075	1610	$1.32\cdot10^{-4}$	$1.32\cdot10^{-4}$
DC (region II)	0.049	0.083	0.644	0.036	0.036
SC (region I)	0.005	0.075	1610	$1.32\cdot10^{-4}$	$1.32\cdot10^{-4}$
SC (region II)	0.049	0.083	0.644	0.036	0.036

Unexpectedly, the conditions for optimal desorbent and salt consumption are equal, both in region I and in region II. This is a result of the high extent of recycling at the low salt concentrations, which decreases both desorbent and salt consumption.

From the table it can be concluded that the ''overall optimum'' is most likely a gradient SMB operated in region I at the minimal feed salt concentration and an inlet salt concentration near c_{int}. These conditions lead to a near-optimum throughput and minimal desorbent consumption and salt consumption. With respect to desorbent consumption, it is very advantageous to operate under gradient conditions; the gradient is able to reduce the desorbent consumption by almost a factor of 8000 for the underlying example.

5. Conclusions

A procedure was developed for the optimization of the SMB separation of dilute mixtures of proteins that obey mass action isotherms. The procedure was illustrated using the separation of BSA and a yeast protein on Q-Sepharose FF as an example. The following general conclusions could be drawn:

1. A gradient is useful to improve throughput,

desorbent consumption and/or salt consumption when the feed is at a salt concentration below two times the concentration of reversal of selectivity (c_{int}).
2. A feed of salt concentration (near) c_{int} can only be separated with a reasonable throughput in a gradient operated at salt concentrations exceeding c_{int} (i.e., region II).
3. When operating in region II and when $z_{BSA}>1$, the addition of salt to the feed improves throughput. In effect, the addition of more salt than strictly necessary for avoiding azeotropic phenomena is preferable. This is truly counterintuitive!
4. Isocratic operation leads to a (local) maximum of throughput in region I.
5. Operation of a gradient in region I reduces desorbent and salt consumption compared to isocratic SMB chromatography.
6. Desorbent and salt use benefit most from the maximization of the recycle of the liquid from section IV to section I. A very small desorbent flow of a concentrated solution is then sufficient to adjust flow-rate and salt concentration.
7. Under maximal recycle, the conditions of minimal desorbent and minimal salt consumption coincide.
8. The preferred way of operating the SMB for separation of dilute mixtures of proteins is at minimal feed salt concentration, at an inlet salt concentration near c_{int}.

6. Nomenclature

c	Concentration in the liquid phase (M)
DC	Desorbent consumption (–)
des	Flow-rate ratio of desorbent (–)
K	Distribution coefficient (–)
m	Flow-rate ratio (–)
P	Throughput (l feed/l sorbent)
q	Concentration in the sorbent phase (M)
S	Selectivity constant (–)
SC	Salt consumption (M)
V, W, X, Y, Z	Optimal point (–)
z	Ionic charge (C/mol)

Greek

α	Discrepancy factor (–)

Super- and subscripts

D	Desorbent
F	Feed
i	Component index (BSA, yp)
I	In section I, inlet
int	At the intersection
iso	In the isocratic situation
max	Maximal
min	Minimal
opt	At the optimal point
p	Protein
s	Salt
W, Y	At the optimal point W or Y

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Appendix A. Optimum flow-rates

This appendix proves that the flow-rate ratios at the optimal point of the region of complete separation (point V, W, X, Y, Z), and the maximal m_4 and minimal m_1 at given salt concentrations c_I and c_F generally lead to the optimal concentration factors, eluent and salt consumption.

The optimal flow-rate ratio at maximum/minimum process variable is found by equating the derivative of the variable on the flow-rate ratio to zero. In this differentiation, the flow-rate ratios are independent variables, as they can be chosen independent of one another (as long as they are chosen within the constraints). When there is no zero in the constrained interval, the optimal flow-rate ratio is found at the limits of the interval.

In Table 5, all derivatives of the optimization functions to m_1 through m_4 are shown. In most cases, no zero occurs in the respective interval; maximal or minimal indicates which extreme of the flow-rate ratio is optimal.

The missing equation in the table is:

Table 5
Derivatives of optimization functions

f	$\frac{df}{dm_1}$	$\frac{df}{dm_2}$	$\frac{df}{dm_3}$	$\frac{df}{dm_4}$
DC	$\frac{1}{m_3 - m_2}$	$\frac{m_1 - m_4}{(m_3 - m_2)^2}$	$-\frac{m_1 - m_4}{(m_3 - m_2)^2}$	$-\frac{1}{m_3 - m_2}$
($c_D < c_{D,max}$)	Minimal m_1	Minimal m_2	Maximal m_3	Maximal m_4
DC	$-\frac{x}{xm_2 - ym_3}$	$\frac{x^2}{(xm_2 - ym_3)^2}$	$\frac{-xy}{(xm_2 - ym_3)^2}$	0
($c_D > c_{D,max}$)	Minimal m_1	Minimal m_2	Maximal m_3	–
SC	$\frac{c_I}{m_3 - m_2}$	$\frac{c_I(m_1 - m_4)}{(m_3 - m_2)^2}$	See main text	$\frac{-xm_2 - c_F m_4}{m_3(m_3 - m_2)}$
($c_D < c_{D,max}$)	Minimal m_1	Minimal m_2		Maximal m_4
SC	$-\frac{xc_D}{xm_2 - ym_3}$	$\frac{x^2 c_D}{(xm_2 - ym_3)^2}$	$\frac{-xyc_D}{(xm_2 - ym_3)^2}$	0
($c_D > c_{D,max}$)	Minimal m_1	Minimal m_2	Maximal m_3	–

Where $x = c_I - c_F$ and $y = c_D - c_F$.

$$\frac{dSC}{dm_3} = \frac{-m_1 c_I m_3^2 + m_4 c_F m_3^2 + 2m_2 m_3 m_4 x - m_4 x m_2^2}{(m_3 - m_2)^2 m_3^2}$$

This equation cannot be solved analytically.

From this table we conclude that it is generally favorable to use the minimal m_1 and m_2, and the maximal m_3 and m_4. However, this does not hold true in case of the salt consumption when the required desorbent concentration exceeds the maximal allowed one. In that case, a complete optimization of both m_1, m_2, m_3, and m_4 is necessary. Note that the equations in Table 5 are independent of the equilibrium constants and hence are applicable to any separation.

Appendix B. General applicability

In this appendix, it will be shown that the approach is not limited to the specific example used as illustration, but is general.

First, we will prove that the curves are of similar shape, independent of the isotherm parameters K_i and z_i. The optimal point is determined by at least one limit on m_3. Rewriting in terms of m_2 makes the equations explicit:

$$m_2 = m_3 \frac{\left(\frac{K_i}{m_3}\right)^{\frac{1}{z_i}} - c_F}{c_I - c_F} \tag{B.1}$$

The maximum or minimum of this equation is found by setting the derivative of m_2 to m_3 to zero:

$$\frac{dm_2}{dm_3} = \frac{\left(\frac{K_i}{m_3}\right)^{\frac{1}{z_i}} \cdot \left(1 - \frac{1}{z_i}\right) - c_F}{c_I - c_F} = 0$$

Irrespective of the values of K_i and z_i, there is only one solution to this equation, which is:

$$m_3 = K_i \cdot \left(\frac{1 - \frac{1}{z_i}}{c_F}\right)^{z_i}$$

It follows that each limit on m_3 only has one maximum when plotted as a function of m_3. When each of the two limits on m_3 has one maximum, the lines can intersect at two points at maximum, irrespective of the values of K_i and z_i. These points of intersection are found by equating the m_3 values.

Complete separation dictates that the SMB can only be operated at combinations of m_2 and m_3 that are inside the curve of the more retained component and outside the curve of the less retained component.

Hence, when there is one point of intersection of the limits on m_3, the shape of the region of complete separation is similar to the region described in the main text. The conditions for the optimal point listed in Table 3 can then be used: the approach is general. When there is no point of intersection, the optimal point is at X, although it should be kept in mind that part of the region of complete separation can be inaccessible. When there are two points of intersection, the region of complete separation is split in two. The optimal point is again at X, but a large part of the region of complete separation is not accessible.

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