ELSEVIER

Journal of Chromatography A, 830 (1999) 293–300

JOURNAL OF CHROMATOGRAPHY A

Optimal design of affinity membrane chromatographic columns

Armando Tejeda[a,*], Jaime Ortega[a], Ignacio Magaña[a], Roberto Guzmán[b]

[a]*Departamento de Biotecnología y Bioingeniería, CINVESTAV/IPN, Ave. IPN No. 2508, México, DF. 07360, México*

[b]*Chemical and Environmental Engineering Department, University of Arizona, Tucson, AZ 85721, USA*

Received 21 July 1998; received in revised form 8 October 1998; accepted 22 October 1998

Abstract

A method for the optimal affinity membrane column design, based in the solution of the Thomas kinetic model for frontal analysis in membrane column adsorption, is presented. The method permits to choose suitable membrane operating conditions, column dimensions and processing time, to maximize the throughput when an operating capacity restriction in the range of 80–95% of the column capacity is used. Two basic design charts were obtained by computer simulation, for residence and processing time calculation, respectively. These charts can be used and manipulated in a wide range of operational conditions, provided that four design specifications related to column axial and radial Peclet numbers, length and pressure drop, are fulfilled. The application of the method was illustrated using experimental data and a simple analytical procedure. The implications of the method and results on the design and optimization of affinity membrane chromatographic columns are discussed.

Keywords: Affinity membranes; Stationary phases, LC

1. Introduction

Recently, affinity membrane technology has evolved as an alternative to affinity column chromatography. It introduces a different approach to exploit the biospecific interactions between a protein and a ligand to economically purify proteins present at very low concentrations in complex solutions, using porous structures with flat-sheet and hollow fiber forms [1–4]. Affinity membranes help to overcome the limitations encountered in conventional commercial processes using chromatographic beads [5–8]. Affinity membranes operate in convective mode, which can significantly reduce diffusion and pressure drop limitations commonly encountered in column chromatography. As a result, adsorption, washing, elution, and regeneration steps are greatly improved and also the probability of deactivation of biomolecules is decreased by shortening their exposure to unfavorable media [9].

Axial and radial diffusion, sorption kinetics, and nonuniformities in membrane porosity and thickness have been shown to affect affinity membrane performance key factors such as, breakthrough curve (BTC) sharpness and residence time. Degradation of membrane performance can be minimized working with axial Peclet numbers greater than 40, and radial Peclet numbers smaller than 0.04. Stacking more than 30 membranes averages out membrane porosity and thickness nonuniformities [10]. Under these conditions, mass transfer resistances are minimized, and the membrane system performance can be

*Corresponding author: Especialidad en Biotecnologlía, Universidad de Sonora, Apdo. Postal 593, Hermosillo, Sonora 83000, México.

0021-9673/99/$ – see front matter
PII: S0021-9673(98)00898-X

predicted using the solution of the Thomas kinetic model for frontal chromatography [11]. Furthermore, if a design constraint on the curve shape is set, an optimal method of design can be obtained [12].

When adsorptive membranes were used to separate proteins from crude solutions in previous works, suspended solids, lipids and aggregated proteins plugged the membrane pores, or caused product loss due to polarization, concentration and rejection at particle layers [13]. To overcome these problems a new generation of large pore-size (10 to several hundred micrometers) membranes have been investigated. In these membrane systems, film mass transfer probably dominate the overall mass transfer resistance, in contrast to previous work, where these effects where not observed because the membrane pore size was too small [14]. Consequently, it is a very important design issue to determine the appropriate system and operating conditions, for a given affinity membrane application, where membrane pore size should balance mass transfer against plugging and polarization problems.

In this work, an affinity membrane column scheme is presented which can be used to determine the basis for a proper design, operation and application of these systems. The scheme permits to choose suitable membrane operating conditions, column dimensions and processing time, to maximize the throughput when an operating capacity restriction in the range of 80–95% of the column capacity is used.

The proposed method is simple, integral and general. It is based in design charts to calculate residence and processing time, takes into account the several hydrodynamic and kinetic factors that affect membrane performance, and can be applied to a wide range of operating and system conditions.

2. Materials and methods

2.1. Affinity membrane column model

Most affinity separations in chromatography are operated in the frontal analysis mode. To describe this behavior the model system for affinity membrane columns was used in the present work [10,12]. This model is based on the isothermal Langmuir sorption of a single solute during flow through a porous membrane (equivalent to an array of fine capillaries parallel with one other) with an average pore diameter of d_p, onto which a ligand is immobilized.

In the analysis, the feed protein concentration is c_o. The protein solution has a concentration $c(z,t)$ with a constant interstitial flow velocity v through a membrane column height L (membrane thickness L_m) and void porosity ε. The concentration of protein in the solid phase is given by $q(z,t)$. The adsorption kinetics is characterized by the association and dissociation rate constants k_1 and k_{-1}, respectively. The maximum adsorption capacity of the membrane is given by the parameter q_m. At equilibrium the dissociation equilibrium constant K_d is given by k_{-1}/k_1.

Optimal operating conditions can be reached working with selected Peclet numbers and appropriated stacked membrane columns, and system performance can then be predicted using the solution of the Thomas no-dispersive kinetic model for frontal chromatography [10–12,15–19].

In the present study the Thomas model was used to develop appropriate design charts through computer simulation. In this work, the design charts along with selected design constrains where used to determine parameters, and operating conditions required for optimum design performance of affinity membrane columns. The analysis is presented for column membrane operating capacity in the range of 80–95%.

2.2. Design constraint

During column operation in frontal mode, for short times solute in the feed is taken up almost completely by the column. After a while, solute breakthrough occurs and the effluent concentration increases with time. Much of the information needed to evaluate column performance is contained in these typical plots of effluent concentration versus time or breakthrough curve (Fig. 1). This curve can be used to determine (1) how much of the column capacity has been used, (2) how much solute is lost in the effluent, and (3) the processing time. This is precisely the performance information needed to optimize processing [20].

A low column capacity utilization causes a de-

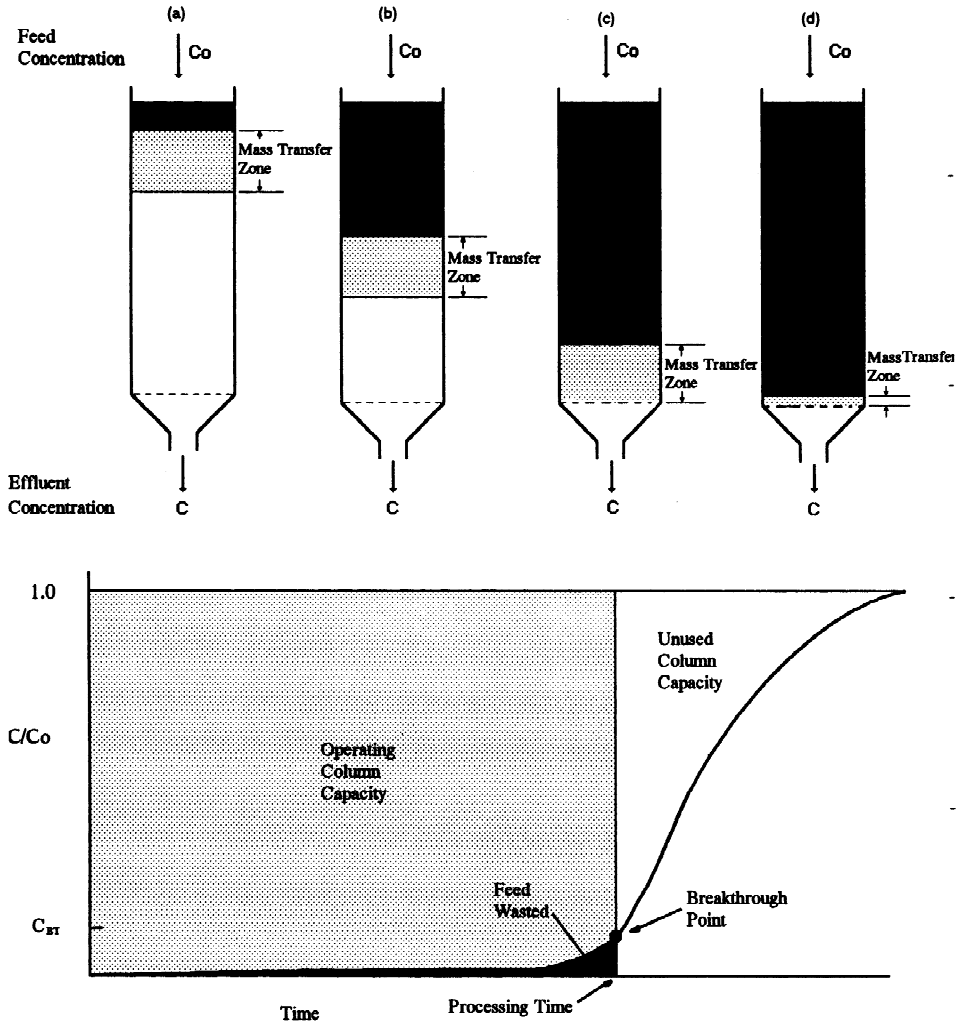

Fig. 1. Column loading process and breakthrough curve for membrane affinity system. For an adsorption step terminated at effluent concentration of breakthrough C_{BT}, a small amount of feed has been wasted, and a portion of the column capacity remains unused.

crease in ligand utilization, or a delay in the saturation time, or a waste of feed solution. Therefore, a high column capacity utilization is a target condition. In the present work, a design constraint was set in order to get an operating capacity of 80, 85, 90, 92.5 or 95% of the column capacity (the range of most interest), at the breakthrough concentration equal to 10% of the inlet concentration or $c/c_o=0.1$. Operating capacity is a parameter more oriented to design, operation and optimization problems in affinity chromatography.

When the Thomas model is used, the effluent concentration in the column depends on three dimensionless groups. The separation factor r, the dimensionless number of transfer units for the adsorption process n, and the throughput or dimensionless effluent volume Γ (a measure of the degree of column saturation). When a operating capacity design constraint is set, the exit concentration becomes a function of Γ and one of the other two variables only [12,21], due to the fact that only one relationship between n and r can meet the specified operating capacity. This relationship depends on the membrane porosity ε and on the ratio of the Langmuir equilib-

rium parameters, maximum binding capacity over the dissociation equilibrium constant q_m/K_d.

2.3. Design charts

Two design charts were obtained by computer simulation using the Thomas model and the design constraint, within the range of reported values [12] of 1–10 000 for the separation factor r and 10–20 000 for the parameter λ_ε given by,

$$\lambda_\varepsilon = \frac{q_m}{K_d}\frac{(1-\varepsilon)}{\varepsilon} \quad (1)$$

The number of transfer units design chart contains the relationships between n and r. For a given pair of r and λ_ε values, the number of transfer units to reach an operating capacity of 95% of the column capacity n_{95} is read from the ordinate of the chart. This value permits to find the number of transfer units n_D, for a design operating capacity Q_D in the range of 80–95% using a design correlation. The suitable residence time of a membrane system can be calculated using this n_D value along with the corresponding dimensionless equation. This residence time will produce a breakthrough curve with the specified operating capacity when the design specifications are met.

The processing time design chart permits to find the required processing time PT given r and the target operating capacity.

2.4. Design specifications

In order to apply the design method, the column membrane system should meet the following design specifications:

(1) Radial Peclet number Pe_r smaller than 0.04,

$$\frac{d_p^2 v}{4DL} < 0.04 \quad (2)$$

where D is the protein diffusion coefficient.

(2) A column consisting of a stack of at least 30 individual membranes,

$$\frac{L}{L_m} \geq 30 \quad (3)$$

(3) Axial Peclet number Pe_z greater then 40,

$$\frac{vL}{D} > 40 \quad (4)$$

(4) Pressure drop ΔP along the column smaller than the value specified by the membrane manufacturer (400 kPa is a typical value), as calculated with the Blake–Kozeny equation [12,22],

$$\frac{150vL\mu(1-\varepsilon)^2}{d_p^2\varepsilon^2} < 400 \text{ kPa} \quad (5)$$

2.5. Computer simulations

Computer programs were used to simulate breakthrough curves. All computer programs were written in Fortran 77 and run on a Pentium 586 PC.

3. Results and discussion

3.1. Design charts

Two design charts for affinity membrane column systems were obtained from the Thomas model and a specified design constraint. The number of transfer units design chart shown in Fig. 2 permits to find the number of transfer units for an operating capacity of 95%, n_{95}, for given values of r and λ_ε. Curves with three defined regions where obtained for λ_ε values greater than 200. For lower values of this parameter, n increases quickly with decreasing r.

Using the results of approximately 900 simulated runs within the studied range of the parameters r, λ_ε, and operating capacity Q_D, the following 98% R^2 correlation with a ± 0.2 variation within 95% confidence limits was developed,

$$n_D = \frac{n_{95}}{20.55 - 0.206(Q_D)} \quad (6)$$

This correlation permits to find the number of transfer units n_D for a design operating capacity Q_D in the range of 80–95%, using given values of n_{95}.

The second chart is the processing time design chart (Fig. 3). This chart permits to find the required processing time PT for given values of r and the design operating capacity. Processing time is in practice independent of the parameter λ_ε. The values of PT on the design chart represent average values of

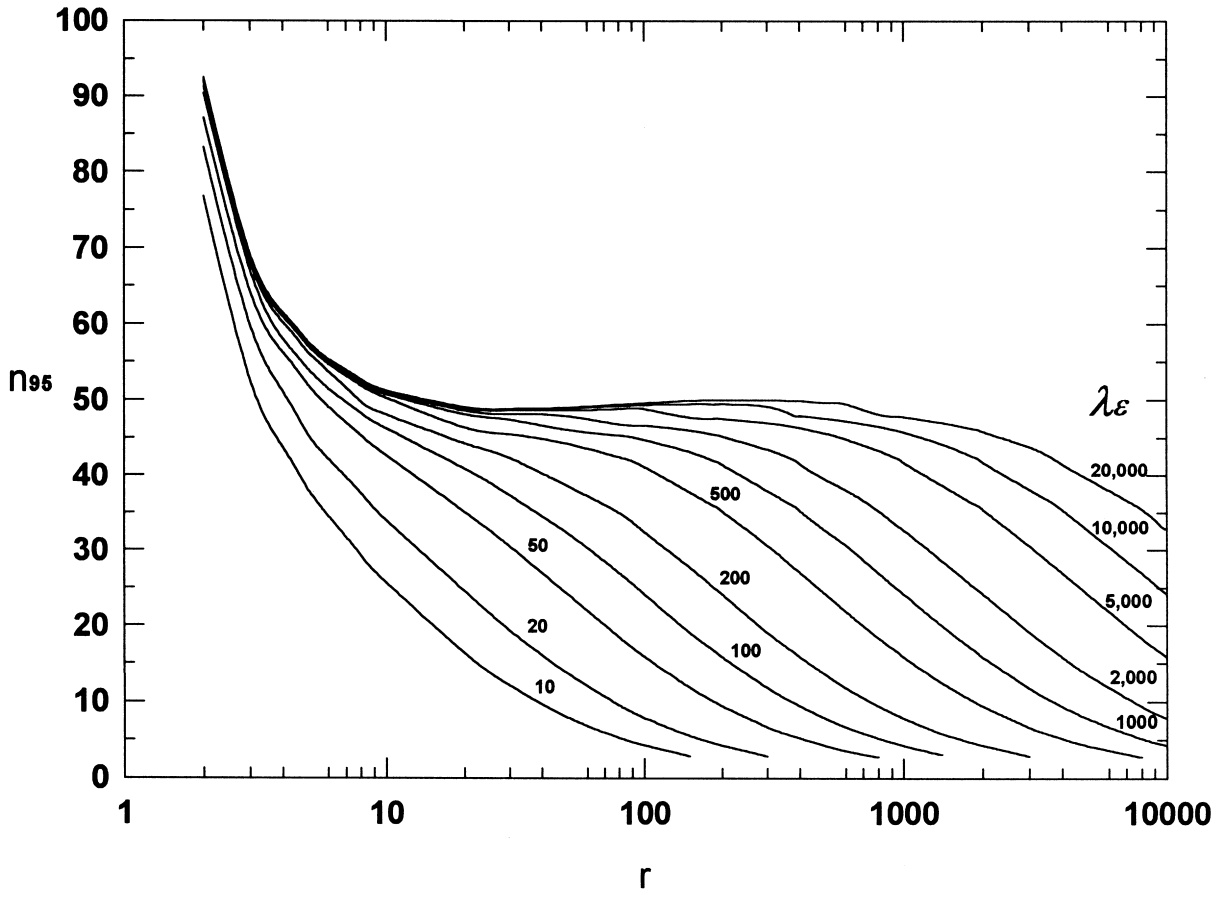

Fig. 2. Number of transfer units design chart calculated from the Thomas model and a constraint of 95% of the membrane column capacity as operating capacity at breakthrough. Using known r and λ_ε values, n_{95} is read from the ordinate and suitable operating conditions for the affinity membrane column can be obtained.

processing times from the simulated runs with a maximum variation of ±3%.

3.2. Design procedure

To illustrate the use of the proposed design methodology for chosing suitable membrane column operating conditions, column dimensions and processing time, experimental data from literature for human γ-globulin purification by adsorption onto an affinity nylon membrane containing immobilized protein A was used [23]. A design operation capacity of 93.5% was chosen. The procedure follows:

(1) Design values specification: the required membrane properties, equilibrium and kinetic parameters, and solution properties design values are shown in Table 1.

(2) Chart parameters calculation: the value of the separation factor r is calculated first using the corresponding dimensionless equation [12] and the design values,

$$r = 1 + \frac{c_o}{K_d} = 21 \quad (7)$$

according to Eq. (1) the value of the parameter λ_ε is 248.57.

(3) Estimation of n_{95} from the design chart (Fig. 2): the number of transfer units n_{95} is read from the ordinate of the chart using the above parameters,

$$n_{95} = 45.1 \quad (8)$$

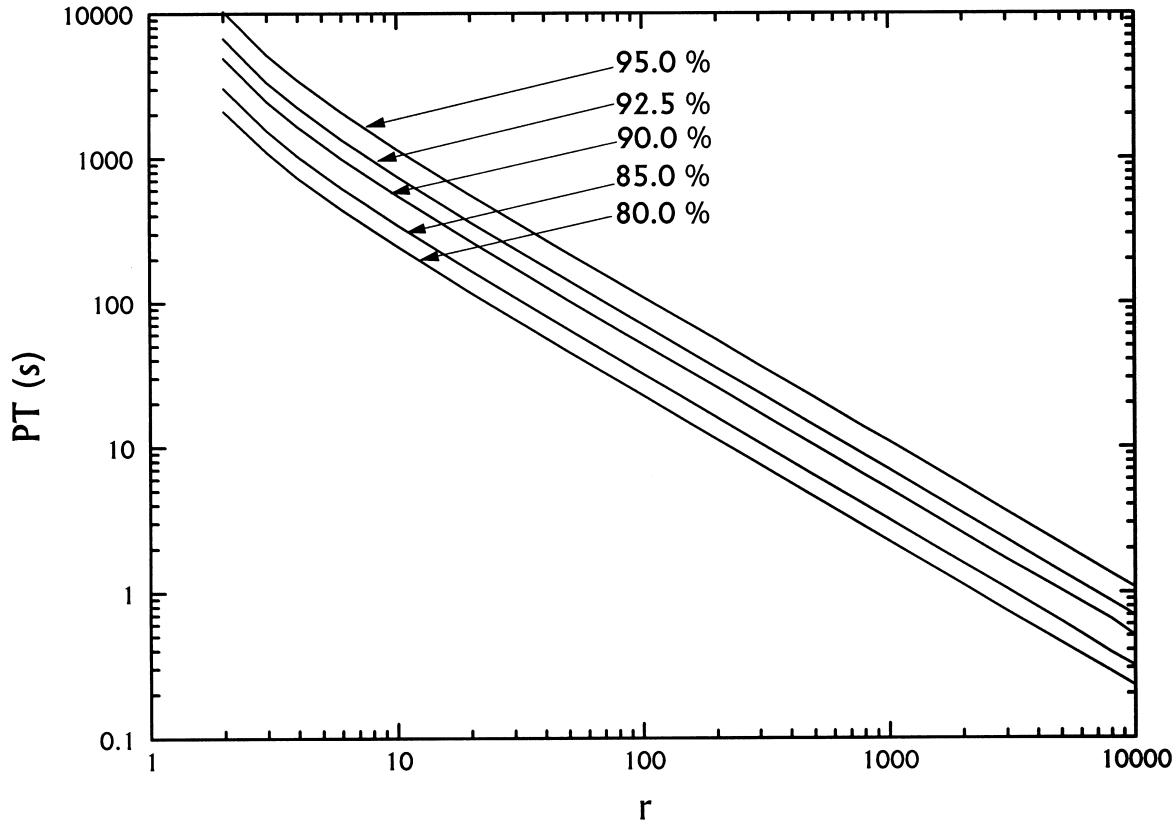

Fig. 3. Processing time design chart calculated from the Thomas model and a constraint of 80, 85, 90, 92.5 and 95% of the membrane column capacity as operating capacity at breakthrough, respectively. Using a known r value and the design operating capacity Q_D, the required processing time PT is read from the ordinate of the chart.

(4) Calculation of the number of transfer units for the design capacity $n_{93.5}$: using Eq. (6) and a design operation capacity of 93.5%, the number of transfer units obtained is,

$$n_{93.5} = \frac{45.1}{20.55 - 0.206 \times 93.5} = 35 \quad (9)$$

(5) Residence time calculation: using the corresponding dimensionless Eq. [12] and design values, the ratio of column height to interstitial flow velocity is,

$$\frac{L}{v} = \frac{\varepsilon n}{(1-\varepsilon) q_m k_1} = 33.2 \text{ s} \quad (10)$$

Accordingly, the above L/v value meets the 93.5% operating capacity restriction.

Table 1
Values of the parameters used in the design procedure

Membrane properties	Equilibrium parameters	Kinetic parameter	Solution properties
$\epsilon = 0.7$	$q_m = 1.933 \times 10^{-5}$ M	$k_1 = 1.27 \times 10^5$ M^{-1} s^{-1}	$c_o = 6.67 \times 10^{-7}$ M
$L_m = 160$ μm	$K_d = 3.33 \times 10^{-8}$ M		$\mu = 0.01$ g cm^{-1} s^{-1}
$\Delta P = 400$ kPa			$D = 4.2 \times 10^{-7}$ cm^2 s^{-1}
$d_p = 3$ μm			

(6) Radial Peclet specification check: using Eq. (2) it is possible to verify if the L/v value can meet the radial Peclet specification. For instance, the calculated value for this particular case is 0.0016, smaller than 0.04.

The residence time in the membrane pores is high enough to prevent radial diffusion limitations. Otherwise, the design method could not be applied, except if a smaller pore membrane is used.

The maximum membrane pore size for the system can be determined from Eq. (2) as well,

$$d_p = \sqrt{\frac{0.04 \times 4DL}{v}} = 15\ \mu m \quad (11)$$

(7) Membrane column length calculation: the membrane column length can be calculated choosing a suitable number of individual membranes for the membrane column module. If 30 individual membranes compose the column, this value can be calculated as,

$$L = 30L_m = 0.48\ cm \quad (12)$$

(8) Interstitial flow velocity calculation: the interstitial flow velocity can be calculated by dividing the column length by the residence time, in this particular case as,

$$v = 0.0144\ cm/s \quad (13)$$

(9) Axial Peclet specification check: using Eq. (4) the values of L and v can be used to verify axial Peclet specification. In this case since the axial Preclet value is calculated as 16 457, it in fact complies with this specification.

The residence time in a membrane pore is low enough to prevent axial diffusion limitations. Otherwise, the design method could not be applied, except if a longer column is used.

(10) Pressure drop specification check: similarly, Eq. (5) and the calculated values of L and v can be used to check the pressure drop specification. The corresponding calculated value is 0.7 kPa. This pressure drop is a permissible operating condition according to the specified membrane properties. Otherwise, the design method could not be applied, except if a wider pore membrane is used.

(11) Flow area or flow-rate determination: the required flow area for a given volumetric flow-rate, or the manageable flow-rate for a given membrane area, can be calculated using the continuity equation. For a 2.5-cm diameter membrane, the manageable flow-rate F is given in this case by:

$$F = \varepsilon v A = 3\ ml/min \quad (14)$$

where A is the membrane column cross-sectional area.

(12) Processing time determination: the processing time PT can be obtained from Fig. 3. For the values of $r = 21$ and $Q_D = 93.5\%$, this parameter is determined as,

$$PT = 400\ s \quad (15)$$

4. Conclusions

A systematic approach for the optimal affinity membrane column design is presented in this work. The approach is based on the solution of the Thomas model for frontal analysis in membrane column adsorption. The analysis permits to find the operating conditions, column dimensions and processing time for an operating capacity restriction in the range of 80–95% of the column capacity.

The solution of the Thomas model under the given design constraint range was developed in a graphic mode for a wide range of operational conditions. In order to properly use this analysis, four design restrictions must be satisfied. The application of this systematic approach was illustrated using experimental data from the literature and a simple procedure. The results successfully show that the approach could be used to help develop the basis for proper design, operation and application of affinity membrane columns in protein separation processes.

5. List of Symbols

A	membrane column cross-sectional area, cm^2
c	solute concentration in the bulk phase, M
c_o	solute concentration in the bulk phase at column inlet, M
d_p	average pore diameter, cm
D	diffusion coefficient, $cm^2\ s^{-1}$

F	volumetric flow-rate, ml min^{-1}
k_1	association rate constant, M^{-1} s^{-1}
k_{-1}	dissociation rate constant, s^{-1}
K_d	dissociation equilibrium constant, M
L	length of membrane column, cm
L_m	membrane thickness, cm
n	dimensionless number of transfer units for overall process
Pe_r	radial Peclet number
Pe_z	axial Peclet number
q	average protein concentration, M
q_m	maximum binding capacity of the membrane, and M based on the solid volume
r	dimensionless separation factor
t	time, s

5.1. Greek letters

ε	void fraction of the membrane column
Γ	dimensionless effluent volume
v	linear velocity, cm s^{-1}

Acknowledgements

This work was supported in part by a grant from Universidad de Sonora–University of Arizona international cooperation agreement. R.G. acknowledges support of National Science Foundation to work in this area of research under grants BCS-9309326 and BES-9415683.

References

[1] X. Zeng, E. Ruckenstein, Ind. Eng. Chem. Res. 37 (1998) 159.
[2] N. Kubota, Y. Konno, K. Saito, K. Sugita, K. Watanabe, T. Sugo, J. Chromatogr. A 782 (1997) 159.
[3] J.E. Kochan, Y. Wu, M.R. Etzel, Ind. Eng. Chem. Res. 35 (1996) 1150.
[4] A. Shiosaki, M. Goto, T. Hirose, J. Chromatogr. A 679 (1994) 1.
[5] E. Klein, Affinity Membranes. Their Chemistry and Performance in Adsorptive Separation Process, John Wiley and Sons, New York, 1991, p. 128.
[6] D.K. Roper, E.N. Lightfoot, J. Chromatogr. A 702 (1995) 3.
[7] J. Thömes, M.R. Kula, Biotechnol. Prog. 11 (1995) 357.
[8] S. Brandt, R.A. Goffe, S.B. Kessler, J.L. O'Connors, S.E. Zale, Bio/Technology 6 (1988) 779.
[9] X. Zeng, E. Ruckestein, Ind. Eng. Chem. Res. 35 (1996) 4169.
[10] H. Liu, J.R. Fried, AIChE J. 40 (1994) 40.
[11] H.C. Thomas, J. Am. Chem. Soc. 66 (1944) 1664.
[12] S. Suen, M.R. Etzel, Chem. Eng. Sci. 47 (1992) 1355.
[13] M. Weissenborn, B. Hutter, M. Singh, T.C. Beeskow, F.B. Anspach, Biotechnol. Appl. Biochem. 25 (1997) 159.
[14] F.T. Sarfert, M.R. Etzel, J. Chromatogr. A 764 (1997) 3.
[15] H.A. Chase, J. Chromatogr. 297 (1984) 179.
[16] B.J. Horstmann, H.A. Chase, Chem. Eng. Res. Des. 67 (1989) 243.
[17] F.H. Arnold, H.W. Blanch, J. Chromatogr. 355 (1986) 13.
[18] P.M. Boyer, J.T. Hsu, Chem. Eng. Sci. 47 (1992) 241.
[19] N.K. Hiester, T. Vermeulen, Chem. Eng. Prog. 48 (1952) 505.
[20] F.H. Arnold, H.W. Blanch, C.R. Wilke, Chem. Eng. J. 30 (1985) B9.
[21] A. Tejeda-Mansir, J.M. Juvera, I. Magaña, R. Guzmán, Bioprocess Eng. (1998) in press.
[22] R.B. Bird, W.E. Stewart, E.N. Lightfoot, Transport Phenomena, John Wiley and Sons, New York, 1960, ch. 6.
[23] M. Unarska, P.A. Davies, M.P. Esnouf, B.J. Bellhouse, J. Chromatogr. 519 (1990) 53.