CONCENTRATION OF α-LACTALBUMIN FROM COW MILK WHEY THROUGH EXPANDED BED ADSORPTION USING A HYDROPHOBIC RESIN

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Abstract - The design of novel applications for the use of new high-density adsorbents in the direct recovery of proteins requires analysis of the fluid dynamics and mass transfer characteristics of the column used with the solid-liquid system. In this article we describe the operating parameters related to the use of a high-density, hydrophobic resin (Streamline® Phenyl) for the recovery of α-lactalbumin from cow milk whey in an expanded-bed adsorption mode of operation. The adsorption isotherm, kinetic curves and chromatographic curves for adsorption and elution were determined for α-lactalbumin recovered from whole whey. The experiments were used to confirm the theoretical model that involves the combination of resistance to the mass transfer in the liquid phase and diffusivity in the adsorbent resin pores. The model solution was obtained through the orthogonal collocation method. A nonlinear multivariable optimization method was used to fit the model to experimental results. A purity of 79% was obtained with the adsorption/elution protocol prior to any polishing procedure.
Keywords: α-Lactalbumin; Milk whey; Expanded bed adsorption; Fluid Dynamics.

INTRODUCTION

Opportunities for the commercial extraction of bioproducts from food waste streams are increasing. Future initiatives to improve the recovery of by-products require additional research to investigate possible uses for valuable waste stream components and to develop cost-effective techniques for their recovery (Beszedits and Netzer, 1982). One example of a potential field for bioproduct recovery is the extraction of proteins from milk whey using chromatographic techniques. A typical composition of cheese whey is shown in Table 1. Most of the purified proteins derived from milk products, such as whey, have important applications in clinical and veterinary medicine, functional food products and cell culture media (Horton, 1995).

In this work we examined the use of a high-density, hydrophobic resin (Streamline® Phenyl) for the recovery of α-lactalbumin (α-LA) from cow milk whey in an expanded-bed adsorption mode of operation. Noppe et al. (1999) report a protocol for the fast and efficient purification of α-LA using defatted cow milk and the same resin as that used here, but with EDTA and an elution step involving a Ca^{2+}-containing buffer. The fundamental aspect of this approach relies on the binding capacity of α-lactalbumins for Ca^{2+} ions. These proteins, which without Ca^{2+} have hydrophobic characteristics, undergo a significant conformational change with

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Ca^{2+} and other metal ions that makes them more hydrophilic. By using Tris-EDTA buffer in the adsorption step the Ca^{2+} ions are removed, making the protein more hydrophobic and increasing its binding to hydrophobic adsorbents. The elution step using Ca^{2+} ions permits recovery of α-lactalbumin because of the reversible change in the hydrophilic character. Although expanded-bed chromatography offers the advantage of reducing the number of steps by saving recovery steps in this application, there is still a need for process engineering data to make the scale-up more reliable. We therefore also examined some important process tools such as adsorption isotherms, adsorption kinetic curves and chromatographic curves for α-LA recovered from whole whey.

Table 1: The main protein components of cheese whey from bovine milk

Protein	Concentration (g/l)	Molecular weight (kDa)	Isoelectric point
β-lactoglobulin	3-4	18.4	5.2
α-lactalbumin	1.5	14.2	4.7 - 5.1
BSA	0.3 - 0.6	69	4.9
IgG, IgA, IgM	0.6 - 0.9	150 - 900	5.8 - 7.3
Lactoperoxidase	0.06	78	9.6
Lactoferrin	0.5	78	8.0

MATERIAL AND METHODS

Milk Serum

Casein partially precipitated bovine milk serum was used in all of the adsorption experiments.

Adsorbent

The expanded bed adsorbent used in these experiments was a STREAMLINE® Phenyl obtained from Amersham Pharmacia Biotech (Sweden). This resin has phenyl groups coupled to the STREAMLINE matrix via uncharged, chemically stable ether linkages, which results in a hydrophobic medium with minimal ionic properties. The average density of the adsorbent was 1.2 $g.ml^{-1}$. The particle size ranged from 100 to 300 μm with an average size of 182 μm measured by the laser light-scattering method using a Mastersizer S detector (Malvern Instruments Ltd.). The mean particle porosity (ε_p) was 0.65, measured by the moment method (Arnold et al., 1985).

Column

The column used was a STREAMLINE 25, with an internal diameter of 25 mm and a total height of 1 meter (Figure 1). Various settled bed heights (H_0) (10.5 cm, 15 cm, 21 cm, 25 cm and 30 cm) were used to study the fluid dynamics and a bed height of 10 cm was used to determine the breakthrough curves.

Buffer System

Equilibrium buffer: 50 mM Tris - 1.5 mM EDTA.
Adsorption buffer: 50 mM Tris - 35 mM EDTA.
Wash buffer: 50 mM Tris - 1.5 mM EDTA.
Elution buffer: 50 mM Tris - 1.5 mM $CaCl_2$.
The pH of all solution buffers was 7.5.

Fluidized Bed Studies

Richardson and Zaki (1954) showed that there was a correlation between bed height and superficial velocity (u) for various settled bed heights. The Richardson and Zaki parameters of Equation 1, n and U_t (terminal settling velocity), were determined from the regression line of a logarithmic plot of porosity versus superficial velocity. All experiments were done using the equilibrium buffer.

$$\frac{U}{U_t} = \varepsilon^n \tag{1}$$

Residence Time Distribution

Residence time distribution was measured to determine the axial dispersion in the liquid phase as a function of bed expansion starting with fixed bed heights of 10 cm and 20 cm. Linear velocity was varied from 3.24 to 4.86 cm/min using the equilibrium buffer. A pulse of the 5 mL tracer fluid (20% aqueous acetone solution) was applied to the column, and the UV absorbance (280 nm) was monitored continuously with a UV spectrophotometer.

Equilibrium and Adsorption Kinetics

Dilutions of casein partially precipitated bovine milk serum containing different concentrations of protein were incubated in a shaking water bath at room temperature (Figure 2). The adsorbent used in these experiments was STREAMLINE® Phenyl. The total protein and α-lactalbumin concentrations in the supernatant were monitored at various time intervals based on absorbance at 280 nm and by HPLC analysis using a Mono Q ionic-exchange column calibrated with α-lactalbumin (85% purity, purchased from SIGMA).
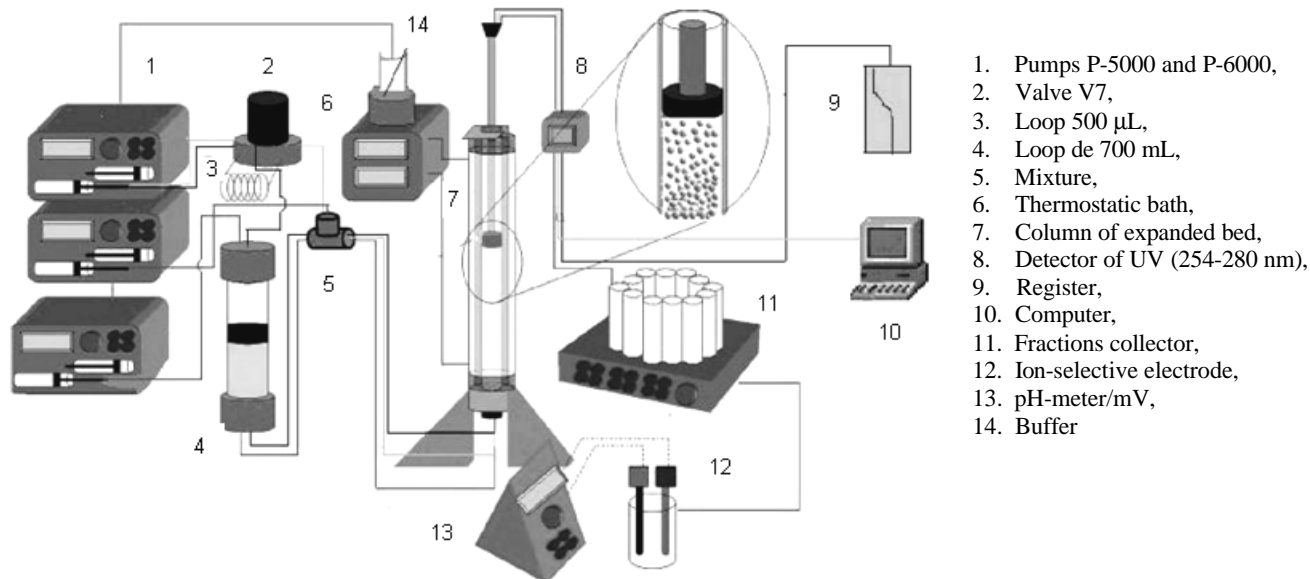
Figure 1: Experimental set-up used for the expanded-bed column experiments.
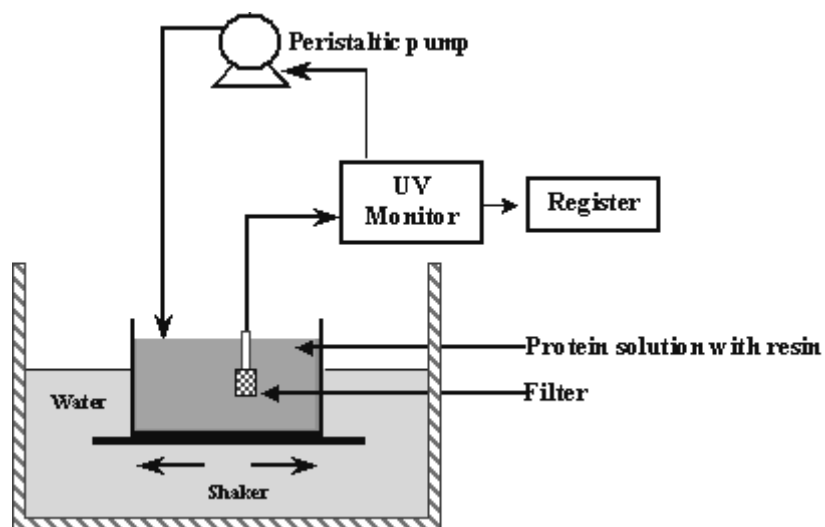
1. Pumps P-5000 and P-6000,
2. Valve V7,
3. Loop 500 μL,
4. Loop de 700 mL,
5. Mixture,
6. Thermostatic bath,
7. Column of expanded bed,
8. Detector of UV (254-280 nm),
9. Register,
10. Computer,
11. Fractions collector,
12. Ion-selective electrode,
13. pH-meter/mV,
14. Buffer

Figure 2: Experimental set-up for batch experiments

Chromatography

The chromatographic experiments were done in the same column (Figure 1) as that used for the expanded-bed experiments (STREAMLINE SP 25 column), coupled to a P-5000 Amersham Pharmacia pump with a UV-monitoring absorbance cell mounted at the column exit. Milk serum was introduced into the column from the bottom using a superficial velocity of 21 cm/min, which caused the 10 cm high fixed bed to expand 2.5-fold.

The expanded-bed adsorption step was done using the frontal mode of operation and was followed by the application of the remaining solution. The expanded bed was washed until the absorbance at 280 nm had returned to the baseline. The elution step was done after the volume in the bed had settled. Samples of the eluted peak were analyzed by SDS-PAGE electrophoresis and HPLC. The HPLC column peaks were calibrated with α-lactalbumin, β-lactoglobulin and casein (all from Sigma).

Mathematical Formulation

A mathematical model that describes protein adsorption on macroporous solids was presented by Horstmann and Chase (1989) and Carrére (1993) and adopted in the present work. It includes the mass transfer in the liquid film, the diffusion in the particle pores (adsorbent resin) and the surface adsorption rate.

The mathematical formulation is the following:

▪ **Mass Balance for a Solid Particle Corresponding to the Differential Equation that Describes the Solute (Protein) Diffusion in the Particle Pores (Adsorbent Resin):**

$$e_p \frac{\partial C_i}{\partial t} = e_p D_{ef} \left[\frac{\partial^2 C_i}{\partial r^2} + \frac{2}{r} \frac{\partial C_i}{\partial r} \right] - \frac{\partial q_i}{\partial t} \qquad (2)$$

where ε_p is the particle porosity, C_i is the protein concentration in the liquid phase inside the particle pores, q_i is the protein concentration in the solid phase, D_{ef} is the effective diffusion coefficient and t and r are the temporal and spatial (radial) variables, respectively.

The initial and the boundary conditions are the following:

$$t = 0 \qquad C_i = 0 \qquad \forall\, r \qquad (3)$$

In the particle center:

$$r = 0 \qquad \frac{\partial C_i}{\partial r} = 0 \qquad (4)$$

On the surface:

$$r = R \qquad \varepsilon_p D_{ef} \frac{\partial C_i}{\partial r} = k_f \, (C_b - C_i = |_{r=R}) \qquad (5)$$

where R is the particle radius and k_f is the external mass transfer coefficient.

According to Equation 5, the rate of mass transfer through the liquid film is related to the overall concentration of protein in the liquid phase C_b and to the concentration of protein in the liquid phase of the particle pores C_i with a radius of R.

▪ **Adsorption Kinetics on the Pore Surface:**

$$\frac{\partial q_i}{\partial t} = K_1 C_i (q_m - q_i) - K_2 q_i \qquad (6)$$

where k_1 and k_2 are the adsorption and desorption kinetics constants, respectively; q_m is the maximum binding capacity of adsorbent and q_i is the quantity adsorbed in a time interval t. Assuming a very fast adsorption rate on the surface, the Langmuir equation at equilibrium ($\partial q_i / \partial t. = 0$) is:

$$q_i = \frac{q_m C_i}{k_d + C_i} \qquad (7)$$

where $k_d = (k_2 / k_1)$.

With this simplification Eq.(2) becomes

$$\varepsilon_p \frac{\partial C_i}{\partial t} = \varepsilon_p D_{ef} \left[\frac{\partial^2 C_i}{\partial r^2} + \frac{2}{r} \frac{\partial C_i}{\partial r} \right] -$$
$$- \frac{\partial C_i}{\partial t} \frac{q_{max} K_d}{(K_d + C_i)^2} \qquad (8)$$

▪ **Mass Balance in the Bulk Liquid Phase with Overall Protein Concentration**

$$\frac{dC_b}{dt} = -\frac{3 v k_f}{R V_1} (C_b - C_i)|_{r=R} \qquad (9)$$

where v is the adsorbent volume and V_1 is the liquid-phase volume.

The initial conditions of Equation (9) are

$$t = 0 \qquad C_b = C_0 \qquad (10)$$

where C_o is the initial liquid-phase protein (BSA) concentration.

Defining the following dimensionless variables:

$$x = \frac{r}{R} \qquad T_i = \frac{C_i}{C_0} \quad T_b = \frac{C_b}{C_0}$$

$$t = \frac{t D_{ef}}{R^2} \quad B_{im} = \frac{k_f R}{e_p D_{ef}} \qquad (11)$$

The set of former equations implies the following results:

▪ **Mass balance on a solid particle:**

$$\left[1 + \frac{q_m k_d}{e_p (k_d + C_0 \Theta_i)^2} \right] \frac{\partial \Theta_i}{\partial \tau} = \left(\frac{\partial^2 \Theta_i}{\partial x^2} + \frac{2}{x} \frac{\partial \Theta_i}{\partial x} \right) \quad (12)$$

$$\tau = 0 \qquad \Theta_i = 0 \forall x \qquad\qquad (13)$$

$$x = 0 \qquad \frac{\partial \Theta_i}{\partial x} = 0 \qquad\qquad (14)$$

$$x = 1 \qquad \frac{\partial \Theta_i}{\partial \tau} = \frac{B_{im}}{\varepsilon_i}\left(\Theta_b - \Theta_i\right)\big|_{x=1} \qquad (15)$$

- Mass balance in the bulk liquid phase:

$$\frac{d\Theta_b}{d\tau} = -\frac{3v}{V1} B_{im}\left(\Theta_b - \Theta_i\big|_{x=1}\right) \qquad (16)$$

$$t = 0 \qquad\qquad \Theta_b = 1 \qquad\qquad (17)$$

When the variable transformation $u = x^2$ is applied to Equations (12) and (15) and the spatial variable is discretized through the orthogonal collocation method (Finlayson, 1980; Villadsen and Michelsen, 1978; Yao C. and Tien, 1992), we have

$$\left[1 + \frac{q_m k_d}{\varepsilon_p (k_d + C_0 \Theta_{ij})^2}\right]\frac{d\Theta_{ij}}{d\tau} = $$

$$(18)$$

$$= 4u_j \sum_{k=1}^{N+1} B_{jk}\Theta_{ik} + 6 \sum_{k=1}^{N+1} A_{jk}\Theta_{ik}$$

$$(J = 1,\ldots,N)$$

$$t = 0 \qquad Q_{ik} = 0 \qquad\qquad k = 1,\ldots, N+1 \qquad (19)$$

$$x = 1 \qquad \sum_{k=1}^{N+1} A_{N+1\cdot k}\,\Theta_{ik} = \frac{B_{im}}{2}\left(\Theta_b - \Theta_{iN+1}\big|_{x=1}\right) \quad (20)$$

where A and B are the matrices of orthogonal collocation.

There are N collocation points in the $0 < u < 1$ range, and one boundary point $u_{N+1} = 1$. The point u = 0 is not included because the symmetry condition requires the first derivative to be zero at u = 0 and this condition is built into the trial function.

The ordinary differential Equations (16) and (18) together with the respective initial conditions (Equations (l7) and (19)) and the boundary conditions (Equations (20) and (16)) form an ordinary differential equation system that can be integrated by known methods, such as the fourth-order Runge-Kutta method (Finlayson, 1980; Yao and Tien, 1992).

RESULTS AND DISCUSSION

Study of Bed Fluid Dynamics

Figure 3 shows that bed height depended on superficial velocity (U) and calculated bed porosity (ε). Richardson and Zaki's (1954) correlation parameters, n and U_t (terminal settling velocity), were determined by linear regression from a logarithmic plot of porosity versus superficial velocity (Table 2). The values for these parameters and the particle terminal velocity agreed with Richardson and Zaki's correlation and with the Stokes equation for low Reynolds number particle-fluid dynamics.
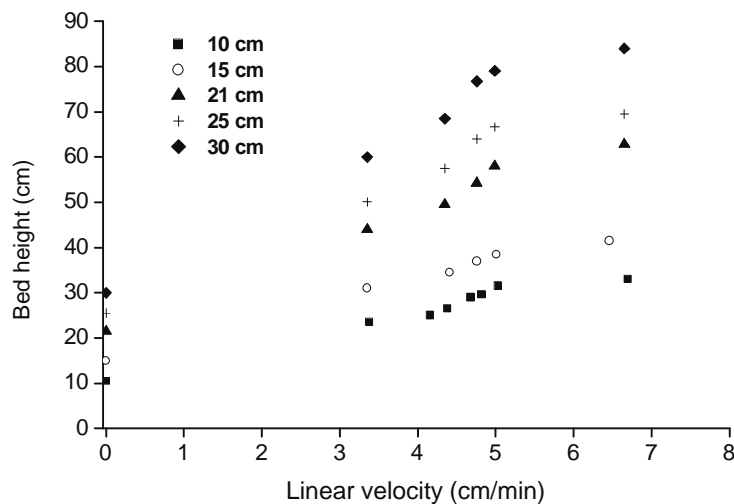

Figure 3: Expansion characteristics of the resin bed

Table 2: Numerical values for the bed expansion parameters

Bed height (cm)	Experimental Richardson & Zaki coefficient (n) (cm/min)	Experimental settling velocity (U_t) (cm/min)	Settling velocity calculated with the Stokes equation (cm/min)
10.0	4.73	17.50	
15.0	4.70	21.00	
21.5	4.78	19.97	21.64
25.5	4.54	19.67	
30.0	4.51	18.95	

Adsorption Equilibrium and Kinetics

The equilibrium isotherm for the total proteins and α-lactalbumin in milk serum are shown in Figures 4 and 5, respectively. A Langmuir-type isotherm was obtained for the total proteins and for the α-lactalbumin, which was adsorbed with other proteins. The data fitted Equation (7) and the parameters k_d and q_m (dissociation constant and maximum capacity of adsorption) are shown in Table 3.

The adsorption kinetics data were used together with the Horstmann and Chase mathematical model to assess the mass transfer parameters in order to scale up the adsorption process. The values of mass transfer resistance parameters D_p and k_f are 4.87×10^{-9} m²/s and 6.29×10^{-3} m/s (Figure 6), which describe the adsorption phenomena in the resin particles relatively well.
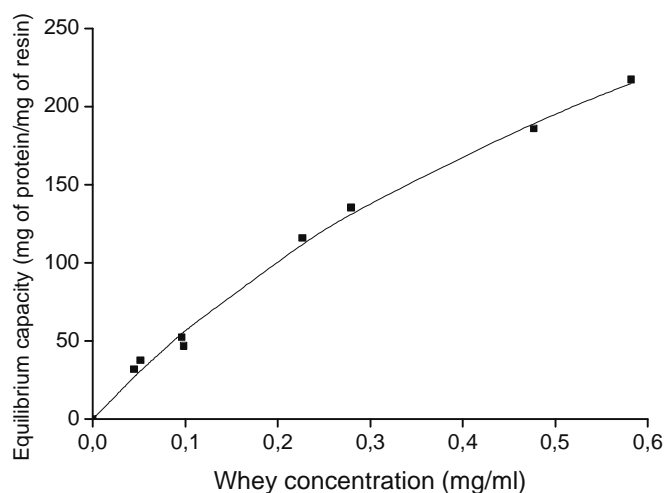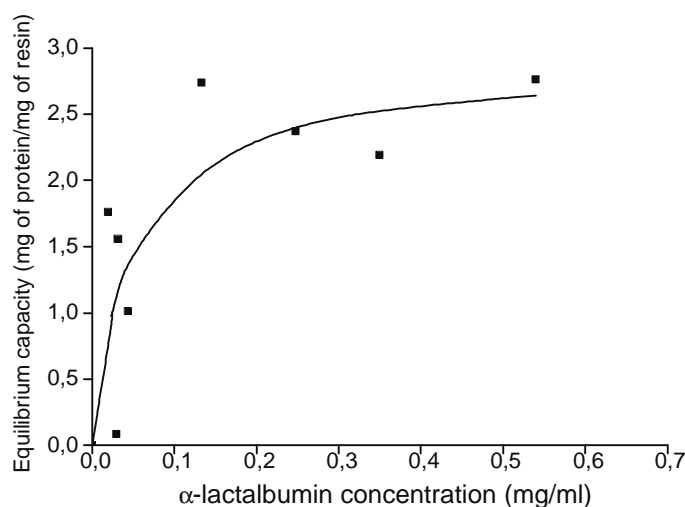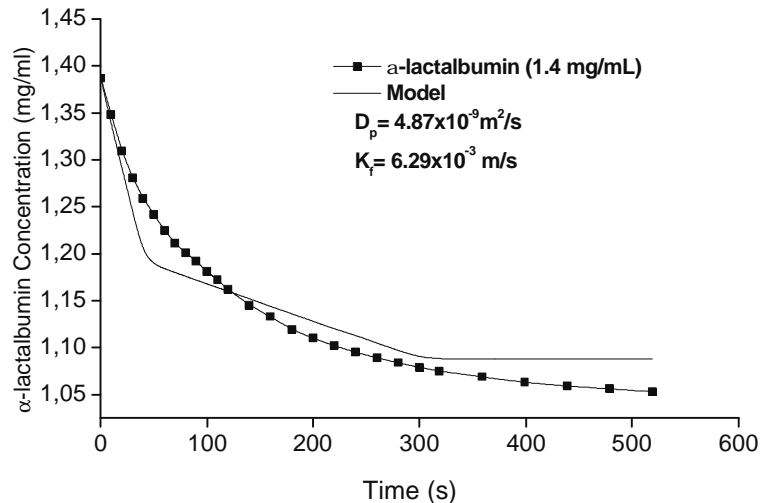

Figure 4: Equilibrium isotherm for total proteins in the whole milk whey

Figure 5: Equilibrium isotherm for α-lactalbumin

Table 3: Equilibrium parameters for the adsorption of a-lactalbumin and whole whey onto Streamline Phenyl resin

	Qm (mg of protein/g of resin)	k_d (mg of protein/g de resin)
α-lactalbumin	19.28	0.61
Whole Bovine whey	508.0	0.79

Figure 6: Adsorption kinetics for α-lactalbumin and the adjusted model

Chromatography

The breakthrough curves for fixed and expanded beds showed a similar behavior (Figure 7). SDS/PAGE 15% polyacrylamide gels were used to identify the proteins in several steps of the process (Figure 8). Lane 1 in Figure 6 shows the molecular mass calibration proteins and lane 3 shows the proteins that were eluted from the resin with the elution buffer. Some α-LA (14 kDa), β-lactoglobulin (18 kDa) and casein (30 kDa) were found in this pool. Lane 4, which is representative of the eluted fraction, contained mainly α-LA together with some traces of β-lactoglobulin and casein. The eluted fraction was analyzed by HPLC in order to determine the α-lactalbumin concentration. The purity of α-lactalbumin after one cycle of adsorption/elution protocol was 79 % with no previous polishing procedure.
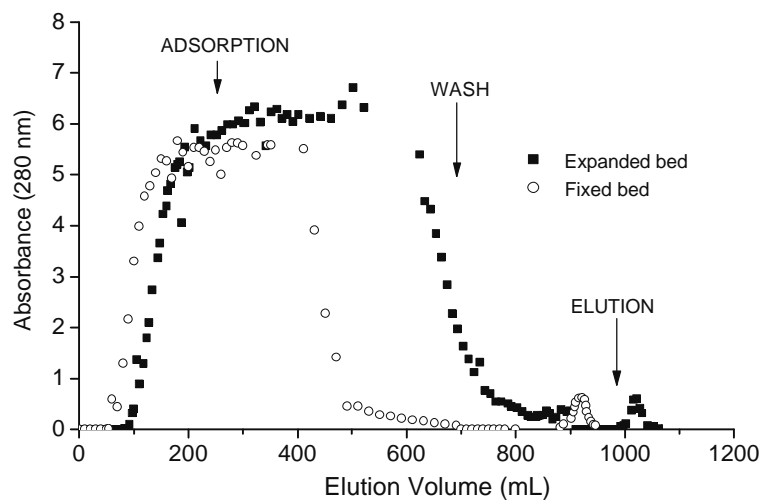
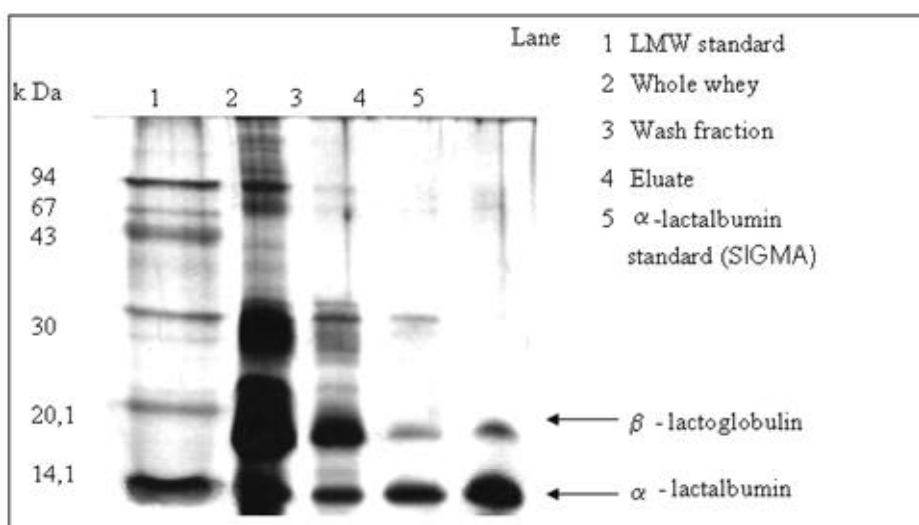**Figure 7:** Breakthrough and elution curves for proteins in milk whey

Figure 8: SDS/PAGE of proteins fractions

CONCLUSIONS

A procedure for the concentration of α-lactalbumin from cow milk whey was developed using a high-density, hydrophobic resin and an expanded-bed column. The expansion characteristics as well as the equilibrium and adsorption kinetic data were determined for future scale-up of the process. The mathematical formulation and the numerical method describe fairly well the adsorption kinetics for α-lactalbumin, but need to be improved. The experimental breakthrough and elution curves and HPLC analysis of the eluate showed that a purity of 79% was obtained for α-lactalbumin after one cycle of the adsorption/elution protocol, prior to any polishing procedure.

ACKNOWLEDGMENTS

NOMENCLATURE

A	matrix of orthogonal collocation	(-)
B	matrix of orthogonal collocation	(-)
B_{im}	Biot number	(-)
C^*	equilibrium concentration,	$\cdot 10^{-3}$ g cm^{-3}
C_b	liquid-phase protein concentration,	$\cdot 10^{-3}$ g cm^{-3}
C_i	particle pore protein concentration,	$\cdot 10^{-3}$ g cm^{-3}
C_o	initial bulk liquid-phase protein concentration,	$\cdot 10^{-3}$ g cm^{-3}
D_{ef}	effective diffusion coefficient,	m^2 min^{-1}
H_o	initial height of the bed	
k_1	adsorption kinetic constant,	cm^{-3}g^{-3} min^{-1}
k_2	desorption kinetic constant,	min^{-1}
k_d	dissociation constant,	$\cdot 10^{-3}$ g cm^{-3}
k_f	external mass-transfer convective coefficient,	m min^{-1}
N	number of orthogonal collocation points	(-)
n	Richardson and Zaki´s parameter	(-)
q^*	equilibrium concentration,	$\cdot 10^{-3}$ g cm^{-3}
q_i	solid-phase protein concentration,	$\cdot 10^{-3}$ g cm^{-3}
q_m	maximal capacity of the solid phase,	$\cdot 10^{-3}$ g cm^{-3}
R	particle radius,	m
r	spatial(radial) variable,	m
R^2	determination coefficient	
t	temporal(time) variable,	min
U	superficial velocity	
u	dimensionless spatial variable	(-)

U_t	terminal setting velocity	(-)
v	solid-phase volume,	cm^3
V_i	liquid-phase volume,	cm^3
x	dimensionless spatial variable	(-)

Greek Letters

Γ^*	equilibrium concentration,	$\cdot 10^{-3}$ g g^{-1}
Γ_m	maximal capacity of the solid phase,	$\cdot 10^{-3}$ g g^{-1}
ε	bed porosity	(-)
ε_p	particle porosity	(-)
τ	dimensionless temporal variable	(-)
Θ	dimensionless concentration	(-)

Indexes

B	index, refers to bulk liquid phase	(-)
i	index, refers to pore liquid phase	(-)
j	index, refers to orthogonal collocation point	(j = 1,…,N)
k	index, refers to orthogonal collocation point	(k =1,…,N+1)

REFERENCES

Arnold, F.H., Blanch, H.W. and Wilke, C.R., Liquid-Chromatography Plate Height Equations, Journal of Chromatography, 330 (1), pp. 159-166 (1985).

Beszedits, S. and Netzer, A., Protein Recovery from food processing wastewaters, B&L Informations Services, Toronto, Ontario, pp. 10-43 (1982).

Carrére, H., Milk Whey Proteins Extraction through Ion Exchange Chromatography in Fluidized Beds: Modeling and Optimization, Ph.D. diss., Institut National Polytechnique de Toulouse, Toulouse, (1993) (in French).

Finlayson, B.A., Nonlinear Analysis in Chemical Engineering, McGraw-Hill, New York (1980).

Horstmann, B.J. and Chase, H.A., Modeling the Affinity Adsorption of Immunoglobulin-G to Protein-A Immobilized to Agarose Matrices, Chem. Eng. Res. Des., vol. 67, pp. 243-254 (1989).

Horton, B.S., Commercial Utilization of Minor Milk Components in the Health and Food Industries, Journal of Dairy Science, 78, pp. 2584-2589 (1995).

Karau, A., Benken, C., Thömmes, J. and Kula, M.R., The Influence of Particle Size Distribution and Operating Conditions on the Adsorption Performance in Fluidized Beds, Biotechnology and Bioengineering, vol. 55, n° 1, pp. 54-64 (1997).

McKenzey, H.A., Milk Proteins and Molecular Biotechnology, vol. 1, Academic Press (1970).

Noppe, W., Haezebrouck, P., Hanssens, I. and De Cuyper, M., A Simplified Purification Procedure of Alpha-Lactalbumin from Milk using Ca2+-dependent Adsorption in Hydrophobic Expanded Bed Chromatography, Bioseparation, vol. 8, nos. 1-5, pp. 153-158 (1999).

Richardson, J.F. and Zaki, W.N., Sedimentation and Fluidization: Part I, Trans. Inst. Chem. Eng. 32, pp. 35-53 (1954).

Villadsen, J.E. and Michelsen, M.L., Solution of Differential Equation Models by Polynomial Approximation, Prentice Hall Inc., New Jersey, pp. 445 (1978).

Yao C.C. and Tien C., Approximation of Intraparticle Mass-Transfer in Adsorption Processes 2 Nonlinear-Systems, Chemical Engineering Science, 47(2), pp. 465-473 (1992).