World Applied Sciences Journal 13 (2): 181-187, 2011
ISSN 1818-4952

Expanded Bed Adsorption of Biomolecules by NBG Contactor: Experimental and Mathematical Investigation

[1]M.H. Shahavi, [1]M. Jahanshahi, [1]G.D. Najafpour,
[1]M. Ebrahimpour and [2]A.H. Hosenian

[1]Nanotechnology Research Institute, Faculty of Chemical Engineering,
Babol University of Technology, Babol, Iran
[2]Mechanical Engineering Department,
Faculty of Engineering, Azad University of Kashan, Kashan, Iran

Abstract: This paper summarizes the critical examination of adsorption performance of the NBG expanded bed contactor operated with Streamline™ DEAE adsorbent under various operating conditions for expanded bed adsorption of BSA nanoparticle and Egg albumin. Scouting of a mechanism of adsorption for these bioproduct on the adsorbent and dynamic binding capacity was studied. Subsequently the batch adsorption data were regressed against the Langmuir model. In order to characterize the adsorption behavior of these bioproduct, breakthrough curves were measured and adsorption of these bioproducts was studied. However, modeling of such expanded bed (NBG) including mass balance of bioproduct in the mobile phase as well as the solid phase was studied for the first time. The overall process yield of recovery of these bioproducts was more than 85% which was a superior result in one-pass frontal chromatography.

Key words: Expanded bed chromatography · Bioproduct · Direct recovery · Dynamic binding capacity

INTRODUCTION

Production of biomolecules by genetically engineered microorganisms, yeasts and animal cells became a very important technique for the preparation of pharmaceuticals. The feed stocks from which proteins are prepared are generally complex, containing solid and dissolved biomass of various sizes and molecular masses, respectively [1]. Despite the nature of the starting material, it is necessary to eventually obtain a pure, defined substance of guaranteed purity and potency. This cannot be achieved with a single purification step but is usually achieved with by a combination of different unit operations that account for the different separation necessities, as is shown in Fig. 1 [2]. Chromatography in EBA mode became a ubiquitous analytical method. Its separation power kept attracting the attention of those interested in producing pure chemicals in large amounts for a variety of purposes [3]. Expanded-bed chromatography allows integrating solid-liquid separation, volume reduction by protein adsorption and partial purification in one unit operation without compromising on separation efficiencies, but saving considerably processing time and capital investment [4]. The process is based on the use of particulate adsorbents with different selectivity's, which reside in a column system [5]. Many of biotechnological products are nanoparticulate [6]. Nano-sized particles can be produced from all peptides, proteins (including monoclonal antibodies and enzymes) and other molecules with suitable reactive side chains [7]. Nanoparticles are of colloidal size. Size can be precisely controlled from 10 *nm* to 120 *nm*. Particles can be efficiently and densely loaded with drugs or other substances such as antibodies, antigens, enzymes or RNA [8]. In recent times there has been increased demand for these nanobioparticles such as protein nanoparticles as drug delivery vehicles, plasmid DNA and viruses as gene theraphy vectors, virus like particles as vaccine component [9]. The feasibility of this technique has been proven with a variety of expression systems (*E. coli*, yeast and mammalian cells) [10].

Corresponding Author: M. Jahanshahi, Nanotechnology Research Institute, Faculty of Chemical Engineering, Babol University of Technology, Babol, Iran, E-mail: mjahan@nit.ac.ir.

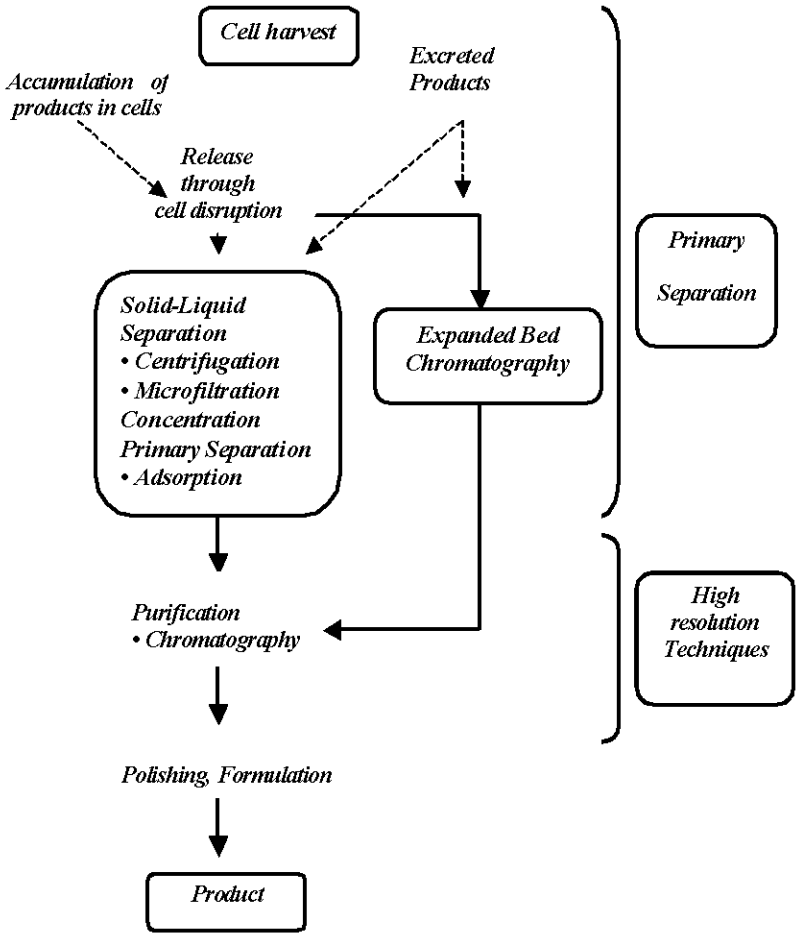

Fig. 1: Process pathways with conventional downstream processes and after inclusion of Expanded bed chromatography.

In previous work we prepared NBG column [11] and Hydrodynamic behaviour of such simple custom expanded bed column was investigated [12]. In this work the feasibility of this technique is shown for the recovery of bioproduct (BSA nanoparticle and Egg albumin) in with a variety of expression systems. However process modeling is also conducted to gain a better understanding of the breakthrough behaviour in NBG column.

MATERIALS AND METHODS

Streamline™ DEAE adsorbent (Amersham Pharmacia Biotech, Uppsala, Sweden) was used in these studies. BSA (fraction V, purity 98%), Tween-20, ethanolamine and glutaraldehyde (25% solution), were commercially supplied by Sigma Aldrich. Egg albumin and other chemicals were bought from Merk (Darmstadt, Germany) and were of analytical grade.

Column Specification: A glass column (NBG column; 1.3 *cm*, ID and 25 *cm* height) [11] with a sintered glass as the liquid distributor and a prolonged top adapter was used for expanded bed experiments. This column filled with Streamline™ DEAE anion-exchange adsorbent (Amersham Biosciences, Uppsala, Sweden) resulting in a certain sediment bed height. Flow rates ranging from 100 to 400 *cm/h* were maintained during the operational procedures. An upward flow of buffer was applied to the column giving a stable expanded bed of certain height. The top adapter was positioned 0.5 ± 0.2 *cm* above the bed surface. A peristaltic pump (model HL-2S, Shanghai Hu Xi Analysis Instrument Factory) was used in Bioproduct purification experiments. The outlet signal was monitored with a flow UV detector (WellChrom fast scanning spectrophotometer K-2600, Knauer, Berlin, Germany) at 280 *nm*. The UV detector was placed as near as possible to the column outlet to reduce the dead volume in the experimental system.

Bioproduct Adsorption Experiment: All adsorption experiments were carried out at 22 ± 1°C. The mixing of adsorbent with feed stocks in simple batch contacting mode is useful for primary bioproduct recovery. Batch binding experiments were performed by incubating 1 *ml* settled volume of the adsorbent with 20 *ml* of bioproducts (0.6, 1.0, 1.5 and 2.5 *mg/ml*) in 0.01 *M* Tris-HCl buffer at pH 7.5 and reaction tubes were placed on a roller incubator and sampled (100μl) at timed intervals. Bioproduct concentrations were measured by absorption using spectrophotometer at wave length of 280 *nm*. In kinetic isotherm experiments, a range of concentrations of bioproducts were mixed with 1 *ml* of the adsorbent and incubated at room temperature for 16 *h*.

Dynamic Adsorption: In order to characterize the adsorption behaviour of these bioproducts breakthrough curves were measured and adsorption of them were studied [13, 4]. All experiments were carried out at 22°C with a settled bed height of 6±0.2 *cm* in column with 1.3 *cm*. Bioproducts solution of 2.5 *mg/ml* in 0.01 *M* Tris-HCl was used. Before applying feedstock solution, the bed was allowed to expand stably at least 30 *min* with the Tris-HCl buffer and then the flow hydrodynamics of the beds was determined [14]. Two operation modes for bioproducts were done: at two fold bed expansion (u = 400 *cm/h*) and the other was at a superficial velocity of 100, 220 *cm/h*. Dynamic binding capacity (DBC) was calculated from bioproducts breakthrough curves [15].

Direct Recovery of Bioproducts Using EBA Purification Process: Expanded bed adsorptions were carried out at 22°C and pH 7.5. After the optimal condition was achieved [12] (ID = 1.3 *cm*, bed expansion degree = 100%) Adsorbent bed pre-equilibrated in the working height (6.0 ± 0.2 *cm*), with equilibration buffer (0.01 *M* Tris-HCl buffer pH 7.5. The feedstock loaded to column until the bioproduct concentration of the outlet was equal to about 15 % of the feed concentration. The elution was profiled with 0.1 *M* NaCl at Tris-HCl buffer to remove the weakly bound. The bioproduct content was measured from time to time during the adsorption, washing and elution periods.

The Equations of the Models of Expanded Bed Chromatography

Modeling the Behavior of Expanded-bed Chromatography: In order to understand the behaviour of the column, the mass-transfer effects for the pore diffusion models were solved to understand the effects on mass-transfer mechanism and intraparticle mass-transfer resistance as functions of hydrodynamic factors.

Mass Balance of Bioproduct in the Mobile Phase: This mass balance is written for a fluid percolating through a bed of spherical particles of radius R:

$$\begin{aligned}
&\frac{\partial C}{\partial t} = D_{ax}\frac{\partial^2 C}{\partial z^2} - u\frac{\partial C}{\partial z} + \frac{3k_{f,e}\varepsilon_s}{\varepsilon_p R_p}\left(C - C_f\right)\\
&IC \Longrightarrow C(z,t=0) = C_0\\
&BC \neq 1 \Longrightarrow C\left(z=0,t\right) = C_0 + \frac{D_{axl}\varepsilon_l}{u}\frac{\partial c}{\partial z}\Big|_{z=0}\\
&BC \neq 2 \Longrightarrow \frac{\partial C}{\partial Z}\Big|_{Z=L} = 0
\end{aligned} \tag{1}$$

Where C is bioproduct concentration in the liquid phase (*mg/ml*) and D_{ax}, liquid phase dispersion coefficient (m^2/s), ε_s effective porosity of adsorbent for the considered bioproduct, R_p: particle radius (m), C_f (t): protein concentration at the particle surface (*mg/ml*), z and t are the abscissa and time, respectively; u is the mobile phase velocity [16].

Mass Balance of Bioproduct in the Solid Phase: The mass balance of bioproduct that is binding to the adsorbent particles is written [15]:

$$\begin{aligned}
&\varepsilon_s\frac{\partial q}{\partial t} = D_S\frac{\partial^2 q}{\partial z^2}\Big|_z + \frac{3k_{f,e}\varepsilon_s}{R_p}\left(C - C_f\right)\\
&IC \Longrightarrow q(z,t=0) = 0\\
&BC \neq 1 \Longrightarrow \frac{\partial q}{\partial z}\Big|_{z=0} = 0\\
&BC \neq 2 \Longrightarrow \frac{\partial q}{\partial z}\Big|_{z=l} = 0
\end{aligned} \tag{2}$$

Where ε_s is effective porosity of adsorbent for the considered bioproduct, q is the concentration of the studied component in the adsorbed phase and Ds is solid phase dispersion coefficient (m^2/s). There is no influence from the bulk flow because the bioproduct only move into the porous particle via a diffusion process.

The film mass transfer coefficient k_f can be calculated as a function of the bed void fraction using the following correlation [17]. Re_p is the Reynolds particle number, Sc is the Schmidt number and D_m the molecular diffusion coefficient of the protein consider.

$$k_{f,e} = \frac{D_m}{d_p}\left[2 + 1.5(1-\varepsilon_l)\mathrm{Re}_p^{1/2} Sc^{1/3}\right] \quad (3)$$

The solid dispersion coefficient is given as a function of the superficial velocity [18]:

$$D_s = 0.04u^{1.8} \quad (4)$$

Pore Diffusion Model: The pore diffusion model is used to explore the kinetics of bioproduct adsorption to the porous particle. Eq. (5) describes the concentration of the bioproduct as a function of the time and the particle radius. Inside the pore, only a diffusion process occurs. The equation and its initial and boundary conditions (*IC* and *BC*) are expressed by [19]:

$$\varepsilon_s \frac{\partial C_i}{\partial t} + \frac{\partial q_i}{\partial t} = \varepsilon_s D_p \left(\frac{\partial^2 C_i}{\partial r^2} + \frac{2}{r}\frac{\partial C_i}{\partial r}\right) \quad (5)$$

$$IC \quad t = 0 \quad C = C_o \quad C_i = 0 \quad q_i = 0$$

$$BC \neq 1 \quad r = 0 \quad \frac{\partial C_i}{\partial r} = 0$$

$$BC \neq 2 \quad r = R \quad \varepsilon_s D_p \frac{\partial C_i}{\partial r} = k_{f,b}(C - C_i)$$

Where C_i is bioproduct concentration at the particle pore (*mg/ml*) and q_i is particle adsorbed bioproduct concentration (*mg/ml*).

The liquid film mass transfer coefficient for the batch adsorption system, k_{fb}, can be calculated using the following correlation [15]:

$$k_{f,b} = \frac{2D_m}{d_p} + 0.31(sc)^{-2/3}\left(\frac{\Delta\rho\mu g}{\rho^2}\right)^{1/3} \quad (6)$$

RESULTS AND DISCUSSION

Equilibration Isotherm: The phenomenon of adsorption is often described by using a curve showing the amount of adsorbate retained at the surface of the adsorbent versus liquid concentration c^*, at thermodynamic equilibrium and at constant temperature. Such a curve is termed "adsorption isotherm". Numerous mathematical models have been proposed to describe these isotherms. The models of Langmuir and of Freundlich are among the simplest and the most frequently used [20]. In this case experimental data were obtained from batch adsorption.

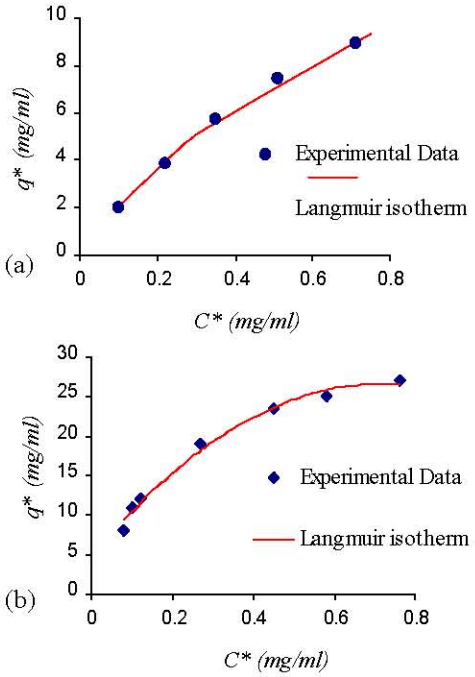

Fig. 2: Adsorption Binding isotherm of a) BSA nanoparticle b) Egg albumin.

The adsorption isotherms of bioproduct to the Streamline™ DEAE are shown in Fig.2. As can be seen, the experimental data are well fitted by the Langmuir equation:

$$q^* = \frac{q_{\max} c^*}{K_d + c^*} \quad (7)$$

(With q^* and C^*, concentration of bioproduct in the solid and liquid phase in equilibrium). By linear fitting of the Langmuir equation, the values of q_{max} and K_d was obtained. Results are given in Table 1.

Breakthrough Curves and Dynamic Binding Capacity: The dynamic binding capacity is usually represented as the total amount of a certain bioproduct in the column subtracting the amount in the dead space per unit adsorbent volume [13]. The dead space is located between the height of the bed and the upper adaptor. The quantity of protein in this space is denoted by Q_d (in *mg*). The dynamic capacity is calculated when the outlet concentration is equal to 40% of the feed concentration. The quantity of protein is $Q_{40\%}$ (in *mg*). The method of Griffith is used [14]:

$$Q_{40\%} = u.C_0 \int_{t=0}^{t(\frac{C}{C_0}=0.4)} \left(1 - \frac{C}{C_0}\right) dt \quad (8)$$

Table 1: Langmuir isotherm adsorption constants and pore diffusion coefficients for BSA nanoparticle and Egg albumin.

Adsorbent	Bioproduct	q_{max} (mg/ml)	K_d (mg/ml)	K_{fb} (10^{-6}m/s)	D_p(10^{11}m^2/s)
Streamline™ DEAE	BSA nanoparticle	21.23	0.95	15.2	3.66
	Egg albumin	39.68	0.28	12.3	1.46

Table 2: Parameters of BTC model and *DBC* for BSA nanoparticle and Egg albumin.

Adsorbent	Bioproduct	u(cm/h)	H/H_0	D_s(10^{-8} m^2/s)	D_{axl}(10^{-6} m^2/s)	K_{fe}(10^{-6} m/s)	DBC (mg/ml adsorbent)
Streamline™ DEAE	BSA nanoparticle	100	1.3	1.58	0.56	8.1	8.59
		220	1.6	6.56	1.79	7.64	7.60
		400	2	19.2	3.90	5.8	6.66
	Egg albumin	100	1.3	1.58	0.56	6.88	11.3
		220	1.6	6.56	1.79	5.34	10.46
		400	2	19.2	3.90	3.3	8.3

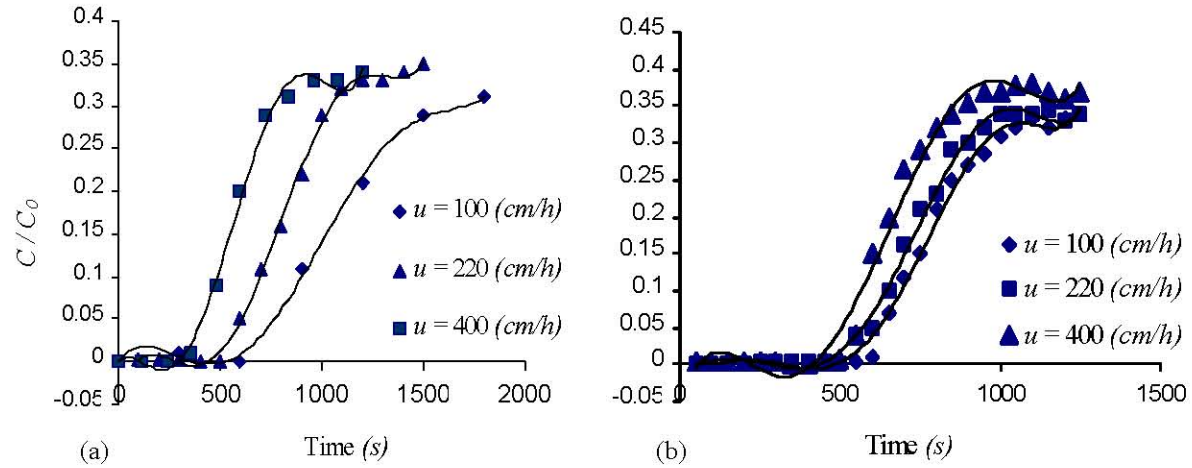

Fig. 3: a) BSA nanoparticle, b) Egg albumin breakthrough curves in the expanded bed adsorption.

$$Q_d = V_d . C_0 \quad (9)$$

$$DBC = \frac{Q_{40\%} - Q_d}{V_d} \quad (10)$$

Where C_0: bioproduct concentration in the feed solution (*mg/ml*) *u*: the flow rate (*ml/h*), V_d: the dead volume between the height of the bed and the upper adaptor (*ml*). Increase the flow velocity results in a significant decrease of dynamic binding capacity. This indicates that the residence time for diffusion of bioproduct into the adsorbent is shortened with increasing flow velocity and resulted in some of the bioproducts being washed out of the column before diffusion in to adsorbent pores.

Parameters for the breakthrough model and the *DBC* of bioproducts to the adsorbents summarized in Table 2 the pore diffusivity D_p obtained by the batch adsorption (Table 1) is used for the calculations. In addition, the liquid phase dispersion coefficient D_{axl} was estimated in previous work [12]. Using independently determined model parameters, the breakthrough curves are predicted and compared to the experimental data. Fig. 3 (a and b) show that the numerical solutions are well fitted to the experimental breakthrough data.

Direct Recovery of Bioproducts Using Eba Purification Process: After having defined optimal running conditions,method scouting was performed using unclarified feed material on Streamline™ DEAE packed in NBG column to a bed height of 6 *cm*. Unclarified feed-stock was used in expanded mode on a Streamline™ DEAE NBG column. Bed expansion/equilibration, feed application and wash and elution were performed at an upward flow velocity of 400 *cm/h*. The buffer used during expansion/equilibration and wash was (0.01 *M* Tris-HCl buffer pH 7.5. Elution was performed in expanded mode. The mode of expanded bed elution could result in the volume increase of the eluted peak compared with the packed bed elution [21]. However, this mode offers the advantage that the top adapter can remain at its original position without the need of downward movement to the top of the settled bed, benefiting in the decrease of

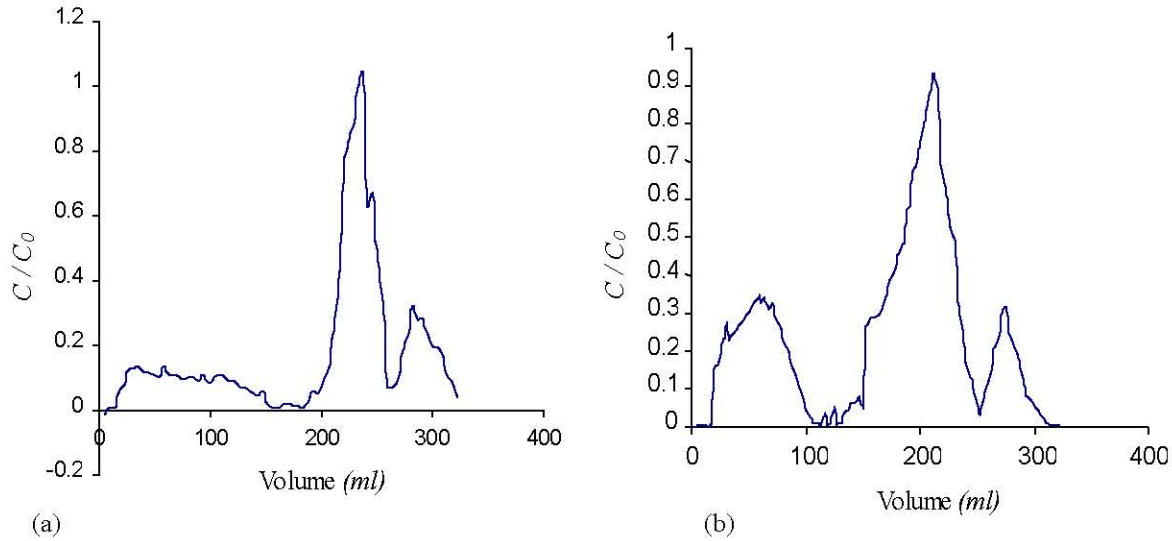

Fig. 4: Full chromatogram of expanded bed recovery of a) BSA nanoparticle and b) Egg albumin in the NBG column.

the processing time. Therefore, elution in expanded bed mode was chosen in this work. The first elution buffer was Tris-HCl buffer containing 0.1 *M* NaCl. In addition, the elution was switched to the buffer containing 1.0 *M* NaCl. The time courses of BSA nanoparticle and Egg albumin purification (washing (first peak), first elution (second peak) and second elution (third peak)) by the NBG column with Streamline™ DEAE adsorbent were shown in Fig. 4 (a and b). The yield of recovery of these bioproduct was more than 85% which was a superior result for a one pass expanded bed chromatography.

CONCLUSION

Expanded bed adsorption chromatography is used to capture the protein product of interest from a crude biological suspension (feedstock of bioproduct) directly, thereby eliminating the need for the removal of the cell debris. While this technique may replace three or four unit operations in a typical downstream process for biological product recovery, the adsorption process is influenced by the interaction between the microbial cells or cell debris and the adsorbent as well as the presence of contaminating solutes. Saving of time is an additional advantage, which can amount to several hours in a large-scale process [22]. In this study it was shown that EBA system (by the NBG column) is successfully used to purify Protein nanoparticle (BSA nanoparticle) and Egg albumin in terms of higher recovery compared with the conventional process. The recovery yield increased more than 85%, showing that the expansion bed carries out the best biomolecules recovery; it occurs due to more particles-nanobiomolecules interaction in expanded bed than in fixed bed by increase to bed void age influence. Batch binding studies of BSA nanoparticle and Egg albumin adsorption upon Streamline™ DEAE indicated that this exhibited an apparent reasonable adsorption and capacity for these bioproduct under common experimental conditions. However in order to characterize the adsorption behaviour of these bioproduct in this column, BTCs were measured. Result showed that increasing the flow velocity results in a significant decrease of dynamic binding capacity. There is clearly much scope for the development of this method and application of such column for the recovery of bioproduct and will be subject of our future publication.

ACKNOWLEDGEMENT

The authors are gratefully acknowledged the support of Nanobiotechnology Research Laboratory, Babol University of Technology, Iran.

REFERENCES

1. Vennapusa, R., S.M. Hunegnaw, R.B. Cabrera and M. Lahore, 2008. Assessing adsorbent-biomass interactions during expanded bed adsorption onto ion exchangers utilizing surface energetics. J. Chromatogr. A., 1181: 9-20.
2. Anspach, F.B., D. Curbelo, R. Hartmann, G. Garke and W.D. Deckwer, 1999. Expanded-bed chromatography in primary protein purification. J. Chromatogr. A., 865: 129-144.

3. Zhao, J., D. Lin and S. Yao, 2009. Expansion and hydrodynamic properties of B-cyclodextrin polymer/tungsten carbide composite matrix in an expanded bed. J. Chromatography. A., 1216: 7840-7845.
4. Jun, Z., Y. Shanjing and L. Dongqiang, 2009. Adsorbents for Expanded Bed Adsorption: Preparation and Functionalization. Chinese J. Chem. Eng., 17: 678-687.
5. Chang, Y.K. and I.P. Chang, 2006. Method development for direct recovery of lysozyme from highly crude chicken egg white by stirred fluidized bed technique. Biochem. Eng. J., 30: 63-75.
6. Jahanshahi, M., S. Williams, A. Lyddiatt and S.A. Shojaosadati, 2004. Preparation and purification of synthetic protein Nanoparticulates: Adsorbent Design Implementation for the Recovery of Nanoparticulate Bioproducts. J. IET. Nanobiotechnol., 151: 176-182.
7. Jahanshahi, M., Y. Sun, E. Santos, A. Pacek, T.T. Franco, A. Nienow and A. Lyddiatt, 2002. Operational intensification by direct product sequestration from cell disruptates: application of a pellicular adsorbent in a mechanically integrated disruption-fluidised bed adsorption process. Biotechnol. Bioeng., 80: 201-212
8. Jahanshahi, M. and M. Ebrahimpour, 2009. Expanded bed chromatography as a Tool for Nanoparticulate Separation: Kinetic Study and Adsorption of Protein nanoparticles. Chromatographia. 70: 1553-1560.
9. Jahanshahi, M., M. Rahimnejad and G.D. Najafpour, 2006. Production of biological nanoparticles from bovine serum albumin for drug delivery. Afr. J. Biotechnol., 5: 1918-1923.
10. Chin Chong, F., W.S. Tan, D.R.A. Biak, T.C. Ling and B.T. Tey, 2010. Direct recovery of recombinant nucleocapsid protein of Nipah virus from unclarified *Escherichia coli* homogenate using hydrophobic interaction expanded bed adsorption chromatography. J. Chromatogr. A., 1217: 1293-1297.
11. Shahavi M.H., GH.D. Najafpour and M. Jahanshahi, 2008. Hydrodynamic behaviour and biochemical characterization of a simple custom expanded bed column for protein purification, Afr. J. Biotechnol., 7: 4336- 4344.
12. Jahanshahi, M., GH.D. Najafpour, M. Ebrahimpour, S. Hajizadeh and M.H. Shahavi, 2009. Evaluation of hydrodynamic parameters of fluidized bed adsorption on purification of nano-bioproducts. Phys. Status Solidi, 6: 2199-2206.
13. Bruce, L.J. and H.A. Chase, 2001. Hydrodynamics and adsorption behaviour within an expanded bed adsorption column studied using in-bed sampling. Chem Eng. Sci., 56: 3149-3162.
14. Tong, X.D. and Y. Sun, 2001. Nd-Fe-B alloy-densified agarose gel for expanded bed adsorption of proteins. J. Chromatogr. A., 943: 63-75.
15. Tong, X.D., X.Y. Dong and Y. Sun, 2002. Lysozyme adsorption and purification by expanded bed chromatography with a small-sized dense adsorbent. Biochem. Eng. J., 12: 117-124.
16. Wright, P.R. and B.J. Glasser, 2001. Modeling mass transfer and hydrodynamics in fluidized-bed adsorption of proteins. AIChE. J., 47: 474-488.
17. Fan, L.S., Y.C. Yang and C.Y. Wen, 1960. Mass transfer in semifluidized beds for solid-liquid systems. AIChE. J., 6: 482-487.
18. Van Der Meer, A.P., C.M.J.P. Blanchard and J.A. Wesselingh, 1989. Mixing of particles in liquid fluidized beds. Chem. Eng. Res. Des., 62: 214-222.
19. Horstmann, B.J. and H.A. Chase, 1989. Modeling the affinity adsorption of immunoglobulin G to protein A immobilised to agarose matrices. Chem. Eng. Res. Des., 67: 243-254.
20. Nameni, M., M.R. Alavi Moghadam and M. Arami, 2008. Adsorption of hexavalent chromium from aqueous solutions by wheat bran. Int. J. Environ. Sci. Tech., 5: 61-168.
21. Hjorth, R., 1999. Expanded bed adsorption: elution in expanded bed mode. Bioseparation, 8: 1-9.
22. Balasundaram, B. and S.T.L. Harrison, 2008. Influence of the extent of disruption of Bakers' yeast on protein adsorption in expanded beds. J. Biotechnol., 133: 360-369.