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Full Length Research Paper

Hydrodynamic behaviour and biochemical characterization of a simple custom expanded bed column for protein purification

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Hydrodynamic behaviour and biochemical characterization of a simple custom expanded bed column made by Nanobiotechnology Group (NBG) was compared with a commercial expanded bed contactor. Hydrodynamic characteristics of the columns (D_{axl} and Bo) with various settled bed height (SBH = 5 - 10 cm, increment of 1 cm) along with variety of column diameters (1.0, 1.6, 2.0 and 2.5 cm) were investigated. All parameters were found to be comparable or superior to those reported for conventional, expanded bed contactors. However, the experimental values of Richardson-Zaki coefficient determined here were close to the value of 4.8, commonly used in the laminar flow regime. The expansion coefficient and terminal velocity of the adsorbent (Streamline™ DEAE) were theoretically calculated based on correlation stated in the literature and also experimentally determined. The adsorbent was also used in batch binding experiments, as well as in commercial and custom assembled expanded bed contactors with various feedstocks comprising bovine serum albumin and egg albumin. This evaluation exhibited good capacities and adsorption/desorption performance of NBG column in compare with commercial expanded bed column. The hydrodynamic behaviour of expanded bed adsorption and the generic application of simple NBG column and its potential for the purification and recovery of protein products are thoroughly discussed.

Key words: Expanded bed adsorption, hydrodynamic performance, protein adsorption, batch binding.

INTRODUCTION

Liquid fluidised bed adsorption (expanded bed adsorption) has emerged as an efficient method for the recovery of biological products from complex feedstocks. The expanded bed adsorption has demonstrated advantages over the traditional methods of recovery, e.g. circumventing the need for clarification of feedstocks before application to a fixed bed chromatography column (Chase, 1994; Lyddiatt, 2002; Annelise et al., 2005; Balasundaram and Harrison, 2008). The hydrodynamic behaviour of fluidised beds/expanded beds applied to chromatographic adsorption is different from the conventional liquid-solid fluidised beds. The conventional chemical engineering view of a fluidised bed is one in which there is a significant degree of mixing, both of the solid and the fluid phases, for example, in gas-fluidised systems (Levenspiel, 1999; Davidson et al., 1985; Kuo and Yiang, 2008). But mixing in liquid fluidised is not as severe as in gas-fluidised systems. Here, the density differences between the solid and the fluid phase are comparatively small and thus, the bed shows a particulate behaviour in which the bed can retain a uniform character (Chase, 1994). In a packed bed, the adsorbent beads are stationary and liquid flow through the bed approximates to plug flow. In contrast, due to the mixing of the adsorbent, a fluidised bed would be expected to show an inferior adsorption performance compared with the packed bed. Therefore, it is highly desirable to investigate the hydrodynamic performance and minimise the degree of mixing in order to mimic the adsorption characteristics found in a packed bed contactor with res-

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pect to capacity and resolution (Thommes, 1997; Jahanshahi et al., 2002; Bermejo et al., 2007).

Column (contactor) and solid phase designs play a major role in fluidised bed/expanded bed adsorption. The growth to full potential of applications for fluidised bed or expanded bed technology in biological product recovery may be considered to be currently limited by the availability of suitable adsorbents and columns in terms of efficiency and cost. The adsorbent design has extensively been studied in earlier publications (Jahanshahi et al., 2003; Jahanshahi et al., 2005; Najafpour, 2007; Jahanshahi et al., 2008). In the other words, such technology has been commercialized with customized contactor designs and extensive development programmes required for their establishment. Such developments have invited re-examination of the simply designed fluidised bed contactor (Nano-Biotechnology Group referred as the NBG contactor, as shown in Figure 1). The NBG contactor has the ability to retain adsorptive fractionation of protein products contained in particulate feedstocks, in respect of suitability for single-pass.

This paper summarises the critical examination of the hydrodynamic performance of the NBG contactor and an equivalent commercial expanded bed contactor operated with Streamline™ DEAE adsorbent under various operating conditions. This investigation includes bed expansion tests, Residence Time Distribution (RTD) measurements comprising determination of Bodenstein number and axial dispersion of the adsorbents across a range of fluid velocities. However, for the purpose of demonstration of the principle, the performance of adsorption of bovine serum albumin in the NBG column is investigated and compared with that of commercial expanded bed column. The capture of egg albumin in batch binding experiments upon Streamline™ DEAE adsorbent and its expanded bed adsorption in both contactors is also demonstrated.

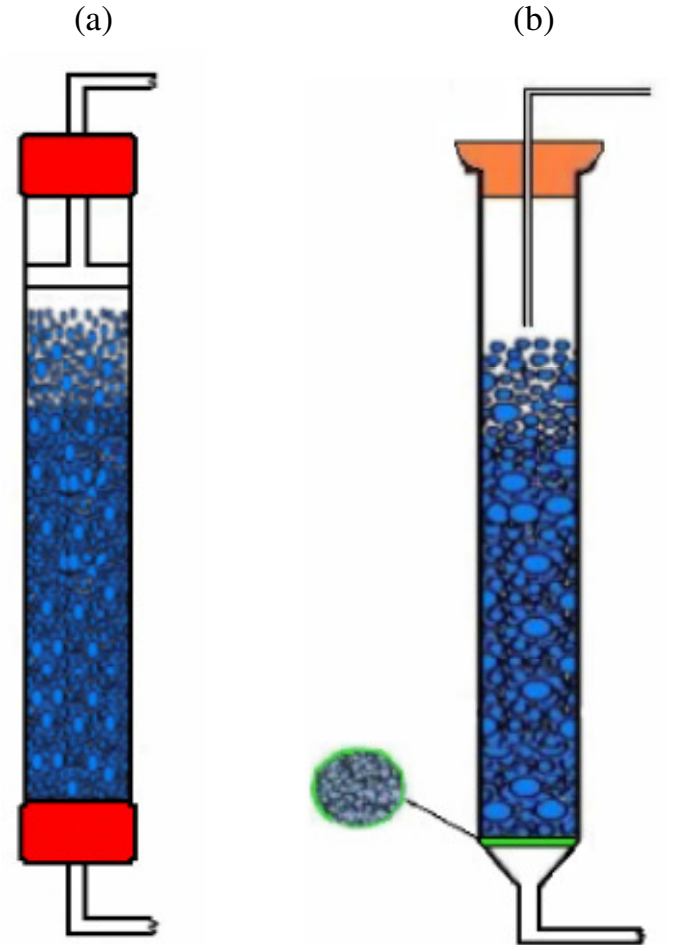

Figure 1. Representation of apparatus for expanded bed experiments. (a) Streamline™ Column (b) Custom built NBG column.

MATERIALS AND METHODS

Materials

Bovine serum albumin was commercially supplied by Sigma and egg albumin was purchased from Merck (Darmstadt, Germany). All reagents were supplied by Merck and the Aldrich Chemical Company (Darmstadt, Germany); they were of analytical grade and used as received. Streamline™ DEAE adsor-bent was purchased from Amersham Biosciences (Piscataway, NJ 08855-1327, USA).

Adsorbent contactors

Two types of contactors were used in this experiment. Streamline™ columns were purchased from Amersham Biosciences and adopted for studies of hydrodynamic and expanded bed recovery performance. In addition, custom built devices which were fabricated by the NBG contactor, Nanobiotechnology Group (Babol University of Technology) which was equipped with a simple sintered glass distributor comprising a 100 µm mesh, were also used. Such contactors comprised a glass column having a hemispherical inlet.

Bed expansion characteristics

The bed expansion characteristics were determined in expanded beds operated with buffer A (10 mM Tris/HCl containing 0.2% (w/v) sodium azide at pH 7.5). The adsorbent was repeatedly washed prior to use with equilibration buffer A and filtered until pH and conductivity reached to equilibrium. A given amount of adsorbent particles was transferred to the columns and allowed to sediment uniformly. Bed expansion of adsorbent was measured and recorded with increasing superficial liquid velocity through the inlet of the column. The superficial flow velocity was subsequently plotted against bed expansion expressed as the percentage ratio of expanded and settled bed heights.

The relationship between superficial liquid velocity (U) and bed voidage (ε) in an expanded bed can be described by the classical correlation first postulated by Richardson and Zaki (1954):

$$U = U_t \varepsilon^n \quad [1]$$

The Richardson–Zaki coefficient (n) can be calculated from the correlations available in the literature (Davidson et al., 1985). The model of Shiller and Naumann is commonly used for the prediction of terminal velocity of the spherical particle (Najafpour, 2007):

$G_a = 18\ Re_t + 2.7\ Re_t^{1.687} \quad 3.6 < G_a < 10^5$ [2]

where Grashof number is given by the following equation:

$$G_a = \frac{\rho \times (\rho_P - \rho) \times g \times d_P^{\ 3}}{\mu^2} \quad [3]$$

and Reynolds number is:

$$Re_t = \frac{\rho \times d_P \times U_t}{\mu} \quad [4]$$

The model of Shiller and Naumann has been successfully used to estimate the particle terminal velocity by Thomas and Yates (1985), Voute et al. (2000) and Jahanshahi et al. (2003, 2006) which are used in the present study as well.

Residence time distribution (RTD) experiments

Residence Time Distribution (RTD) measurements were performed using a negative step signal method (Levenspiel, 1999; Amersham Bioscience, l998). A bed of the adsorbent particles was fully expanded using buffer A at the test flow rate. A dilute acetone solution (1% v/v) was used as the input to the column in a system fluidised with buffer A. The UV absorbance of the acetone was measured by spectrophotometer at wave length of 280 nm in the exit stream from the column using a UV monitor.

The Bodenstein number (B_o) and axial dispersion coefficient (D_{axl}) which express the state of liquid dispersion and fluidisation behaviour, was calculated according to the following equations (Karau et al., 1997):

$$\frac{\delta^2}{\overline{t^2}} = \frac{2}{B_o} - 2\left(\frac{1}{B_o}\right)^2 \times \left[1 - \exp(-B_o)\right] \quad [5]$$

$$B_o = \frac{U \times H}{\varepsilon \times D_{axl}} \quad [6]$$

The axial dispersion in the liquid phase can also be interpreted in a form more familiar to researchers in the chromatography based on the theoretical plate number (Pai et al., 2000). For the small derivations from the plug flow the axial dispersion of the liquid phase can be expressed as follows (Mullick and Flickinger, 1999; Lan et al., 1999; Bierau, 2001; Jahanshahi et al., 2002; Najafpour, 2007):

$$D_{axl} = \frac{U \times H}{2 \times \varepsilon \times N} \quad [7]$$

Batch equilibrium experiments

Batch binding experiments were performed by incubating 1 ml settled volume of the adsorbent with 20 ml egg albumin (0.6, 1.0, 1.4 and 2.0 mg/ml) and reaction tubes were placed on a roller incubator and sampled (100 µl) at timed intervals. Protein concentrations were measured by absorption using spectrophotometer at wave length of 280 nm. In kinetic isotherm experiments, a range of concentrations of egg albumin were mixed with 1 ml of the adsorbent and incubated at room temperature for 12 h and protein concentration was calculated as described above. The Langmuir isotherm, which permits an estimate of how much product an adsorbent is capable of adsorbing (q_m) and how tightly the product binds to the immobilised ligand (K_d), applied (Ivonov et al., 2006). This model is based on the following Equation:

$$q^* = \frac{q_m \times C^*}{(K_d + C^*)} \quad [8]$$

where $K_d = K_2/K_1$ (the dissociation constant of the equilibrium reaction, where K_1 and K_2 are the rate constants governing the forward and backward direction of a particular reaction). The terms q^* and C^* represent the equilibrium concentration of the biomolecules for the adsorbent solid phase and liquid phase, respectively. The maximum binding capacity is represented by q_m whilst K_d is the dissociation constant of the solute-adsorbent complex, that represents a measure of the affinity between the defined biological products and the adsorbent solid phase.

Fluidised/expanded bed adsorption

All fluidised bed adsorption experiments were undertaken according to a common protocol. Adsorbent was packed into the chosen contactor, and equilibrated with buffer A. Prior to feedstock application, the bed was fluidised at 100% expansion ($H/H_o = 2$, where H and H_o represent expanded and settled bed heights, respectively) in equilibration buffer A. The experiment was continued in a single pass operation with the application of serum bovine and egg albumin as feedstocks to the fluidised adsorbent at a given linear flow velocity. After feedstock application, the fluidised bed was washed with buffer A, until A_{280} reached baseline values. Subsequently, product desorption was achieved in fluidised bed mode with 0.5 M NaCl in buffer A. Appropriate fractions were collected and assayed for protein content as described before.

RESULTS AND DISCUSSION

Bed expansion

The bed expansion contributes to the adsorption efficiency as a composite function of liquid distribution, liquid properties (viscosity, density), particle characteristics and the configuration of the column in terms of wall and distributor effects (Thömmes, 1997). The adsorbent expansion in the NBG contactor might be expected to exhibit unstable fluidization behaviour because of the channelling generated by the simple distributor. This was not visibly apparent in the present study (Figure 2). In support of this point, Lan et al. (1999) concluded that distributor design along with a sufficient SBH, dissipate jet stream generated by the flow distributor.

In order to estimate the variation of the bed expansion as a function of flow velocity throughout the bed, the Richardson–Zaki equation was used. The experiments were operated in buffer A and relevant graphs are shown in Figures 2 and 3. The theoretical terminal velocity was derived from Shiller and Nanmann model. The experimental values of terminal velocity (U_t) can be determined by fitting data into the Richardson–Zaki equation. The values for the terminal velocity were derived from the

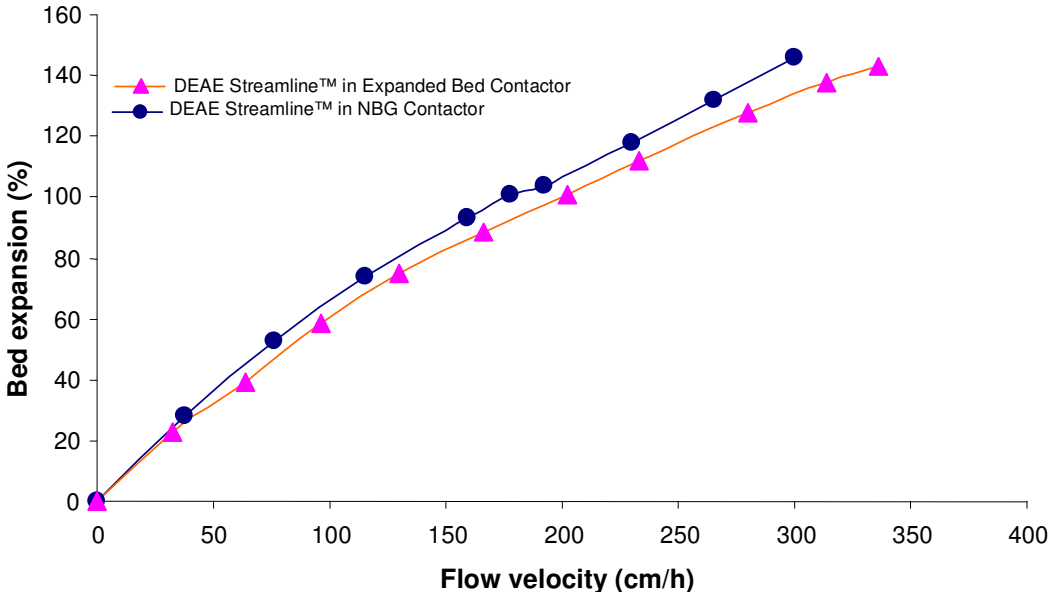

Figure 2. Bed expansion of the adsorbent as a function of linear flow velocity in expanded bed and NBG contactors.

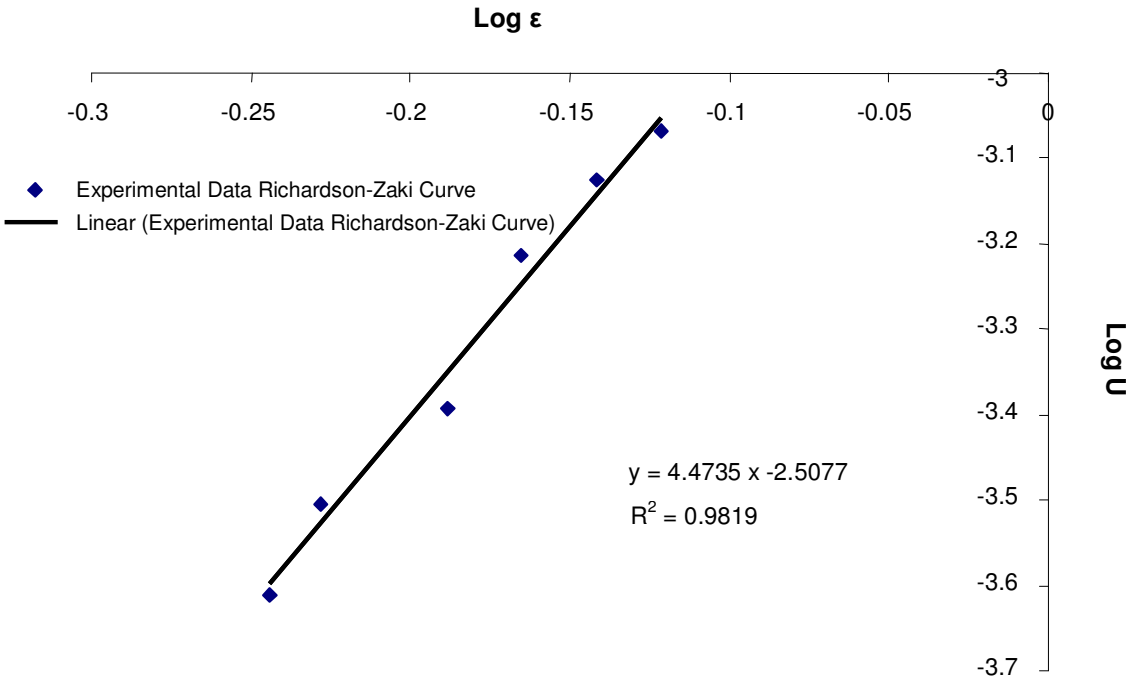

Figure 3. Flow velocity as function of bed voidage for calculation of Richardson–Zaki coefficient (n) and terminal velocity (U_t).

equations mentioned earlier (Bed expansion characteristics). The summary of bed expansion characteristics is presented in Table 1. The experimental values of the Richardson–Zaki coefficient determined here were about 4.7 and approximated to the value of 4.8, commonly used in the laminar flow regime (Richardson and Meikle, 1961; Tong and Sun, 2002). The experimental values of the expansion coefficient and terminal velocity of the adsor-

Table 1. Experimental and theoretical values of expansion coefficient and terminal velocities for the adsorbent in the NBG contactor.

Type of data	U_t (m/s)	U_m (m/s)	n
Theoretical data	3.25262 E-03	3.74482 E-05	4.74542
Experimental data	3.106 E-03	3.6762 E-05	4.735

Table 2. Bo and D_{axl} for different settled bed height (a)

Bo	D_{axl}	T (s)	EBH (cm)	SBH (cm)
8.400639	0.12579	114.43	10	5
9.519199	0.008893	106.53	12	6
16.60508	0.147457	155.02	14	7
18.946599	0.13098	180.78	16	8
20.58865	0.168619	185.15	18	9
18.36429	0.200093	178.57	20	10

Table 2. Bo and D_{axl} for different expanded bed height (b).

Bo	D_{axl}	T (s)	EBH (cm)	SBH (cm)
7.91374	0.000516	342.21	8	6
3.638651	0.003578	122.66	10	6
9.252633	0.003021	105.13	12	6
7.706067	0.004355	91.568	14	6
1.943883	0.023601	41.161	16	6

bent in the NBG contactor agreed more closely with the results calculated from literature correlation than the corresponding values for the commercial contactor. There was a small difference between the experimental data and calculated data (Table 1) attributed to the assumption of settled bed voidage (ε_o = 0.4) and the difference of particle size distribution in each of the beds. The discrepancies between these results are similar to those reported in the literature. For example, Thömmes et al. (1995) found that the calculated terminal velocity was about 10-fold higher than that experimentally determined in the fluidised bed of controlled pore glass. That was due to the porous nature and irregular shape of the glass matrix.

Mixing of the liquid phase (RTD experiments)

Measurement of the axial dispersion, that is, the deviation from plug flow movement of fluid elements in an adsorbent bed (both packed and fluidised), is commonly performed by residence time distribution (RTD) analysis of step or pulse signals (Levenspiel, 1999). This method was employed in the present research work, in order to characterise and compare the NBG contactor with a commercial expanded bed contactor. Measurements were made with regard to degree of axial dispersion and bed stability when materials were fluidised under similar conditions. The values of theoretical plate number (N), axial dispersion coefficient (D_{axl}) and Bodenstein number (B_o) were estimated and summarised in Table 2. Comparison of the results confirmed the superior hydrodynamic properties, that is, diminished mixing and stable fluidisation throughout the bed. For example, the Bodenstein number of the adsorbent in the NBG, which represents the axial dispersion and fluidisation behaviour in the passage of fluid elements through the contactor, was as good as that of commercial contactor.

Figure 4 summarises the variation of D_{axl} with the degree of bed expansion for the adsorbent operated in buffer A. The D_{axl} curve increases with increased superficial flow velocity (Hjoth, 1999; Jahanshahi et al., 2002). This might suggest that in a given contactor configuration every individual adsorbent is characterised by a range of flow velocities at which the degree of axial dispersion is at a minimum. The measured B_o values were judged (Table 2), superior fluidisation characteristics were exhibited which gave justification for further experimentation in the fluidised bed adsorption of bioproducts.

Equilibrium kinetic studies

A batch binding characteristics of Streamline™ DEAE

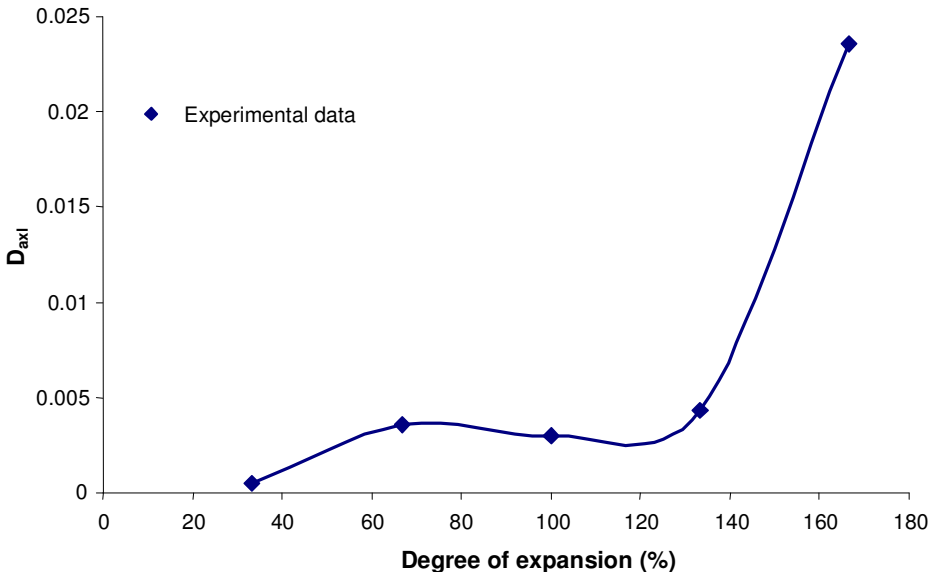

Figure 4: Axial dispersion coefficient as a function of bed expansion.

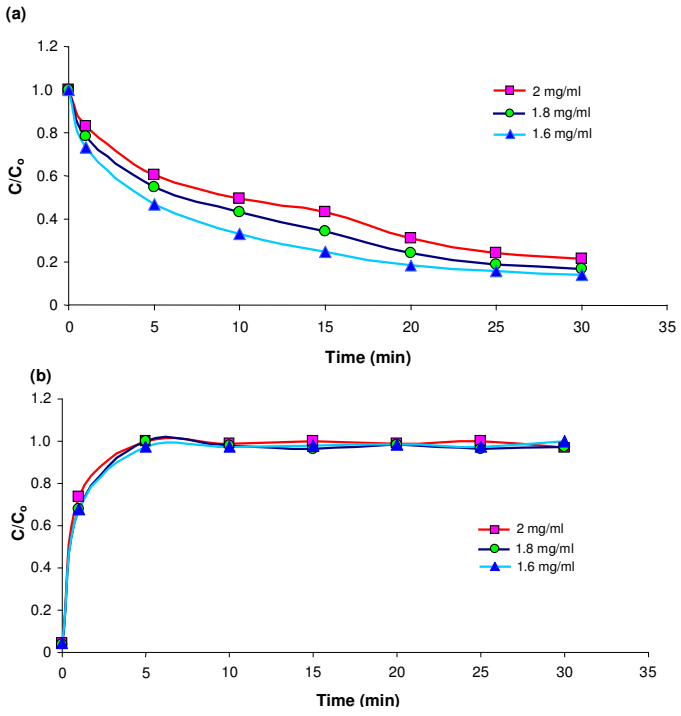

Figure 5. Batch binding of egg albumin to the Streamline™ DEAE adsorbent. (a) adsorbent (b) desorption

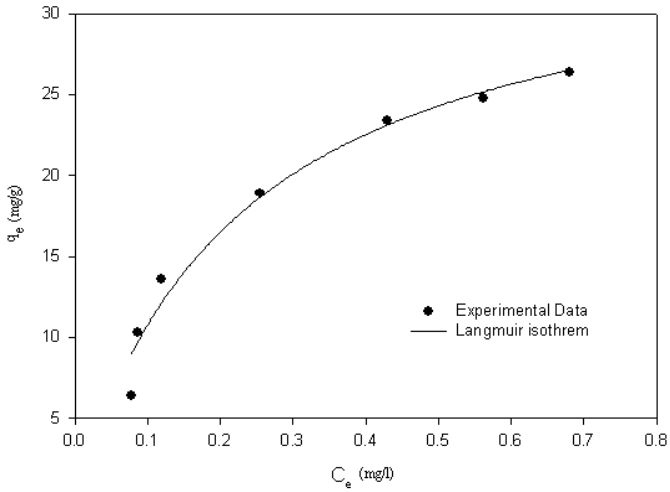

Figure 6. Adsorption Binding isotherms of egg albumin to the Streamline™ DEAE adsorbent.

was undertaken with egg albumin. The uptake of different concentrations of egg albumin upon the adsorbent is depicted as a function of different time-scales in Figure 5. It can be seen from Panel A that the uptake of egg albumin is, particularly over the first minute of reaction. Panel B in Figure 5 indicates that proteins desorption approached a steady state value approximately after 5 min of reaction time. Full equilibrium adsorption isotherm is given in Figure 6 and the extracted kinetic data given in Table 3. A good adsorption for the adsorbent is also indicated by the estimated value for q_m (Table 3). Enhanced rates of binding and the attainment of higher binding capacities may be influenced by the lowered diffusion resistance to the attainment of saturation imparted by the Streamline™ DEAE, a standard adsorbent for expanded bed adsorption, and for good penetration of egg albumin (Jahanshahi et al., 2005, 2008; Najafpour, 2007).

Table 3. Egg albumin adsorption equilibrium and kinetic parameters.

	q_m	C^*/K_d	R	Rsqr	Adj Rsqr
Langmuir isotherm	35.4415	4.3604	0.99	0.97	0.97

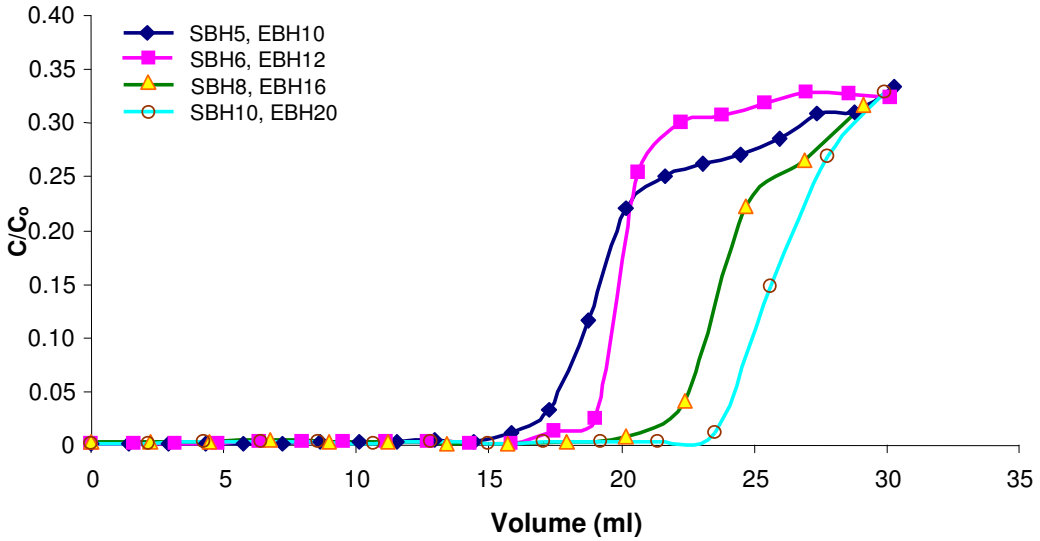

Figure 7. Breakthrough curve of egg albumin with Streamline™ DEAE adsorbent.

Expanded bed adsorption

The expanded bed adsorption performance of the adsorbent was investigated in the NBG contactor at different settled bed heights operated with bovine serum albumin and egg albumin at given flow velocities. As it can be seen from Figure 7 the dynamic capacity (estimated from the protein challenge required to achieve 10% breakthrough of the protein, $C/C_o = 0.1$) of SBH of 10 was the highest value recorded for the adsorbent under study, and the shape of all breakthrough curves closely approximated to that of a packed bed of that material operated similarly with the same feedstock (data not shown). This observation confirmed the relatively low degree of axial mixing and high stability of the fluidised bed in the NBG contactor. Very small SBH exhibited a lower dynamic capacity and a shallower increase in product breakthrough, which could be attributed to channelling resulting from minor bed instabilities, as it has been demonstrated in hydrodynamic assessments before. Full chromatogram of fluidised bed recovery of egg albumin also is displayed in Figure 8.

In addition, a step-by-step fluidised bed adsorption of bovine serum albumin in the commercial column as well as in the NBG column has been carried out. The step elution of Streamline™ DEAE in the commercial column resulted in broad peaks of protein concentration, whereas a similar step yielded a sharper peak for the NBG contactor such that the protein was eluted in a more concentrated form (Figure 9). However, in this comparative experiment, the NBG contactor exhibited the lowest breakthrough and potential for fore-shortening overall operational time with respect to loading, washing and elution stages, and might possess potential processing advantages by virtue of the distributor design.

Conclusion

A simple unique fluidised bed contactor (NBG), which was equipped with a glass tube fitted with a simple sintered glass distributor comprising a 100 µm mesh, has been prepared and used herein. In respect of physical performance, the bed expansion response of NBG was similar or better than commercial expanded bed contactor. In experiments undertaken to assess the hydrodynamic behaviour of fluidised bed systems (estimation of N, D_{axl} and B_O etc), the performance of the NBG was relatively constant across a range of expanded bed height and fluid velocities. This study was completed with that of Streamline in different column diameters and SBH which deteriorated within the same range of study.

Batch binding studies of egg albumin adsorption upon Streamline™ DEAE indicated that this exhibited an apparent reasonable adsorption and capacity for the protein under common experimental conditions. In addition, adsorption and desorption equilibria were achieved in a relatively short contact time (Figure 4). In fluidised

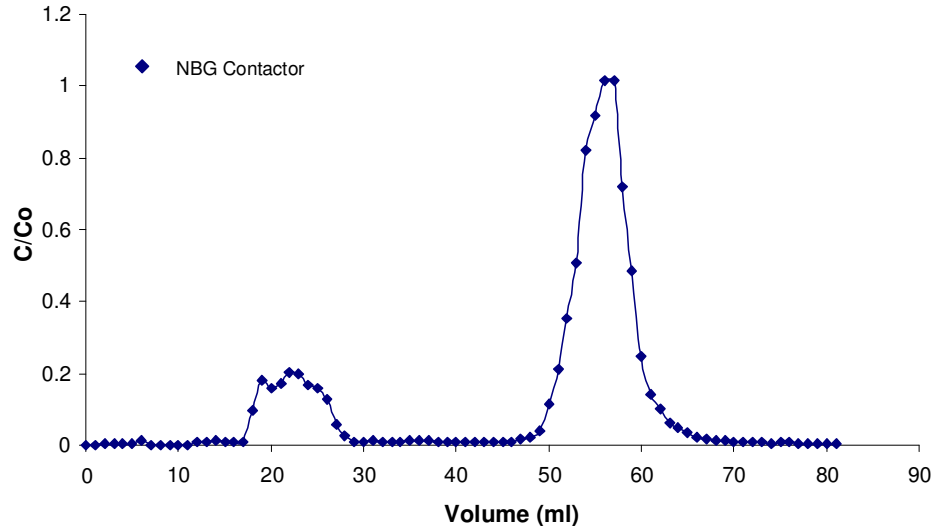

Figure 8. Full chromatogram of fluidized bed recovery of egg albumin in NBG contactor.

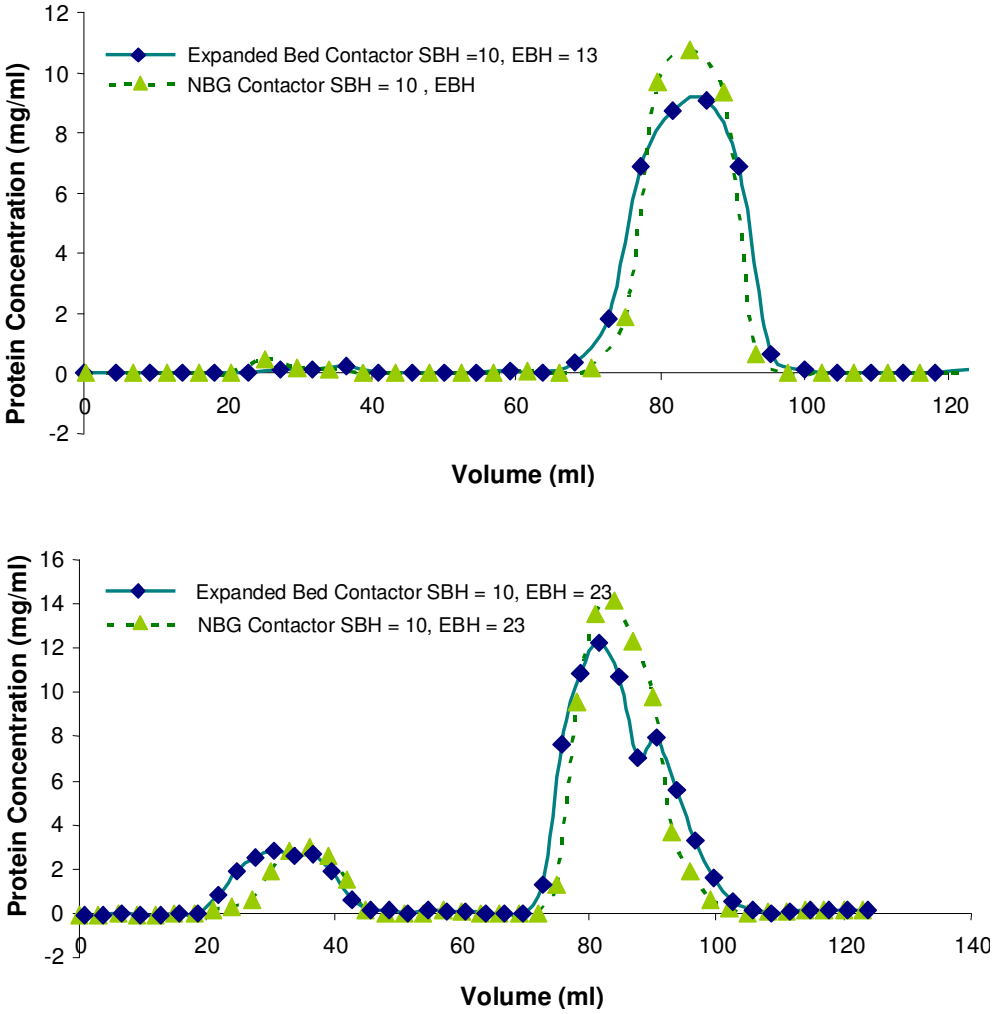

Figure 9. Full chromatogram of fluidised bed recovery of BSA in expanded bed and NBG contactors different expanded bed height.

bed experiments, the rapid achievement of baseline values of the feedstock in the effluents following the initiation of washing and elution steps confirmed and additional advantage of the NBG design (Figure 8). Furthermore, the ability to operate the adsorbent at flow velocities up to 2 or 3 times that of other column without diminution of adsorption or washing efficiencies because of its distributor is a clear practical advantage.

It has been believed that the simple, economic design of the NBG contactor compares favourably in hydrodynamic and biochemical performance with commercial expanded bed contactor. It can be concluded from the work reported herein that the prototype NBG contactor has many of the properties required as a column for protein purification and deserves further study in terms of refined geometric design, operational longevity and suitability for validated manufacturing operations which can be subjected to future publications.

ACKNOWLEDGEMENT

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Nomenclatures and greek letters

B_o, Bodenstein number; ***C****, Equilibrium concentration of the biomolecules in liquid phase; **D_{axl}**, Axial dispersion coefficient in liquid phase; **DEAE**, Diethyl amine ethyl; **d_p**, Diameter of the particle; **g**, Acceleration of gravity; **G_a**, Grash of number; **H**, Fluidised bed height; **K_d**, Adsorption equilibrium constant; **n**, Richardson-Zaki coefficient; **N**, Theoretical plate number; **NBG**, Nanobiotechnology Group; ***q****, Equilibrium concentration of the biomolecules in adsorbent solid phase; **q_m**, Maximum loading of adsorbate; **RTD**, Residence time distribution; **Re_t**, Terminal Reynolds number; **U**, Liquid flow velocity; **U_t**, Particle terminal velocity; **ε**, Bed voidage; **$ε_o$**, Settled bed voidage; **μ**, Viscosity of the liquid; **ρ**, Density of liquid phase; **$ρ_p$**, Density of solid phase; δ^2, Residence time variance.

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