Protein Adsorption and Hydrodynamic Stability of a Dense, Pellicular Adsorbent in High-Biomass Expanded Bed Chromatography

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Abstract A dense, pellicular UpFront adsorbent (ρ = 1.5 g/cm^3, UpFront Chromatography, Cophenhagen, Denmark) was characterized in terms of hydrodynamic properties and protein adsorption performance in expanded bed chromatography. Cibacron Blue 3GA was immobilised into the adsorbent and protein adsorption of bovine serum albumin (BSA) was selected to test the setup. The Bodenstein number and axial dispersion coefficient estimated for this dense pellicular adsorbent was 54 and 1.63 × 10^{-5} m^2/s, respectively, indicating a stable expanded bed. It could be shown that the BSA protein was captured by the adsorbent in the presence of 30% (w/v) of whole-yeast cells with an estimated dynamic binding capacity (C/C_0 = 0.01) of approximately 6.5 mg/mL adsorbent.

Keywords: hydrodynamic stability, protein adsorption, expanded bed chromatography, bovine serum albumin, yeast cells

Direct recovery of proteins from particulate-containing feedstock, including fermentation broth, cell lysates, and mammalian cell cultures by expanded bed chromatography is well established [1-5]. Expansion of adsorbent particles in the expanded bed chromatography permits free flow through of particulate materials (*i.e.*, cells and cell debris) while simultaneously capturing the desired proteins. The integrated technique of expanded bed chromatography has eliminated the complex solid/liquid operations such as centrifugation and filtration used in conventional downstream processing approaches [6].

The success of expanded bed chromatography depends largely on the characteristics of the adsorbent used. Generally, the adsorbent must have minimal nonspecific interaction with biomass as well as little particle aggregation within the adsorbent. Preferably, the adsorbent particle is small to reduce the diffusion resistance [7], and the density of adsorbent must be larger than the feedstock to prevent the particle entrainment. The expanded bed formed in the column must also be hydrodynamically stable [8]. In a previous study, we showed that an increase in the biomass concentration resulted in elevated feedstock density and viscosity that raised the voidage in the column [1]. The bed expanded excessively and the column bed collapsed. Commercially available adsorbents with an average density of 1.2 g/mL are not suited for industrial scale expanded bed chromatography [9]. High-density adsorbents (ρ>1.2 g/mL) are needed for the purification of feedstock with a higher biomass concentration.

The focus of this work was the hydrodynamic stability of the expanded bed and the protein adsorption performance of a pellicular, dense adsorbent (ρ = 1.5 g/mL) developed by UpFront Chromatography A/S (Copenhagen, Denmark) [2]. Cibacron Blue 3GA dye-ligand (Sigma, St. Louis, MO, USA) was immobilized onto the adsorbent particles according to Dean and Watson [10] to be used as a ligand for direct adsorption of protein from unclarified yeast feedstock. The immobilized dye-ligand concentration was determined using a method adapted from Chamber [11]. The bovine serum albumin (BSA) was chosen as a model protein in this study.

UpFront agarose beads are a pellicular adsorbent with a particle size in the range of 151 to 323 μm and a density of 1.5 g/mL. They consist of 6% cross-linked agarose and non-porous core particles [2]. The bed expansion characteristics of UpFront adsorbent were determined in a 20-mm inner diameter UpFront FastLine column. Even flow distribution in the column was achieved by agitation with a magnetic stirrer at the base of the contactor [1].

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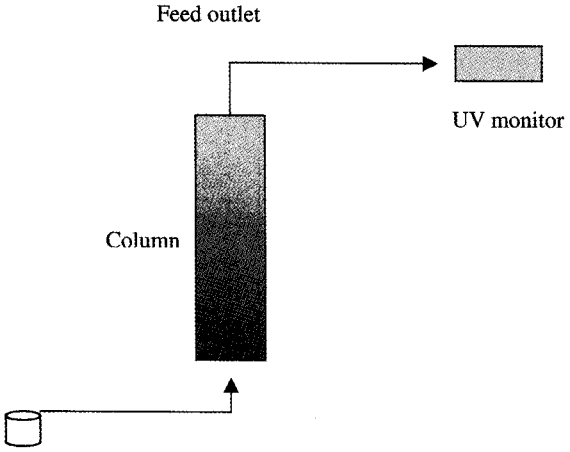

Fig. 1. Schematic diagram of the expanded bed system.

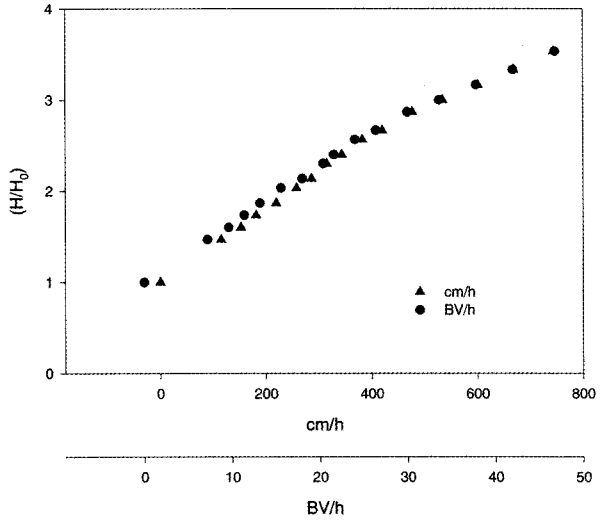

Fig. 2. Bed expansion of the dense, pellicular UpFront adsorbent.

The column was loaded with UpFront adsorbent (50 mL) to a settled-bed height (H_0) of 15 cm and connected to a peristaltic pump (Watson-Marlowe Company Ltd., UK) that provided fluid. The flow velocity was then increased stepwise until a stable bed height was obtained. The bed was allowed to stabilize for 5 min to ensure that that no channels built up and the beads made small movements (Fig. 1).

The residence time distribution (RTD) test is a tracer stimulus method that can be used to assess the degree of longitudinal axial mixing (dispersion) in the expanded bed by defining the number of theoretical plates. Diluted acetone solution [12,13] was used as a tracer input for the fluid entering the column (frontal mode). Ultraviolet absorbance was detected via fast protein liquid chromatography utilizing a UV-M detector (Amersham Pharmacia Biotech, Uppsala, Sweden). The bed was first fully expanded with working buffer (50 mM Tris-HCl, pH 7.5) and allowed to stabilize for 20 min to achieve steady-state. The working buffer was then replaced with 1% (v/v) acetone, and the UV-absorbance of the fluid column outflow was measured (positive step input signal). After the UV signal became stable around a maximal value (approx. 40 min) the diluted acetone solution was again replaced with working buffer (negative step input signal), which was passed through the bed until a stable baseline UV signal (0%) was recorded.

UpFront adsorbent was loaded into the column to a 15-cm sedimented-bed height and fluidized with binding buffer (50 mM Tris-HCl, pH 7.5) for 4 h at a flow rate of 225 cm/h. Using the same flow rate, feedstock containing 30% (w/v) Baker's yeast suspension and 3.0 mg/mL BSA was loaded into the column. The protein concentration of the effluent was monitored and the experiment stopped if it exceeded 95% of the initial protein concentration. Sorption performance was expressed as the ratio of the breakthrough capacity at 1% breakthrough to the equilibrium capacity (*i.e.*, the maximum BSA binding capacity of the adsorbent under the experimental conditions).

Our data demonstrate that the bed expanded only 3.5-fold in working buffer solution at flow velocities as high as 800 cm/h (Fig. 2). Earlier experiments with Streamline DEAE adsorbent – a lower density adsorbent manufactured by Amersham Pharmacia Biotech – had necessitated a much lower flow rate of approximately 350 cm/h to achieve the same bed expansion [1]. Streamline DEAE (ρ = 1.2 g/mL) is an adsorbent designed for expanded bed operation consisting of non-porous crystalline core materials and 6% crosslinked agarose. We demonstrated in this study that UpFront adsorbent expansion was relatively low compared to that of Streamline adsorbent despite an elevated biomass concentration (30% (w/v), Fig. 2). The Streamline adsorbent bed was unstable once the feedstock biomass concentration reached 20% (w/v). Because bed stability is critical to the performance of an expanded bed adsorption operation, UpFront adsorbent was better suited to process feedstock with a higher biomass concentration at a shorter processing time and a higher flow velocity. The Streamline Direct CST I and Streamline Direct HST (new generation of adsorbents introduced by Amersham Pharmacia Biotech) which have higher particle density (ρ = 1.8 g/mL) and salt-tolerant functional groups (*i.e.*, improved adsorption and fluidization performance) may also can be employed in facilitating expanded bed operations.

The Richardson-Zaki correlation [14] describes the effects of bed voidage (ε), on the settling velocity rate (U_t). It can be used to predict the liquid velocity required to produce a given degree of expansion during fluidization. It can also be used to predict and model the expansion behavior of expanded bed adsorption columns. The bed expansion characteristics of UpFront adsorbent in the presence of buffer and 30% (w/v) yeast cells are shown in Fig. 2. The bed height increased linearly with the fluid velocity and could be described by the Richardson-Zaki equation:

$$U = U_t \varepsilon^n \quad (1)$$

Table 1. Bed expansion characteristics of UpFront adsorbent in 30% (w/v) yeast feedstock

Richardson-Zaki coefficient, n	5.3
Terminal settling velocity, U_t (cm/h)	1585.0

where U is the liner velocity (cm/h), ε is the bed voidage, U_t is the terminal settling velocity (cm/h), and n is the Richardson-Zaki exponent. The bed voidage could be estimated via Eq. 2:

$$\frac{H}{H_o} = \frac{(1-\varepsilon_0)}{(1-\varepsilon)} \tag{2}$$

where ε_0 is the settled-bed voidage, and H/H_0 is the ratio of the expanded and sediment bed height, respectively. The settled-bed voidage ε_0 was assumed to be 0.4 [15]. The bed values of n and U_t were determined experimentally from bed expansion data by finding the slope (n) and the y-intercept (U_t) of log U *vs.* log ε [16]. The values for U_t and n for binding buffer containing 30% (w/v) biomass were 1,585 cm/h, and 5.3, respectively (Table 1). The terminal velocity U_t of the yeast containing buffer was lower (3,162 cm/h, Fig. 3) than the value determined in a previous study [2] using binding buffer as the fluid phase. This reduction might have been the result of a higher viscosity due to more yeast biomass. The adsorbent bed of the 30% (w/v) yeast feedstock was judged to be stable since its n value was corresponded to the theoretical value of 4.8, which is commonly used to indicate the flow is in laminar flow regimes [17].

To assess the degree of longitudinal axial mixing (dispersion) in the expanded bed we used the RTD test, a tracer stimulus method (Fig. 3). The performance of expanded bed chromatography depends very much on the stability of the bed expansion while maintaining a low axial mixing level. A 1% (v/v) acetone-buffer solution was used as the tracer input for fluid entering the column. The number of theoretical plates was calculated from the mean residence time of the tracer and the variance of the tracer output signal, representing the standard band broadening of a sample zone. The number of theoretical plates and height equivalent to a theoretical plate (HETP) were calculated using the following equations:

$$N = t_m^2/\sigma^2 \tag{3}$$

$$HETP = L/N \tag{4}$$

where t_m is the mean residence time, σ is the standard deviation, and L is the height of the expanded bed. The variance (σ) was used to give σ_θ^2 (Eq. 5) and to estimate the Bodenstein number (Eq. 6) [18], which is the ratio of convection to dispersion mass transport as defined in Eq. 8 [19].

$$\sigma_\theta^2 = \sigma^2/t_m^2 \tag{5}$$

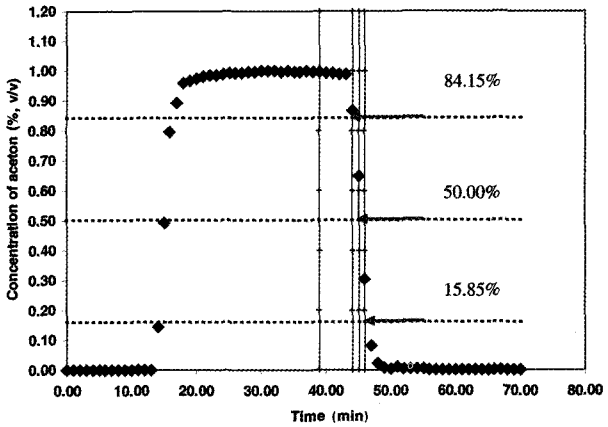

Fig. 3. Residence time distribution (RTD) test of the dense, pellicular UpFront adsorbent. The standard deviation was calculated by taking half the distance between the points 15.85 and 84.15% of the maximum acetone concentration [12].

Table 2. Theoretical plate numbers and height equivalents (HETP), Bodenstein numbers, and dispersion coefficient (D_{ax}), for a dense, pellicular adsorbent expanded bed column

Column volume (cm^3)	100.5
Linear flow rate (cm/h)	764.0
Sedimented height (cm)	15.0
Expanded bed height (cm)	29.0
Bed voidage, ε	0.7
Theoretical residence time, t (min)	2.5
Mean residence time, t_m (min)	5.0
Standard deviation, σ	1.0
Number of theoretical plates, N	25.0
Height equivalent to a theoretical plate (HETP) (cm)	1.28
Bodenstein number	54.0
Dispersion coefficient, D_{ax} (m^2/s)	1.63×10^{-5}

The mean residence time is a function of the distance between the maximum acetone concentration (MAC) to 50% of the MAC [12] using an RTD test. The theoretical residence time is the hydrodynamic residence time calculated from the column volume and the applied flow rate.

$$\sigma_\theta^2 = 2/B_o + 8/B_o^2 \tag{6}$$

$$B_o = uH/\varepsilon D_{ax} \tag{7}$$

where u is the superficial fluidizing velocity, ε is the bed voidage, and D_{ax} is the axial dispersion coefficient.

Anspach *et al.* [20] reported that expanded bed with Bodenstein numbers>40 coincide with low axial mixing in the column and an adsorption performance similar to a packed bed. The Bodenstein number and axial dispersion coefficient estimated for the here-tested dense pellicular adsorbent were 54 and 1.63×10^{-5} m^2/s, respectively (averaged from two RTD tests, Table 2). The axial dispersion coefficient was consistent with little axial mixing and comparable in magnitude to the findings of Tong and

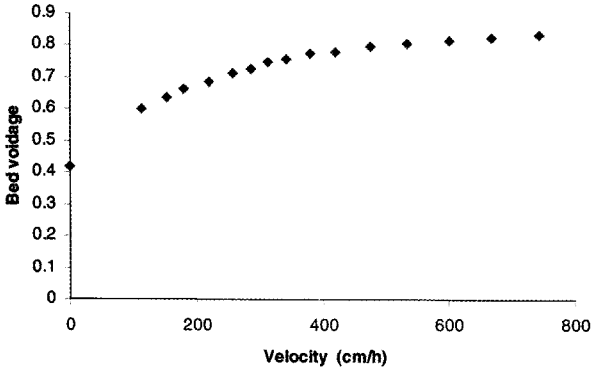

Fig. 4. Flow velocity versus bed voidage.

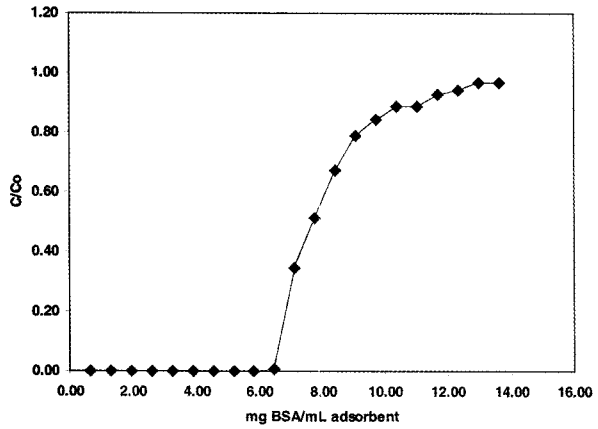

Fig. 5. Protein adsorption of a dye-ligand immobilized dense, pellicular UpFront adsorbent in the presence of 30% (w/v) yeast cells.

Sun [21]. We therefore conclude that the expanded bed with the dense pellicular adsorbent was suitable for expanded bed adsorption operations. The mean residence time (t_m = 5 min) obtained here (Table 2) was longer than the theoretical residence time (t = 2.5 min), which may be a reflection of axial dispersion in the expanded bed column [22]. It has been reported that the tracer (*i.e.*, acetone) can in some cases diffuse into the adsorbent [22]. This may have contributed to the longer mean residence time observed in our study.

The axial dispersion coefficient strongly depends on bed voidage and flow rates [23]. The bed voidage characteristics of UpFront adsorbent in the presence of 30% (w/v) yeast cells are shown in Fig. 4. There was no significant variation (<15%) in bed voidage when the velocity was increased from 200 to 600 cm/h, which, again, may be the result of low axial dispersion within the expanded bed. It is known that low-degree dispersion can be achieved by using an adsorbent with a relatively high density [23]. Our data support this notion.

Protein (BSA) adsorption with dense, pellicular adsorbent immobilized dye-ligand from feedstock containing 30% (w/v) yeast cells was tested in an UpFront contactor. Localized small circular movements of the dense pellicular adsorbent particles were clearly visible, indicating a stable expanded bed had been obtained [8]. As can be seen in Fig. 5, BSA protein was successfully captured by the dye-ligand immobilized dense pellicular adsorbent in the present of 30% (w/v) biomass. The dynamic binding capacity at C/C_0 = 0.01 was close to 6 mg/mL settled adsorbent. The relatively high density of this pellicular adsorbent (1.5 g/mL) allowed the processing of feedstock with a high biomass concentration without diluting the feedstock. This would reduce processing time and, hence, minimize product modification and/or inactivation due to the long processing time [7,24].

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