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Performance comparison of suspended bed and batch contactor chromatography

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Abstract

In some applications, the purification and recovery of biomolecules is performed via a cascade of batch adsorption and desorption stages using agitated contactors and related filtration devices. Suspended bed chromatography is a recent process-scale innovation that is applicable to these separations. This hybrid technique exploits the benefits of combining batch adsorption in an agitated contactor with elution in an enclosed column system. To some extent, the process is similar to batch contactor chromatography but can be fully contained and significantly quicker. The process has two steps; first the fluid containing the sample is mixed with the adsorbent in a stirred tank. Second, the slurry suspension is transferred directly into a specialized column, such as an IsoPak column. The media with the adsorbed product is formed as a packed bed, whilst the suspension liquid is passed out of the column. The product is then eluted from the packed bed utilizing standard column-chromatography techniques. The performance of the suspended bed and the agitated contactor operations are demonstrated both by full-scale experimental results and process simulations. The purification of ovalbumin from a hen-egg white feedstock by anion-exchange chromatography was used as a case study in order to prove the concept. With the availability of both pump-packed systems and shear-resistant media, suspended bed chromatography is a better alternative for a range of applications than the traditional batch separations using agitated contactors.

Keywords: Suspended bed chromatography; Batch contractor chromatography; Adsorption; Preparative chromatography; Proteins; Ovalbumin

1. Introduction

Preparative and process-scale chromatography has established itself as an important unit operation within the downstream processing of bio-pharmaceuticals [1]. The applications of process chromatography include a range of separations for different biopolymers, including proteins, peptides, nucleic acids and carbohydrates. Ion exchange is a chromatography technique that is widely applied in downstream processing and can be carried out using agitated or column contactors. The two process approaches have been compared elsewhere [2].

There is a current trend towards process intensification aimed at the implementation of techniques that afford higher product throughput and lower operational costs. In order to address these needs, there has been recent progress in the development of

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equipment for reproducible and automated pump-driven packing and unpacking of columns for large-scale chromatography [3]. The use of such equipment for the large-scale purification of ovalbumin from egg-white feedstock has been reported [4]. Suspended bed chromatography relies on the use of such equipment in order to provide an alternate solution applicable to the downstream purification of biopharmaceuticals [5]. In this way, the equipment primarily intended for column packing and unpacking can be additionally employed to conduct other integrated downstream operations.

Calculation of non-linear chromatographic band profiles is a useful tool that is applicable to the analysis, design, scale-up and optimization of chromatography operations [6]. Once the model is in place, different process conditions can be systematically investigated in order to determine the optimal outcome of the process [7]. The simulation of overloaded chromatographic band profiles has been successfully applied to the separation of drugs [8], enantiomers of pharmaceutical importance [9], simulated moving bed separations [10] and proteins [11–14]. However, the overwhelming majority of this simulation work refers to small-scale separations. In this paper, we use the general rate model of chromatography [6] to analyse the large-scale separation of egg-white proteins via different techniques, namely batch adsorption in agitated contactors, column chromatography and suspended bed chromatography.

2. Experimental

2.1. Materials and equipment

Cell debris remover (CDR) and Express-Ion Q were obtained from Whatman (Maidstone, UK). An IsoPak column (50×44 cm I.D.) and an associated recirculating slurry preparation station equipped with a 750-l vessel were obtained from Millipore (Stonehouse, UK). Tris(hydroxymethyl)aminomethane (Tris) was obtained from Merck (Poole, UK). All other chemicals were of analytical reagent grade. Fresh, large hen eggs were obtained from Barradale Farms (Headcorn, UK).

2.2. Procedures

2.2.1. Adsorption data

A batch technique for measuring the equilibrium data was applied [6]. Express-Ion Q was equilibrated with 0.025 *M* Tris–HCl buffer (pH 7.5). The adsorption of an initial 2.0 g/l solution of ovalbumin was carried out in a batch stirred vessel (20 ml) using different masses of dry media over a 180-min period. After equilibration, the media were separated and the absorbance of the solution was read at 280 nm. From the final, equilibrated, concentration of ovalbumin in the solution, the amount adsorbed was calculated by material balance.

2.2.2. Batch kinetics

Express-Ion Q was equilibrated with 0.025 *M* Tris–HCl buffer (pH 7.5). The adsorption of 2.0 g/l ovalbumin was carried out in a batch stirred tank (250 ml) using 370 mg dry mass of the media over a 60-min period, with continuous monitoring of the adsorbate solution at 280 nm.

2.2.3. Feedstock preparation

Egg-whites (55 l) were separated from 1440 fresh hen eggs and diluted to 10% (v/v) with 0.025 *M* Tris–HCl buffer (pH 7.5). The egg-white suspension was clarified using a total of 35 kg of pre-equilibrated CDR in a batch mode. Spent CDR was removed by centrifugation through a 1.6×0.6 mm slotted screen (EHR 500 basket centrifuge, Robatel and Mulatier, Lyons, France) and the sample was clarified through Grade 541 filter paper (Whatman). The clear solution (550 l) containing 5 mg/ml of total protein was used for chromatography on Express-Ion Q.

2.2.4. Column chromatography

Express-Ion Q (20 kg) was equilibrated with 0.025 *M* Tris–HCl buffer (pH 7.5) to give a final slurry concentration of ca. 30% (w/v) using the recirculation slurry preparation station associated with the IsoPak column. The height of the 44 cm I.D. Isopak column was adjusted to 16 cm, and the column was pump-packed in upflow from the slurry tank at a pressure of ca. 10 p.s.i. (1 p.s.i.=6894.76 Pa) according to the column manufacturer's guidelines. The packed bed of Express-Ion Q (44×16 cm I.D.)

had a volume of ca. 24.3 l and a packing density of 0.227 kg/l.

Egg-white feedstock (500 l) was loaded onto the column and non-bound material was removed by washing with 150 l of 0.025 *M* Tris–HCl buffer (pH 7.5). Bound material was eluted using a 400-l linear gradient of 0–0.5 *M* NaCl in 0.025 *M* Tris–HCl buffer (pH 7.5). The Express-Ion Q was re-equilibrated with 0.025 *M* Tris–HCl buffer (pH 7.5). The flow-rate was maintained at 150 cm/h throughout. All procedures were carried out in downflow at room temperature (15–20°C).

2.2.5. Suspended bed chromatography

Express-Ion Q (16.25 kg) was equilibrated with 0.025 *M* Tris–HCl buffer (pH 7.5) and collected by centrifugation through a 1.6×0.6 mm slotted screen (EHR 500 basket centrifuge). Egg-white feedstock was transferred to the IsoPak slurry preparation vessel (750 l), and the equilibrated Express-Ion Q was added to the feedstock. The ion exchanger was maintained in suspension by continuous, closed loop recirculation (30–40 l/min) of the slurry through the IsoPak slurry preparation unit. Adsorption was continued in suspended-bed mode for 30 min.

The height of the 44 cm I.D. IsoPak column was preset to 16.3 cm (ca. 24.8 l) and the feedstock/media suspension was pumped through the column upflow from the slurry tank at a constant packing pressure of ca. 10 p.s.i., according to the column manufacturer's guidelines. During this stage of column packing, depleted feedstock was exhausted to waste. The column's upper bed support was used as a filter to retain the media. Bed consolidation continued until ca. 100 l of the suspension remained, at which time, the exhaust liquid was recirculated through the system to ensure that all of the adsorbent was packed into the column. Then, another 30 l of depleted feedstock were directed to waste. Additional pre-equilibrated media (2.8 kg) were suspended in the residual 70 l of depleted feedstock, and this slurry was pumped into the column to fill any voidage present. In this way, column packing was completed.

The packed column of Express-Ion Q (44×16.3 cm I.D.) had a volume of ca. 24.8 l and a packing density of 0.229 kg/l. Non-bound material was removed by washing with 150 l of 0.025 *M* Tris–HCl buffer (pH 7.5). Bound material was eluted using a 400-l linear gradient of 0–0.5 *M* NaCl in 0.025 *M* Tris–HCl buffer (pH 7.5). All procedures were carried out in upflow at room temperature (15–20°C). The ascending-flow mode ensures that none of the unbound or desorbed material contacts the fresh adsorbent used to replenish the bed, which may originate secondary, unwanted, effects. The flow-rate was maintained at 300 cm/h during column operations. Following elution, the IsoPak column was pump-unpacked, without disassembly.

2.3. Assays

Pooled fractions at various stages of chromatography were assayed for total protein and ovalbumin content by fast protein liquid chromatography (FPLC) [15].

3. Theoretical

3.1. Multicomponent equilibrium

Within the framework of the steric mass-action (SMA) ion-exchange equilibrium formalism, the steric hindrance of salt counterions upon protein binding is accounted for via the steric factor of the protein, σ_i [14]. In a system of n proteins and one salt, the stoichiometric exchange expression for the i-protein can be written as:

$$C_i^* + \nu_i\overline{Q_1^*} \overset{K_i}{\leftrightarrow} Q_i^* + \nu_i\overline{C_1^*} \tag{1}$$

in the above equation, $i=2,3,\ldots,n+1$. Note that subscript 1 corresponds to the salt. The equilibrium present in Eq. (1) is characterized by the constant K_i, and the protein exhibits a characteristic charge equal to ν_i. The equilibrium concentrations of a solute in the mobile and stationary phases are defined by C_i^* and Q_i^*, respectively.

In the above-mentioned system, the equilibrium is described by the expression:

$$K_i = \frac{Q_i^*}{C_i^*} \cdot \left[\frac{C_1^*}{\Lambda - \sum_{i=2}^{n+1} (\nu_i + \sigma_i) Q_i^*} \right]^{\nu_i} \tag{2}$$

where Λ is the total capacity of the ion-exchange media. Note that, for the salt, $K=1$, $\nu=1$ and $\sigma=0$ [14]. Within the framework of the SMA formalism, ideality of both phases at equilibrium is assumed. Although this is hardly to be the case for the real situation, it is possible that the activity coefficients of the protein are roughly constant in the range of studied concentrations. As a result, the contribution of the non-ideality is probably lumped within the parameters present in Eq. (2), as has been shown to be the case for a simpler case of low-molecular-mass organics in a reversed-phase system [16].

3.2. Calculation of concentration profiles

3.2.1. Stirred tank process

The separation process in a stirred tank is governed by two mass transfer resistances: (i) film resistance and (ii) intraparticle resistance. In order to account for the mass transfer occurring inside the particle, the following conservation equation can be written:

$$\frac{\partial Q_i}{\partial t} = \frac{D_{\text{eff}}}{R^2} \cdot \left(\frac{\partial^2 Q_i}{\partial \rho^2} + \frac{2}{\rho} \cdot \frac{\partial Q_i}{\partial \rho} \right) \tag{3}$$

where $Q_i(\rho,t)$ is the solute concentration inside the particle, a function of both radial position (ρ) and time (t). The radial position is normalized with respect to the particle radius (R). The kinetics of intraparticle mass transfer is accounted for via an effective diffusion coefficient (D_{eff})

The above equation has to be solved subject to initial and boundary conditions. The initial concentration profile in the particle is given by:

$$Q_i(\rho,0) = \Xi(\rho) \tag{4}$$

The balance of solute flux across the film gives the first boundary condition at the surface of the particle:

$$\left(\frac{\partial Q_i}{\partial \rho} \right)_{(1,\theta)} = Bi\left(C_i^f - C_i^s\right) \tag{5}$$

where C_i^f and C_i^s are the liquid-phase concentrations in the bulk fluid and particle surface, respectively. The Biot number is defined by:

$$Bi = \frac{Rk_{\text{f}}}{D_{\text{eff}}} \tag{6}$$

where k_{f} is the film mass transfer coefficient. The value of k_{f} was estimated following Armenante and Kirwan [17].

The second boundary condition is defined by the existence of equilibrium at the surface of the particle:

$$C_i^s = \frac{Q_i(1,\theta)}{K_i} \cdot \left[\frac{C_1^s}{\Lambda - \sum_{i=2}^{n+1} (\nu_i + \sigma_i) Q_i(1,\theta)} \right]^{\nu_i} \tag{7}$$

The third boundary condition is given by the fact that there is no transfer at the center of the particle:

$$\left(\frac{\partial Q_i}{\partial \rho} \right)_{(0,\theta)} = 0 \tag{8}$$

On the other hand, the conservation equation related with the fluid phase in the tank is given by:

$$\frac{\mathrm{d}C_i^f}{\mathrm{d}t} = -3\frac{V_{\text{m}}}{V_{\text{f}}} \cdot \frac{k_{\text{f}}}{R} \cdot \left(C_i^f - C_i^s\right) \tag{9}$$

where V_{m} and V_{f} are the volumes of the media and fluid in the tank, respectively.

The initial condition for Eq. (9) is determined by the initial concentration of the solute (C_i°) in the bulk liquid:

$$C_i^f(0) = C_i^\circ \tag{10}$$

The set of Eqs. (3)–(10) describe the separation process in the stirred tank. The solution of these equations was found via orthogonal collocation on finite elements [6]. Following discretization, the resulting stiff system of differential–algebraic equations was numerically integrated using the MATLAB routine ode15s. This routine uses a quasi-constant step size implementation of numerical differentiation formulas in terms of backward differences.

3.2.2. Fixed-bed process

In addition to fluid film and intraparticle mass transfer resistances, the process in the column is influenced by axial dispersion. The packed bed is, however, assumed to be radially homogeneous, i.e., the process parameters are constant over the cross section.

The nondimensional mass balance in a column slice gives the concentration in the fluid stream as a function of both position and time, $C_i^f(z,\theta)$ as:

$$\frac{\partial C_i^f}{\partial \theta} + \frac{\partial C_i^f}{\partial z} + 3\frac{1-\epsilon}{\epsilon}\cdot\frac{k_f\tau}{R}\cdot\left(C_i^f - C_i^s\right) = \frac{1}{Pe}\cdot\frac{\partial^2 C_i}{\partial z^2} \tag{11}$$

In the above equation, the nondimensional position (z), nondimensional time (θ), residence time (τ) and Peclet number are defined by:

$$z = x/L \tag{12}$$

$$\theta = t/\tau \tag{13}$$

$$\tau = L/u \tag{14}$$

$$Pe = uL/D_a \tag{15}$$

In the above equations, ϵ is the porosity of the packed bed, x is the actual position in the direction of the flow, L is the column length, u is the interstitial velocity and D_L is the axial dispersion coefficient.

The initial condition corresponding to Eq. (11) is given by the profile of solute concentration along the column:

$$C_i^f(z,0) = \Phi(z) \tag{16}$$

The first boundary condition is defined by the 'close–close' Danckwerts condition at the column inlet:

$$\left(\frac{\partial C_i^f}{\partial z}\right)_{(0^+,\theta)} = -Pe\left[C_i^f(0^-,\theta) - C_i^f(0^+,\theta)\right] \tag{17}$$

Note that, in Eq. (17), the shape of the injection profile at the column inlet defines the first term within the bracket, i.e.:

$$C_i^f(0^-,\theta) = \Psi(\theta) \tag{18}$$

The second boundary condition is defined by the stop of the mass transfer at the column outlet:

$$\left(\frac{\partial C_i^f}{\partial z}\right)_{(1,\theta)} = 0 \tag{19}$$

The set of Eqs. (3)–(8) and (11)–(19) describe the separation process in the chromatography column. The numerical solution of these equations was found in the same way as described above for the set of the stirred tank equations. The axial dispersion and fluid-film mass-transfer coefficients in the packed bed were estimated following Athalye et al. [18]. Experimental measurements of column efficiency for IsoPak columns were found to be in good agreement with these correlations [19].

4. Results and discussion

4.1. Equilibrium data

It has been previously shown that, under the experimental conditions applied in this study, only ovalbumin is effectively bound to the media [4,20]. Note that hen egg-white predominantly contains ovalbumin [21]. Accordingly, the modeling efforts in this paper will be restricted to the description of the chromatographic behavior of ovalbumin, the target protein.

Experimental ion-exchange equilibrium data for ovalbumin are presented in Fig. 1. As can be seen from this figure, the SMA formalism properly correlates the experimental data. The identified values of the model parameters along with the estimated errors are also presented in the caption of Fig. 1. The four parameters of the model were simultaneously fitted to the experimental data. The physical significance of

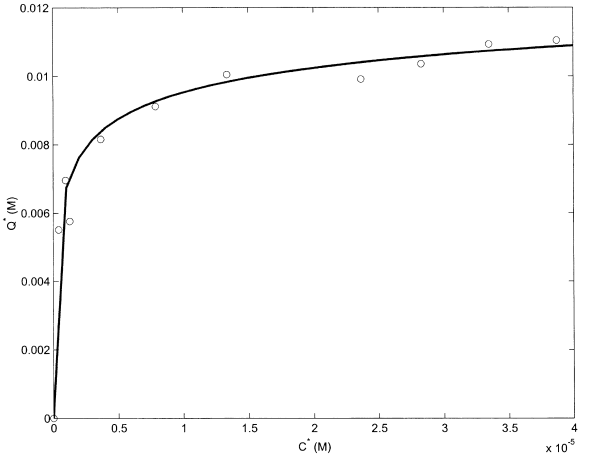

Fig. 1. Ion-exchange equilibrium data of ovalbumin (circles) and correlation via the SMA formalism (line) in 0.025 M Tris–HCl buffer (pH 7.5). Equilibrium parameters: $\Lambda = 1 \pm 0.1$ M, $K = (2.5 \pm 1.7)\cdot 10^{-5}$, $\nu = 6.1 \pm 0.1$ and $\sigma = 53 \pm 6$.

these parameters is described elsewhere [14]. The analysis of the fitted values shows that the errors associated with the determination of some parameters, especially the equilibrium constant, are not small. The identification of the media capacity and the steric factor is associated with smaller errors. Finally, the estimate of the characteristic charge was made with little error. Accordingly, an equivalent fit of the experimental adsorption data can be obtained with different combinations of the presented parameter estimates within the boundaries defined by the associated errors.

It is noteworthy that the identified value of Λ is close to the known capacity of the Express-Ion Q anion exchanger. Note that the small-ion capacity, the column packing density and the column voidage associated with this media were reported to be equal to 0.93 mequiv./dry g, 0.22 dry g/ml and 75% (v/v), respectively [20]. Combining these values, a capacity of 0.82 *M* is obtained. This value is reasonably close to Λ due to the approximate nature of the values for the packing density and the voidage. Also, the identified value ν for the target protein is close to a previously reported value for ovalbumin in ion-exchange systems [11].

4.2. Batch kinetics

The experimental kinetics of uptake for ovalbumin in a stirred tank are presented in Fig. 2. Note that the equilibrium concentration is achieved in about 10 min. The stirred tank model described above properly accounts for the experimental data. As a result, a value of 10^{-8} cm^2/s was estimated for the intraparticle diffusion coefficient, D_{eff}. This value of D_{eff} is close to values reported for ovalbumin in other ion exchangers [11,12]. The identified value of D_{eff} was used in all of the calculations associated with the operation of either the stirred tank, column or suspended-bed chromatography.

4.3. Column chromatography

A large-column separation of the feedstock is presented in Fig. 3. As seen from this figure, the location of the predicted ovalbumin peak is in good agreement with the experimental data. On the other hand, there is some disagreement between the model

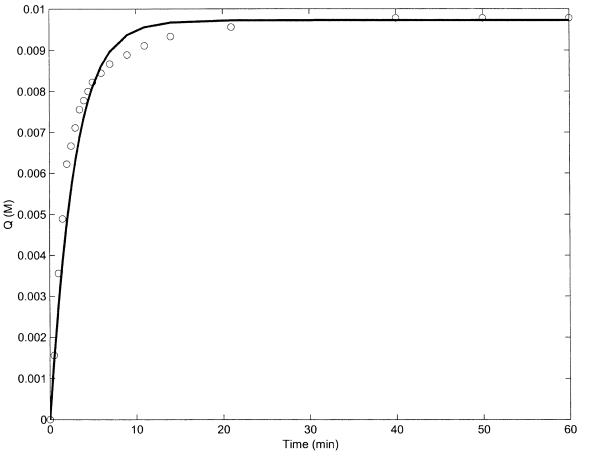

Fig. 2. Kinetic uptake of ovalbumin by Express-Ion Q (circles) and correlation via the intraparticle diffusion model (line) in 0.025 *M* Tris–HCl buffer (pH 7.5).

predictions and the shape of the experimental ovalbumin band and gradient profile. Part of this disagreement is due to the fact that, for the sake of comparison, the ordinate of both experimental and theoretical profiles was normalized with respect to the profile maximum.

Two factors contribute to the disagreement in the case of the protein band. The first is that the

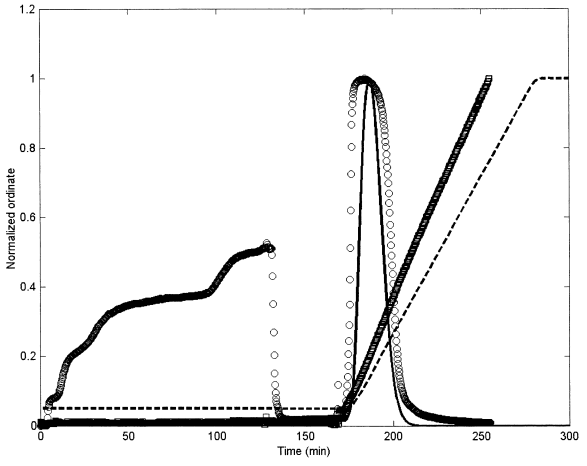

Fig. 3. Experimental (symbols) and calculated (lines) traces corresponding to the column chromatography purification of ovalbumin from egg whites. The traces presented are the experimental UV signal (circles), experimental conductivity signal (squares), calculated ovalbumin profile (solid line) and calculated salt profile (dashed line). For experimental conditions, see text.

experimental UV signal is truncated as a result of detector saturation and the calculated band is not. The second effect is the nonlinear nature of the relationship between protein concentration and protein UV absorption at higher concentrations. Note that, despite the disagreement between the shape of the calculated and experimental band profiles for ovalbumin, FPLC analysis of the desorbed protein demonstrated it to be predominantly ovalbumin, with some ovomucoid and ovoglobulin content; observations that are in keeping with those previously reported for Express-Ion Q [4,5,20].

Although the experimental conductivity often maintains a linear relationship with the salt concentration, the proportionality coefficient is not necessarily equal to one. As a result, the applied normalization will not produce an experimental conductivity profile and a theoretical salt profile with the same slope. Note, however, that both the experimental and the calculated gradient profiles presented in Fig. 3 start at the same time.

4.4. Suspended-bed chromatography

A separation performed using suspended-bed chromatography is presented in Fig. 4. Note that the model also fairly predicts the outcome of the process in this case.

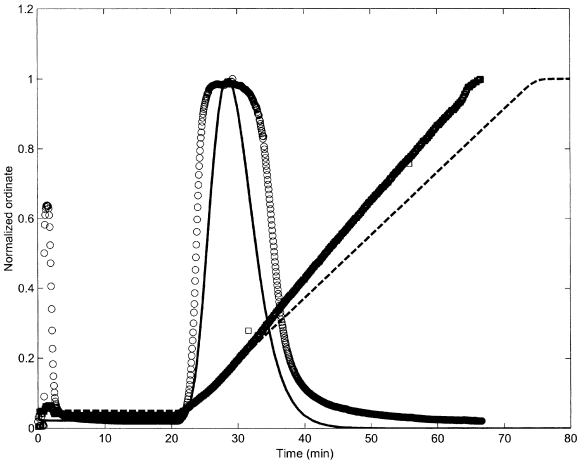

Fig. 4. Experimental (symbols) and calculated (lines) traces corresponding to the suspended-bed chromatography purification of ovalbumin from egg whites. The traces presented are the experimental UV signal (circles), experimental conductivity signal (squares), calculated ovalbumin profile (solid line) and calculated salt profile (dashed line). For experimental conditions, see text.

The experimental UV traces from both column- and suspended-bed chromatography are presented in Fig. 5. A normalized abscissa is provided to account for the differences in hold-up time (t_o) and combined loading and washing time ($t_{load+wash}$). It is seen from this figure that the large-scale experimental data has good consistency. The overall time is shorter in Fig. 4 since the loading step is done in batch mode, while in Fig. 3, the loading is performed in column mode. Moreover, the linear velocity in Fig. 3 is 150 cm/h while in Fig. 4, it is 300 cm/h.

Since there is a good agreement between experimental and calculated profiles for both the traditional column chromatography and the suspended-bed approach, it is possible to use the models employed in this study in order to calculate the expected outcome for a full-scale stirred-tank process.

4.5. Performance comparison

The calculated concentration profiles for the stirred tank, the column chromatography and the suspended-bed approaches were used in order to produce the process parameters presented in Table 1. The adsorption and desorption efficiencies are defined as:

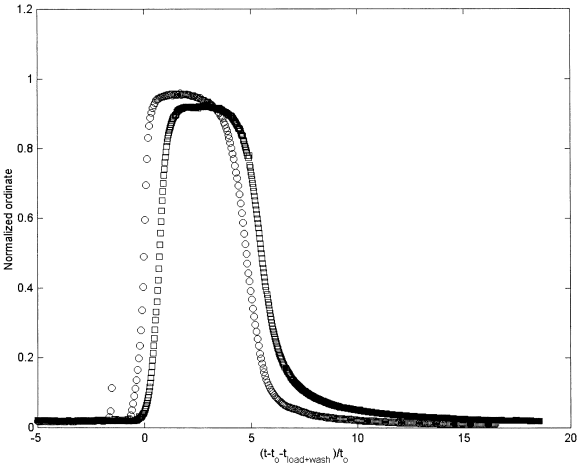

Fig. 5. Experimental UV signals corresponding to the column-chromatography mode (circles) and the suspended-bed mode (squares). For experimental conditions, see text.

Table 1
Performance comparison of the agitated contactor and the suspended-bed chromatography approaches

		Agitated contactor[a]	Suspended-bed chromatography	Column chromatography
Adsorption efficiency (%)	Experimental	69.0–70.3		ca. 100.0
	Calculated	71.0		100.0
Desorption efficiency (%)	Experimental	86.0	ca. 100.0	ca. 100.0
	Calculated	87.9	93.0	97.4
Eluent volume (l)		187	135	125
Process time (min)		270	170	204

[a] Experimental data taken from Ref. [2].

$$\text{Adsorption efficiency (\%)} = 100 \cdot \frac{\text{Protein adsorbed}}{\text{Protein loaded}} \quad (20)$$

$$\text{Desorption efficiency (\%)} = 100 \cdot \frac{\text{Protein desorbed}}{\text{Protein adsorbed}} \quad (21)$$

The experimental values of the adsorption and desorption efficiencies were calculated from the experimentally determined values of the protein content for pooled fractions at various stages of the chromatography. The analysis of the pooled fractions was performed via FPLC, as described elsewhere [15]. Note that there is a fair agreement between the experimental and calculated values of the efficiencies for all of the operation modes.

The desorption efficiency of the suspended-bed approach is higher than the corresponding value obtained with the agitated contactor, which is probably related to the fact that the stirred-tank elution is a single-stage process versus a multi-stage process happening during column elution. The overall yield of the process is simply:

$$\text{Yield} = \text{Adsorption efficiency} \times \text{Desorption efficiency} \quad (22)$$

From the above expression and the results presented in Table 1, it is clear that the yield of the suspended-bed approach is higher than the yield obtained with the stirred-tank approach, thus, the former procedure is more efficient than the latter.

Another important factor in favor of the suspended-bed approach is that the volume of eluent containing the target is less in the suspended-bed mode than in the agitated contactor mode (see Table 1). This means that the recovered target is more concentrated and, accordingly, less material goes to the next step downstream, which has an economic benefit. Finally, the processing time required by the suspended-bed chromatography process is shorter than the time required by a traditional batch contacting process using stirred tanks. Note that the reported processing time does not include the time required for feedstock conditioning, an upstream process step.

Finally, Table 1 also presents the performance parameters for the column chromatography case. These results show that traditional column chromatography is clearly the most efficient technique for the specific separation under consideration. The performance parameters for the column mode have been included in Table 1 for the sake of completeness and to give an overall perspective about three possible operation techniques. In the general case, we should expect that the suspended-bed approach occupies, in terms of overall efficiencies, an intermediate position between traditional column chromatography and agitated contactor chromatography. Note, however, that the emphasis in this paper is the comparison between the suspended-bed approach and the batch contactor mode.

It is important to realize that suspended bed chromatography is not intended as a universal solution to all the separation problems encountered in the downstream processing of biopharmaceuticals. Rather, it provides a solution to some specific applications where batch contactor chromatography is being considered as the viable process technique.

One of these applications is the initial capture of the target in a process step involving large volumes of dilute, clarified feedstock, especially if the step uses a small amount of media exhibiting high selectivity for the target. The technique should be especially effective when the volume ratio of feedstock to media is in the range of 50–100. Note that, in this case, the loading of the protein could take a long time if the process is conducted using traditional column chromatography. On the other hand, the adsorption stage in an agitated contactor is typically completed in 30–60 min, regardless of scale [2]. In general, a column process that requires a long loading cycle could be a good candidate for suspended-bed chromatography. Other scenarios with long column-loading cycles are those related with low flow-rates due to slow kinetics or potential bed compression.

Another key scenario where the suspended bed technique could be useful is when the target is unstable in the feedstock, for example, as a result of enzymatic degradation. In this case, the process time associated with the capture step should be minimized in order to maximize the recovery of the target.

Finally, in applications where the target is of high value and the media is of relatively low cost, the media can be disposed after recovering the target via suspended bed. In this case, the need for media cleaning-in-place (CIP) is eliminated from the process.

Agitated contactor processes are open, labor intensive, difficult to automate and problematic to validate [2]. An important issue is that, within the scope of these processes, there is a significant component of manual operation associated with the harvest and transfer of media between stirred tanks and filtration devices. Manual operations with media usually involve media losses and direct exposure of operators to loaded media. It is clear that suspended-bed chromatography reduces the number of manual operations as a result of the level of automation present in the packing unit.

5. Conclusion

Suspended-bed chromatography is a contained, hybrid technique for specific applications in downstream processing. It uses standard, commercially available, equipment and media. In addition to column packing and unpacking operations, the pump packing system can be used efficiently as a batch contactor for adsorption and as a column contactor for desorption. Any moderately robust media resistant to pumping, agitation and slurry transfer can be used. The Millipore IsoPak system and Whatman ion-exchange cellulose media were used through this work, with excellent results. Suspended bed combines the benefits of batch adsorption in terms of short contact times with the advantages of column elution in terms of resolution, recovery and containment.

Suspended-bed chromatography saves operation time, increases the yield and decreases the eluent volume with respect to the traditional series of stirred tank operations and associated fluid/solid separations. Moreover, elution in the suspended-bed mode is less challenging, easier to automate/validate/sanitize and less labor-intensive than the corresponding elution in a train of stirred tanks and associated filtration devices. Media losses and operator exposure to loaded media are also minimized in the suspended bed as a result of the media containment and implemented automation.

The rate model of chromatography properly predicts the operation of the suspended bed unit. It should be a useful tool for the analysis, design, scale-up and optimization of this novel hybrid technique. Suspended bed chromatography constitutes an additional useful technique to be considered when designing a downstream purification.

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