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New Expanded Bed Adsorption Model for Purification of Bovine Serum Albumin

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Abstract Based on the static and dynamic adsorption of Streamline DEAE, a modified tank-in-series model including particle size distribution was used to describe the adsorption performance of bovine serum albumin in an expanded bed. The calculated results indicated that the suggested model was able to simulate breakthrough curves under various conditions.

Key words: Adsorption, expanded bed model, particle size distribution, bovine serum albumin

Processes for producing pharmaceutical or diagnostic products involve the purification of proteins from a variety of sources. Typically, such purification processes contain multiple unit operations to achieve the process solution. Each step in the recovery process affects the overall process economy by increasing the operational cost and process time, and also by causing a loss in product yield, which can account for up to 80% of the final product cost. Therefore, a significant reduction in processing costs can be achieved by combining unit operations. Expanded bed adsorption is one such technique that can combine the steps of clarification, concentration, and initial purification into one unit operation [3].

It has already been demonstrated that expanded bed technology not only shortens the processing time but also increases the product yield. Therefore, increasing numbers of researchers have been using an expanded bed to purify a desired product [1, 4, 8]. However, they have invariably focused on the application of these systems, with little attempt to study their fundamental adsorption properties. Compared to conventional packed bed matrices for protein purification, the adsorbents of an expanded bed should have a high density in order to achieve the high throughout required in industrial applications of adsorption chromatography. Moreover, the adsorbents should have a comparatively wide particle size distribution to reduce any back-mixing in the column. Therefore, an understanding of the adsorption properties of an expanded bed system is very important to provide an effective design, scale-up operation, and control of this purification technique.

A few reports in recent literature describe the adsorption mechanism of an expanded bed. Kim *et al.* [15] studied the adsorption mechanism of a high density mixed mode adsorbent. Karau *et al.* [9] investigated the influence of particle size distribution on the adsorption performance in expanded bed, and a modified packed bed model (modified Hall's model) was used to describe the influence [14]. However, the particle size distribution was not considered in the modified model. Koh *et al.* [10] simulated the experimental breakthrough data of L-phenylalanine on a parallel diffusion model. Owen and Chase [11] developed a model to describe the performance of continuous counter-current expanded bed adsorption. However, both models used in these reports are based on the assumption that the adsorbent particles are of uniform size. In fact, the reason that the adsorption performance of an expanded bed was similar to that of a packed bed could be attributed to the formation of a classified fluidized bed, when adsorbent particles with a certain size and/or density distribution were used. Therefore, a reasonable adsorption model for an expanded bed should include the size distribution of the adsorbent particles. Yet, there are no such models found in the recent literature.

Currently, most commercially available adsorbents for an expanded bed are produced by Amersham Pharmacia Biotech, and are developed from a cross-linked agarose base matrix by inclusion of an inert core material to provide the required density. Because commercially available matrices provide a reliable basis for a parameter study, Streamline DEAE was chosen as the model adsorbent and BSA as the model protein. In our previous paper, the influence of

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operating conditions on the breakthrough curves had been investigated [7]. In this paper, a modified tank-in-series model including adsorbent size distribution is proposed to describe the adsorption performance in an expanded bed, which will aid in optimizing operating parameters with respect to minimizing costs, the use of raw materials, and scale-up.

MATERIALS AND EXPERIMENTAL METHODS

Materials and Apparatus

The Streamline DEAE was purchased from Amersham Pharmacia Biotech. The size distribution data of Streamline DEAE were determined by a Coulter LS-230 laser size analyzer. The bovine serum albumin (BSA) (fraction V) was purchased from Sino-American Biotech. All other chemicals used were of analytical grade and obtained from commercial sources. The Streamline 25 column (25 mm× 100 mm) and peristaltic pump (Watson-Marlow, 504S) were purchased from Amersham Pharmacia Biotech. The effluent concentrations were detected by an ÄKTA explorer 100 system obtained from Amersham Pharmacia Biotech.

Adsorption Isotherm and Diffusion Coefficient

The adsorption isotherm was carried out at different temperatures as previously described. The batch uptake profiles of the BSA adsorption were performed as described by Horstmann and Chase [6].

Breakthrough Curves

The breakthrough curves were determined by frontal adsorption experiments, using BSA as the model protein in a 20 mM sodium phosphate buffer (pH 7.0). After the sample application step, the bed was washed in the packed bed mode until the signal in the UV detector reached the baseline. Then, the bed was eluted with 1 M NaCl in a 20 mM phosphate buffer (pH 7.0). The resin was regenerated by adding a mixture of 0.8 M NaOH and 1 M NaCl with a minimum contact time of 4 h. An aqueous solution of 2-propanol (30% v/v) was used to remove lipophilic substances from the matrix. The buffer and protein solutions were prepared daily and degassed.

Modified Tank-in-Series Model

When the adsorbents were expanded in the column, the smaller, lighter particles moved to the top of the expanded bed, whereas the larger, heavier particles moved to the bottom, resulting in a stable, uniform expansion. With the stable fluidization of the expanded bed, only small circulatory movements of the adsorbent were observed. Therefore, the expanded bed could be divided into several tanks, each tank being regarded as a stirred tank system (Fig. 1), and the adsorbent particle diameter in each specific tank was the same.

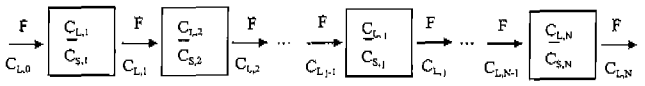

Fig. 1. Scheme of model of tank-in-series.
$C_{L,j}$: protein concentration of liquid in tank j, mg/ml, $C_{s,j}$: average protein concentration in adsorbent in tank j, mg/ml, F: volumetric velocity, ml/s.

For any tank, the mass balance equation can be given by

$$V_{L,j}\frac{dC_{L,j}}{dt}+V_{s,j}\frac{d\bar{C}_{s,j}}{dt}=F(C_{L,j-1}-C_{L,j}) \quad (1)$$

when $t=0$, $C_{L,j}=0$, $j=1, 2, \ldots, n$

when $j=1$, $C_{L,j-1}=C_{L,0}$

For diffusion within the adsorbent particle, the point concentration of the solution is given by

$$\varepsilon_p\frac{\partial C_i}{\partial t}=\varepsilon_p D_p\left(\frac{\partial^2 C_i}{\partial r^2}+\frac{2}{r}\frac{\partial C_i}{\partial r}\right)-(1-\varepsilon_p)\frac{\partial q_i}{\partial t} \quad (2)$$

The second-order reaction model developed by Chase was used to describe the equilibrium adsorption behavior [2]:

$$q_i=\frac{q_m C_i}{K_d+C_i} \quad (3)$$

As the binding rate is instantaneous compared to the protein transfer rate,

$$\frac{\partial q_i}{\partial t}=\frac{\partial q_i}{\partial C_i}\frac{\partial C_i}{\partial t} \quad (4)$$

and

$$\frac{\partial q_i}{\partial C_i}=\frac{q_m K_d}{(K_d+C_i)^2} \quad (5)$$

Substituting Eq. (4) into Eq. (3) gives

$$\frac{\partial q_i}{\partial t}=\frac{q_m K_d}{(K_d+C_i)^2}\frac{\partial C_i}{\partial t} \quad (6)$$

Substituting Eq. (5) into Eq. (2) gives

$$\left(1+\frac{1-\varepsilon_p}{\varepsilon_p}\frac{q_m K_d}{(K_d+C_i)^2}\right)\frac{\partial C_i}{\partial t}=D_p\left(\frac{\partial^2 C_i}{\partial r^2}+\frac{2}{r}\frac{\partial C_i}{\partial r}\right) \quad (7)$$

the rate of mass transfer through the external films related to the bulk liquid concentration in the pore liquid at the surface of the particle

$$r=R_j \qquad \left.\frac{\partial C_i}{\partial r}\right|_{r=R_j}=\frac{k_f}{D_p\varepsilon_p}(C_{f,j}-C_i)|_{r=R_j} \quad (8)$$

at the center of the adsorbents

$$r=r_{0j} \qquad \frac{\partial C_i}{\partial r}=0 \quad (9)$$

and the rate of change of the bulk concentration is given by

$$\frac{dC_{s,j}}{dt}=\frac{3}{R_j}D_p\frac{\partial C_j}{\partial r}\bigg|_{r=R_j} \quad (10)$$

Equations (1), (7), and (10) are general forms of the modified tank-in-series model. The appropriate set of equations can be solved using the orthogonal collocation method.

Result and Discussion

Equilibrium Adsorption Isotherms and Diffusion Coefficient

The adsorption isotherm for the binding of BSA to the adsorbent at pH 7.0 was obtained from Hu *et al.* [6]. A Langmuir isotherm was used to simulate the experimental data. At different temperatures, the equations were as follows:

$$q_i=\frac{50.6C_i}{0.11+C_i} \quad (5°C)$$

$$q_i=\frac{55.2C_i}{0.07+C_i} \quad (15°C)$$

$$q_i=\frac{61.4C_i}{0.04+C_i} \quad (30°C)$$

The diffusion coefficient was obtained by fitting uptake curves using Eq. (9).

In order to solve the modified tank-in-series model, the distribution of adsorbents in the expanded bed should be known. However, it is difficult to obtain this data because of the complexity of the adsorbent movement in an expanded bed, so the only distribution data available was reported by Hasson [5]. Recently, Willoughby *et al.* [13] used a modified column to measure the particle size distribution in an expanded bed adsorption system, thereby providing data for an improved model. In contrast, the two-phase theory was applied to an expanded bed by Thelen and Ramirez [12] to predict the hydrodynamic properties of the system, indicating that the adsorbent distribution in an expanded bed column can be predicted by a theoretical model. The experimental conditions selected in the current study were similar to those of Hasson, so that the data could be applied to the model. From bottom to top, the expanded bed height was divided into four sections, in which the adsorbent fraction was 41%, 28%, 19%, and 12%, respectively, plus the average diameter of the adsorbent therein was 238 μm, 186 μm, 164 μm, and 144 μm respectively. Breakthrough curves under different operating conditions were obtained from Hu *et al.* [7], and the modified tank-in-series model was used to fit the curves to obtain the liquid film mass transfer coefficients (k_f). Good fits were obtained

Table 1. Parameters obtained by regression of the modified tank-in-series models.

Operation conditions	D_p($\times10^7$ cm^2/s)	k_f($\times10^4$ cm/s)
Initial concentration (mg/ml)		
0.5	0.90	2.0
1.0	0.86	2.7
2.0	0.70	3.2
Temperature (°C)		
5	0.70	1.5
15	0.86	2.7
30	1.00	4.0
Sedimented height (cm)		
11.5, 15, 22.5	0.86	2.7

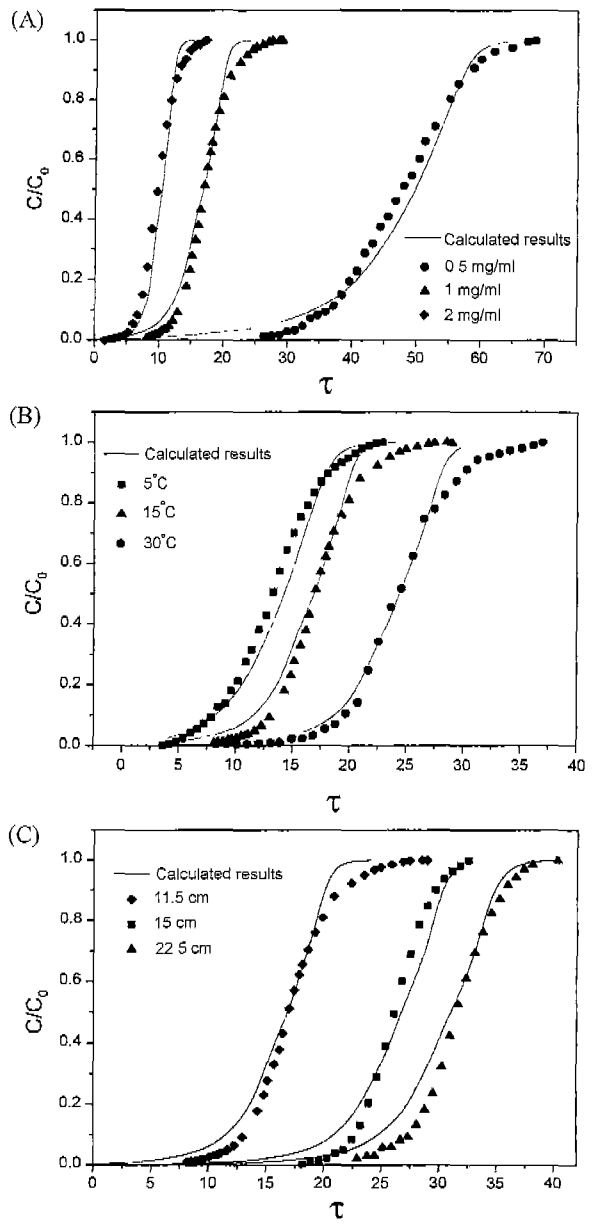

Fig. 2. Breakthrough curves regressed by the modified tank-in-series model at different initial concentrations (A), temperatures (B), and sedimented bed heights (C).

for each experiment and the results are summarized in Table 1. The Hall's model developed by Karau *et al.* [9] was also used to simulate the breakthrough curves for comparison.

Figure 2(A) shows the simulation for breakthrough at three different concentrations within a range of 0.5–2 mg/ml at a fixed flow rate (22 ml/min). The value of k_f obtained by data fitting increased as the concentration increased. Figure 2(B) shows the simulation for breakthrough at three different temperatures within a range of 5–30°C at a fixed flow rate. Since an increase in temperature improves the protein transfer within the liquid film, and the value of k_f is a lumped constant which includes the mass transfer effect, an increase in k_f was anticipated with an increase in temperature. The simulation results supported this assumption.

As k_f was obtained from the simulation, this parameter was then used to predict the breakthrough curves at three different sedimented bed heights ranging from 11.5 cm to 22.5 cm.The simulation results are illustrated in Fig. 2(C). Although these results were not as good as those obtained by curve fitting, the absolute prediction of the adsorption characteristics in an expanded bed was still possible if the distribution data of the adsorbent in the expanded bed were available.

In order to compare the modified tank-in-series model with the modified Hall's model, the relative errors between the simulation results and the experimental data are illustrated in Fig. 3. The relative errors with the modified tank-in-series model (±10%) were smaller than those of Hall's model (±20%). It should be noted that there are only two parameters (N_{film} and N_{pore}) in the Hall's equation, and a multi-parameter model usually fits experimental data better than a single-parameter model. In view of this, the modified tank-in-series model would appear to be more accurate for describing the adsorption performance in an expanded bed.

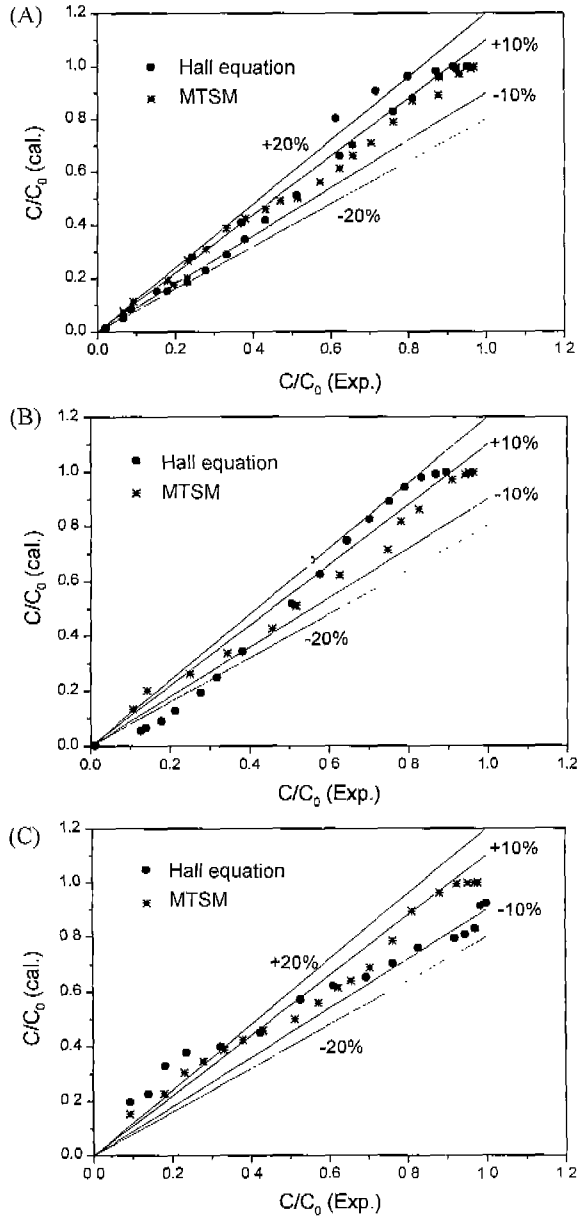

Fig. 3. Comparison of the calculated protein concentration at outlet relative to experimental data at different initial concentrations (A), temperatures (B), and sedimented bed heights (C).

CONCLUSION

A modified tank-in-series model was developed to describe the adsorption performance in an expanded bed. When compared with other models used for an expanded bed, the presently proposed model is the first to consider the adsorbent size distribution and thus is closer to the real conditions of an expanded bed. The simulation accuracy was better than that of the modified Hall's model. However, the model used distribution data in the expanded bed, which is not easy to obtain. A theoretical model for adsorbent distribution in an expanded bed under different operating conditions will be further investigated.

NOMENCLATURE

C_i : Point concentration of liquid inside particle, mg/ml
$C_{L,j}$: Protein concentration of liquid in tank j, mg/ml
$\overline{C}_{s,j}$: Average protein concentration in adsorbent in tank j, mg/ml
D_p : Effective particle diffusion coefficient, cm^2/s
F : Volumetric velocity, ml/s
K_d : Dissociation constant, mg/ml
k_f : Liquid film mass transfer coefficient, cm/s
q_i : Point concentration of solute in adsorbent, mg/ml
q_m : Maximal protein concentration in adsorbent, mg/ml
R_j : Average particle radius of adsorbent in tank j, cm

r : Radial coordinate, cm
r_{0j} : Particle radius of inert core of fraction j, cm
t : Time, s
$V_{L,i}$: Volume of liquid phase in tank j, ml
$V_{s,i}$: Volume of solid phase in tank j, ml

Greek Symbols

ε_p : Particle porosity
τ : Dimensionless time=$D_p t/\delta^2$

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