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Comparison of the Adsorption Characteristics of Expanded Bed Adsorbent with Conventional Chromatographic Adsorbent

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Abstract−The static and kinetic adsorption characteristics of Streamline DEAE and DEAE-Sepharose FF were studied under various operating conditions. The adsorption isotherms for the two types of adsorbents were obtained and found to fit well to a Langmuir-type expression. The adsorption kinetics of Streamline DEAE at different concentrations, temperatures, and viscosities were studied and a mathematical model including particle size distribution was developed to describe the adsorption performance of Streamline DEAE. Comparing the uptake curves of Streamline DEAE with DEAE-Sepharose FF, it could be concluded that Streamline DEAE achieves equilibrium faster to get equilibrium than DEAE-Sepharose FF, indicating that Streamline DEAE could be used in higher flow rate systems.

Key words: Adsorption, Expanded Bed, Kinetics, Model

INTRODUCTION

Expanded bed adsorption is one type of integrated technology now being considered for the purification of desired products directly from cell suspensions, crude cell lysates, and refolded mixtures. Expanded bed adsorption combines classification processing, such as microfiltration and centrifugation, and partial purification processing into a one-unit operation, providing product isolation while minimizing the number of processing operations required for product recovery [Chase, 1994]. Therefore, the use of expanded bed adsorption in the separation processes of biotechnology has received considerable attention in the past ten years and a wide range of applications have been proposed [Chase and Dreager, 1984; Chase, 1994; Clemmitt and Chase, 2000; Hjorth, 1997; Hu et al., 1999; Mullick and Flickinger, 1999; Owen and Chase; 1999]. However, most of the studies have been focused on the application of this technique, with little attempt to investigate the fundamental adsorption properties.

Streamline adsorbents are a set of adsorbents specially designed for the expanded bed adsorption procedure by Amersham Pharmacia Biotech [Hjorth, 1997]. Compared with the conventional chromatographic matrices, they have high-density inert cores that allow them to achieve the high throughput required in industrial applications of adsorption chromatography. Moreover, they have a comparatively wide particle size distribution that reduces the back-mixing in the column. Most of the application studies on expanded bed adsorption used this type of adsorbent for the separation process [Chase and Dreager, 1984; Chase, 1994; Clemmitt and Chase, 2000; Hjorth, 1997; Hu et al., 1999]. However, the fundamental adsorption research for the adsorbent was scarce.

Although there have been a large number of mathematical models concerning protein adsorption [Chase, 1984; Graham and Fook, 1982; Horstmann and Chase, 1989; Johnston and Hearn, 1990; Johnston et al., 1991; Skidmore et al., 1990; Tsou and Graham, 1985] and some reports about the effect of the adsorbent size on the bioproduct separation [Choi, 1990], the effect of surface pressure on the adsorptive behavior of a globular protein [Cho et al., 1999] and the effect of overflow parameter on the frequency response of a continuous flow adsorber [Park et al., 2000], there have been no reports until now concerning the adsorption model that includes both size distribution and heterogeneous structure. Most researchers have assumed Streamline adsorbents as the conventional adsorbent in order to use a traditional adsorption model [Chang and Chase, 1996; Karau et al., 1997; Wright et al., 1999]. Unlike traditional adsorbents, the particle size distribution was designed purposely for the Streamline adsorbent. Therefore, a realistic adsorption model concerning a Streamline adsorbent should take into account the particle size distribution.

In this paper, Streamline DEAE and DEAE Sepharose FF were chosen as the model adsorbents and Bovine serum albumin (BSA) as the model protein. The static and kinetic adsorption performance of the two adsorbents was studied under various conditions. Moreover, a heterogeneous adsorption model including particle size distribution was developed in order to describe the kinetic adsorption performance of Streamline DEAE.

MATERIAL AND METHODS

Streamline DEAE and DEAE-Sepharose FF were purchased from Amersham Pharmacia Biotech. The particle size distribution data of Streamline DEAE was determined by Coulter LS-230 laser size analyser. Bovine serum albumin (BSA) (fraction V) was purchased from Sino-American Biotech. All other chemicals used were of analytical grade and were obtained from commercial sources.

1. Adsorption Equilibrium Isotherms

Flasks of 25 ml total volume were filled with 0.5 ml resin and 14.5 ml BSA solution in a 20 mM sodium phosphate buffer (pH 7.0). After the 20 h incubation, the protein content in the supernatant was determined by UV adsorption (280 nm). The amount of

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protein adsorbed was calculated from the difference between the initial and final concentrations.

2. Adsorption Kinetics

Adsorption kinetics was measured via protein absorbency at 280 nm by using the apparatus described by Horstmann and Chase [Horstmann and Chase, 1989]. The adsorbent was equilibrated in the required buffer, and made to a 1:1 (v/v) suspension. Twenty ml of buffer was circulated in the system by means of a peristaltic pump. The initial optical density of the buffer was recorded as the blank sample, and then a small volume of a concentrated solution of BSA (8 mg/ml) in buffer was added. The total volume was kept at 50 ml adjusted by buffer. After the optical density reached equilibrium, the value was recorded and 2 ml of the adsorbent suspension was added. The optical density of the solution decreased with time as BSA was adsorbed to the adsorbent until the density became constant. The equilibrium concentration was determined by applying the constant optical density to the correlation curve between optical densities and BSA concentrations. A series of such experiments was carried out at different initial concentrations of BSA, temperatures and solution viscosities. Adding a quantity of glycerol modified the solution viscosity.

3. A Model for Adsorption Kinetics

In order to propose the adsorption kinetics model of Streamline DEAE, the assumptions proposed by Hostmann and Chase were used [Horstmann and Chase, 1989]. Compared to the conventional chromatographic adsorbent, an additional assumption was made due to the specific properties of Streamline DEAE. The adsorbent particle was regarded as spherical with an inert core. Moreover, the thickness of the agarose shell was constant, and the size distribution of the adsorbent was a function of that of the inert core.

For diffusion within the adsorbent particle, the point concentration of a solution was given by Eq. (1).

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

The second-order reaction model developed by Chase [Chase, 1984] was used to describe the equilibrium adsorption behavior expressed by Eq. (2).

$$\frac{\partial q_i}{\partial t} = k_1 C_i (q_m - q_i) - k_{-1} q_i \tag{2}$$

As described above, Eq. (2) became Eq. (3) at equilibrium, which is the form of the Langmuir equation.

$$q_i = \frac{q_m C_i}{K_d + C_i} \tag{3}$$

Where q_m is maximum capacity and K_d dissociation constant.

According to this assumption [Horstmann and Chase, 1989], the binding rate was instantaneous compared to the protein transfer rate as given by Eqs. (4) and (5).

$$\frac{\partial q_i}{\partial t} = \frac{\partial q_i}{\partial C_i} \frac{\partial C_i}{\partial t} \tag{4}$$

and

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

Substituting Eq. (5) into Eq. (4) gave

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

Substituting Eq. (6) into Eq. (1) gave

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

The rate of mass transfer through the external film related the bulk liquid concentration in the pore liquid is expressed by Eq. (8) at the surface of the particle.

$$\left. \frac{\partial C_i}{\partial r} \right|_{r=R_j} = \frac{k_f}{D_p \varepsilon_p} (C_L - C_i)|_{r=R_j} \tag{8}$$

The rate becomes zero at the surface of the inert core of the particle.

$$\left. \frac{\partial C_i}{\partial r} \right|_{r=r_{0,j}} = 0 \tag{9}$$

Based on the data of particle size distribution, the adsorbents were divided into several fractions, and the adsorbent diameter within each specific fraction was assumed to be uniform. In a stirred tank system, the rate of change of bulk concentration was given by Eq. (10).

$$\frac{dC_L}{dt} = -\sum_j \frac{3\nu_j k_f}{R_j V} (C_L - C_i)|_{r=R_j} \tag{10}$$

Eqs. (7) to (10) were the general forms of the new model. When $r_{0,j}=0$, and j=1, the model was changed to the traditional liquid film and pore diffusion model, so the new model was named the modified liquid film and pore diffusion model (MFPDM). The model was solved with Matlab 5.0 by the orthogonal collocation method [Finlayson, 1980]. The values of D_p and k_f were obtained by the regression of experimental data.

If the protein-binding rate is assumed to be the rate-limiting step, a model named as kinetic constant model can be obtained by integration of Eq. (2) [Johnston and Hearn, 1990; Skidmore et al., 1990], and is given by Eq. (11).

$$C(t) = C_0 - \frac{\nu}{V} \left[\frac{(b+a)\left\{ 1 - \exp\left(-\frac{2a\nu}{V} k_1 t \right) \right\}}{\left(\frac{b+a}{b-a} \right) \left\{ 1 - \exp\left(-\frac{2a\nu}{V} k_1 t \right) \right\}} \right] \tag{11}$$

where

$$a^2 = b^2 - \left(\frac{C_0 V}{\nu} \right) q_m \tag{12}$$

$$b = \frac{1}{2} \left(\frac{C_0 V}{\nu} + q_m + \frac{K_d V}{\nu} \right) \tag{13}$$

The model was used to simulate the experimental results for comparison.

RESULTS AND DISCUSSION

1. Particle Size Distribution of Streamline DEAE

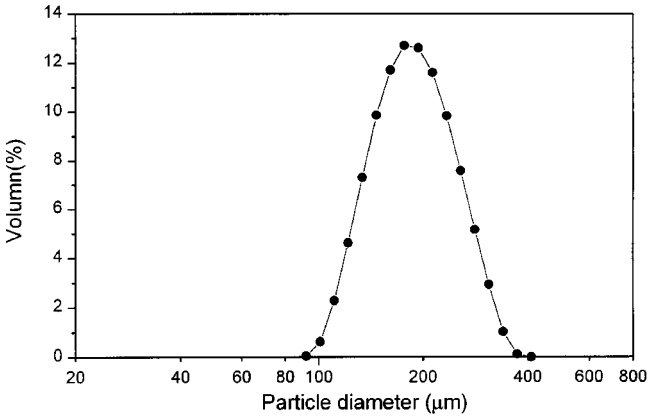

Fig. 1. The particle size distribution data of Streamline DEAE measured by Coulter LS-230 laser size analyser.

Table 1. The static adsorption data at varied temperatures (pH 7.0)

Temperature (°C)	STREAMLINE DEAE		DEAE-Sepharose FF	
	q_m (mg/ml)	K_d (mg/ml)	q_m (mg/ml)	K_d (mg/ml)
15	55.2	0.07	56.5	0.065
25	58.3	0.045	59.8	0.041
30	60.4	0.037	62.2	0.031
35	63.5	0.028	65.5	0.022

Fig. 1 shows the particle size distribution of Streamline DEAE measured by Coulter Laser L-230. The graph shows that the particle size distribution was similar to the logarithmic Gaussian distribution and the largest volume of solid was given by particles of diameter of approximately 200 μm. The corresponding mean diameter of the particle was 202.8 μm, which was in good agreement with the result provided by Amersham Pharmacia Biotech.

2. Equilibrium Adsorption Isotherm

Equilibrium adsorption isotherm experiments of Streamline DEAE and DEAE-Sepharose FF were conducted at four different temperatures ranging from 15 °C to 35 °C. The equilibrium adsorption performances of the two types of adsorbents could be described by the Langmuir type equation and the adsorption parameters are summarized in Table 1. It can be concluded from Table 1 that the disassociate constant K_d and maximum adsorption capacity q_m of Streamline DEAE are similar to those of DEAE-Sepharose FF. The reason might be that both of the functional groups of the adsorbents are diethylaminoethyl, which results in a similar binding force and selectivity to BSA. Moreover, though the particle diameter of Streamline DEAE is larger than that of DEAE-Sepharose FF (65-145 μm), the inert core in Streamline DEAE decreases the content of agarose, leading to a similar maximum adsorption capacity. Furthermore, the value of K_d decreased with the increase in temperature while the q_m increased, indicating that the adsorption performance on BSA of the two adsorbents is improved with the increase of temperature.

3. Adsorption Kinetics

3-1. Effect of Various Operating Conditions on the Kinetic Adsorption Performance

Fig. 2 shows how the uptake curves varied with the change of initial concentration. The effect of protein concentration on the adsorption kinetics is difficult to observe directly and is illustrated by the kinetic model, which is discussed in the following section. Fig. 3 shows the effect of solution viscosity on the kinetic adsorption performance. As shown in the graphs, the uptake curves become shallower and require more time to reach equilibrium as the solution viscosity increases. However, as reported by Chase and Dreager [Chase and Dreager, 1992], the maximum adsorption capacity was not affected by the change of viscosity because the final concentration in the solution was the same under the different conditions. Fig. 4 shows the effect of temperature on the kinetic adsorption performance. Based on the experimental data of equilibrium adsorption isotherms, it can be anticipated that the uptake curves will become steeper with the increase in temperature. Moreover, the viscosity of the solution decreases and the movement of the protein molecules increases with the increase in temperature, which improves the transfer performance of the protein. The experimental data supports this assumption.

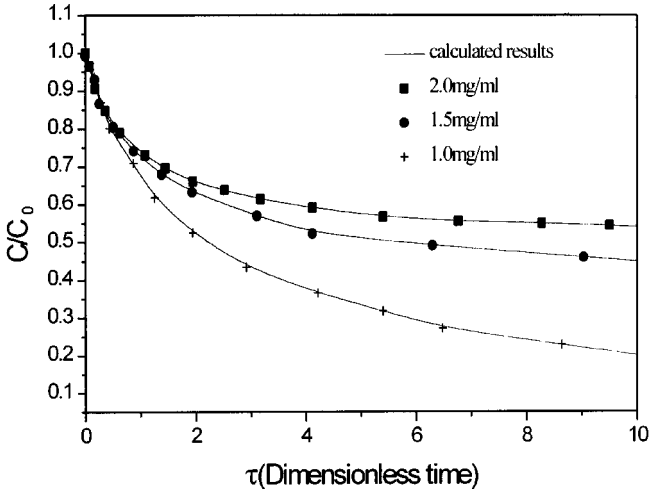

Fig. 2. The adsorption profile for BSA by the modified film and pore diffusion model at varied initial concentrations.

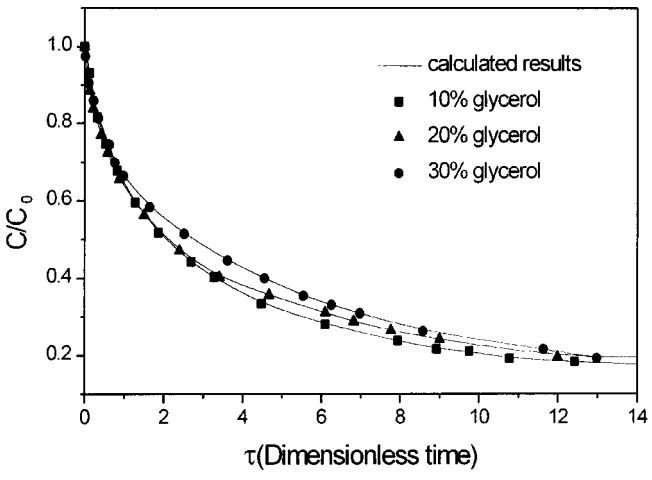

Fig. 3. The adsorption profile for BSA by the modified film and pore diffusion model at varied viscosities.

3-2. The Simulation Results of the Two Models

Two types of models, a kinetic constant model and a modified

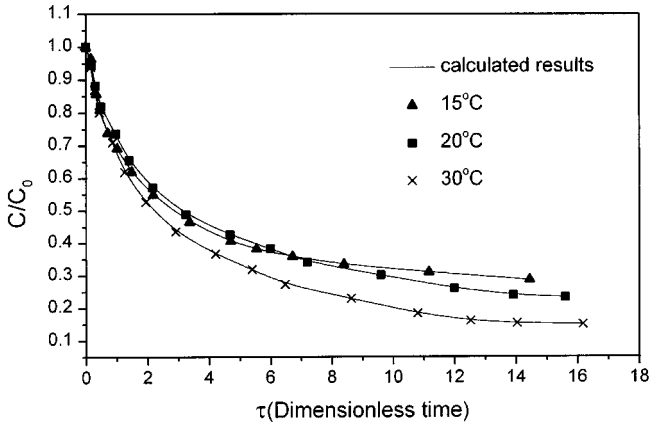

Fig. 4. The adsorption profile for BSA by the modified film and pore diffusion model at varied temperatures.

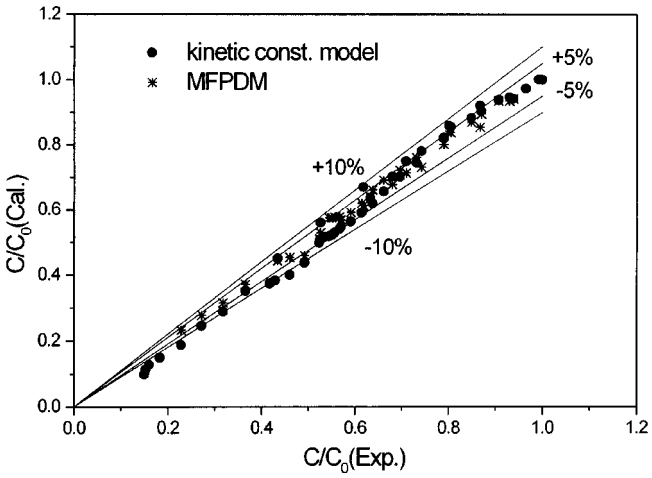

Fig. 5. Comparison of calculated concentration of protein with experimental data at varied initial concentrations.

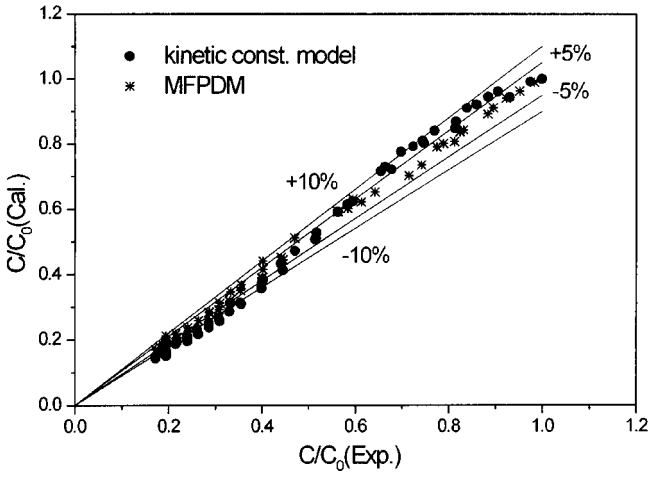

Fig. 6. Comparison of calculated concentration of protein with experimental data at varied viscosities.

film and pore diffusion model (MFPDM), were used to simulate the uptake curves. For MFPDM, the adsorbent was divided into four sections. The average diameter for the four sections was 133.7 μm, 169.3 μm, 203.3 μm and 262.4 μm, respectively, and the volume percentage was 24.7%, 24.4%, 24.2%, and 26.6%, respectively. The value of liquid film mass transfer coefficient, k_f, estimated by the correlation used by Horstman and Chase [Horstmann and Chase, 1989] was not suitable for streamline DEAE due to its particular structure. Therefore, in this paper, the value of k_f was obtained by the regression of experimental data. The thickness of the agarose shell was assumed to be 60 μm in this study. The simulation results are listed in Table 2. The kinetic constant parameter k_1, the effective pore diffusivity D_p, and the liquid film transfer coefficient, k_f, changed under various conditions. The reason the values of all three parameters were dependent on the operating conditions was that all the parameters were lumped constants because of a simplification of the models. The dependency of the three parameters on the operating conditions was reported by Johnston and Hearn [Johnston and Hearn, 1990] and Horstmann and Chase [Horstmann and Chase, 1989].

The comparisons of relative errors of simulation obtained by the modified film and pore diffusion model with those of the kinetic constant model are shown in Figs. 5, 6 and 7. As shown in these figures, the relative errors of the modified film and pore diffusion model (±5%) are smaller than those of the kinetic constant model (±10%), indicating that the modified film and pore diffusion model

Table 2. The parameters obtained by the regression of the two models

Operation conditions	D_p ($\times 10^7$ cm^2/s)	k_f ($\times 10^6$ cm/s)	k_1 ($\times 10^3$ ml/mgs)
Initial concentration (mg/ml)			
1.0	0.9	0.5	1.0
1.5	0.8	0.7	0.96
2.0	0.7	1.0	0.90
Composition of glycerol (w/w)			
10% glycerol/buffer	0.65	0.3	0.92
20% glycerol/buffer	0.6	0.2	0.83
30% glycerol/buffer	0.55	0.1	0.64
Temperature (°C)			
15	0.86	0.45	0.93
20	0.9	0.5	1.0
30	1	0.6	1.3

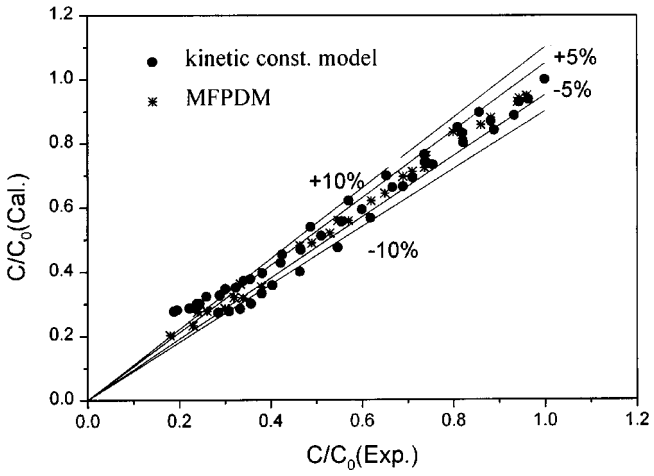

Fig. 7. Comparison of calculated concentration of protein with experimental data at varied temperatures.

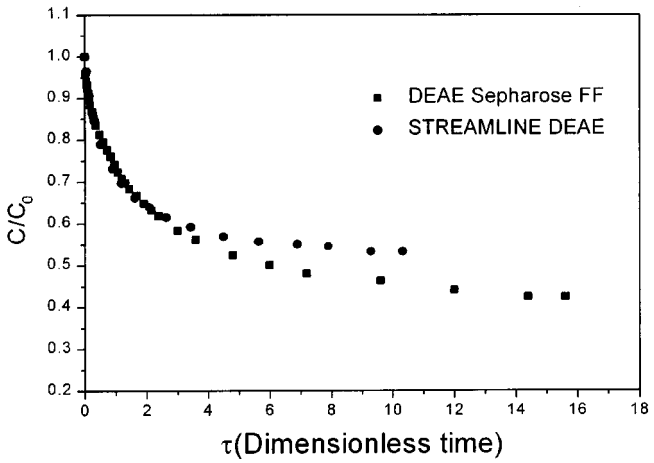

Fig. 8. Adsorption profiles for BSA by DEAE Sepharose FF and STREAMLINE DEAE.

can be more accurate in describing the kinetic adsorption performance of Streamline DEAE.

3-3. Comparison of Kinetic Adsorption Performance of Streamline DEAE and DEAE-Sepharose FF

Fig. 8 shows the kinetic adsorption curves of Streamline DEAE and DEAE-Sepharose FF. As shown in the figure, the uptake curves of both of the two adsorbents are the same at the initial adsorption stage, although the difference becomes larger and larger as the adsorption time elapses. It can be explained as follows. The transfer resistance is primarily focused on the side of the liquid film at the initial stage and the liquid film transfer resistance does not depend on the particle size or the structure of adsorbent, so there is no difference in the uptake curves at the beginning of the adsorption stage. However, at the final stage of adsorption, the main transfer resistance governing the adsorption performance is pore diffusion; the diffusion distance in the Streamline DEAE is shorter than that in DEAE-Sepharose FF due to the inert core in Streamline DEAE. Therefore, Streamline DEAE is faster in reaching equilibrium than DEAE-Sepharose FF. This result indicates that Streamline DEAE can be used in high-flow-rate separation processes.

CONCLUSIONS

The aim of these investigations was to show the adsorption performance of Streamline adsorbent and to develop a mathematical model to describe the specific properties of this kind of adsorbent. The equilibrium adsorption isotherm and adsorption kinetics of Streamline DEAE and DEAE-Sepharose FF were studied under different operating conditions. The adsorption isotherms for the two types of adsorbents were found to fit well to the Langmuir type expression and the values of two parameters, K_d and q_m, were very similar between the two types of adsorbents as shown in Table 1. The kinetic adsorption characteristics of Streamline DEAE at different concentrations, temperatures, and viscosities were investigated and a mathematical model including particle size distribution was developed in order to describe the adsorption performance. The simulation results indicated that the model could better describe the kinetic adsorption performance. Further work is being undertaken on the adsorption performance in expanded beds and the model developed in this paper will be used to describe the adsorption performance in expanded beds.

NOMENCLATURE

$C(t)$: liquid phase concentration at the time t [mg/ml]
C_0 : initial liquid phase concentration [mg/ml]
C_L : liquid phase concentration [mg/ml]
C_i : point concentration of liquid inside particle [mg/ml]
D_p : effective particle diffusion coefficient [cm^2/s]
k_1 : forward rate constant for surface reaction [ml/mg·s]
k_{-1} : reverse rate constant for surface reaction constant [s^{-1}]
K_d : disassociation constant [mg/ml]
k_f : liquid film mass transfer coefficient [cm/s]
q_i : point concentration of solute in the absorbent [mg/ml]
q_m : maximal protein concentration in the absorbent [mg/ml]
R_j : particle radius of the fraction j [cm]
r : radial co-ordinate [cm]
r_{0j} : particle radius of inert core of the fraction j [cm]
t : time [s]
V : volume of liquid [ml]

Greek Letters

δ : thickness of agarose shell [cm]
ε_p : particle porosity
ν_j : volume of adsorbent of the fraction j [ml]
τ : dimensionless time=$D_p t/d^2$

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