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Modeling a Protein Foam Fractionation Process

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Abstract

A simple staged model for the protein foam fractionation process is proposed in this article. This simplified model does not detail the complex foam structure and gas-liquid hydrodynamics in the foam phase but, rather, is built on the conventional theoretical stage concept considering upward bubbles with entrained liquid and downward liquid (drainage) as countercurrent flows. To simulate the protein concentration distribution in the liquid along the column by the model, the bubble size and liquid hold-up with respect to the position must be known, as well as the adsorption isotherm of the protein being considered. The model is evaluated for one stage by data from the semibatch foam fractionation of egg albumin and data from the continuous foam fractionation of bovine serum albumin. The effect of two significant variables (superficial gas velocity and feed protein concentration) on enrichment is well predicted by the model, especially for continuous operation and semibatch operation when initial concentration is high.

Index Entries: Staged model; foam fractionation; egg albumin; bovine serum albumin.

Introduction

Foam fractionation is a method for separating chemicals in solution according to the differences in surface activities. It is quite effective in recovering proteins from dilute solutions owing to the hydrophobic and hydrophilic nature of protein molecules. The advantages of this technique are that it is highly efficient for very dilute solutions when other separation techniques do not work and that the capital and operation costs are low compared with other conventional separation techniques *(1)*. This process is also easy to scale up *(2)*. Several studies between 1937 and 1959 *(3)* have described both the concentration and purification of enzymes and other proteins. One of the earliest applications of foam fractionation was to use air as the carrier gas to concentrate small amounts of protein in starch wash

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water from the effluent of an industrial starch manufacturing process *(3)*. The theory underlying how surfactants and proteins work in foam fractionation is summarized in the classic book *Progress in Separation and Purification*, by Lemlich *(4)*. A survey of the recent literature indicates a renewed interest in using foam fractionation to separate proteins and enzymes *(2,5–9)*. The current interest seems to indicate the need for a low-cost, highly effective purification method for biological products, particularly in the first concentration step that is used to remove about 90% of the water from dilute solutions. Reviews by Uraizee and Narsimhan *(1,10)* and Lockwood et al. *(11)* further support these studies by providing theoretical understanding and empirical observations of protein foam fractionation.

The primary mechanisms underlying foam fractionation are as follows: First, surface-active substances such as proteins preferentially adsorb on bubble surfaces (generated by bubbling gas through a solution). Second, the surface-active substance is carried upward by the rising bubbles, which accumulate above the solution surface. Finally, when this swarm of bubbles (the foam phase) collapses, a concentrated protein solution (foamate) remains and is recovered from the low liquid content foam phase.

The application of foam fractionation to biological products is not commonly used for two main reasons *(10)*. The first is the lack of sufficient understanding of the complex principles governing the process, both in the gas-liquid dispersion hydrodynamics and protein adsorption (which makes prediction of the performance difficult). The second is the presumed denaturation of the biological molecules during the process.

Malysa and Warszynski *(12)* studied the dynamic effects (motion) associated with foam-layer formation and stability, and observed that the competition between the rate of equilibrium coverage reestablishment (disequilibration induced by motion) by surface diffusion (increasing the stability of film) and the rate of film drainage (decreasing the stability of film) determines the volume and stability of the foam layer. Bhakta and Ruckenstein *(13)* have reviewed the theoretical work on static foam decay and compared various models in terms of their ability to describe drainage, coalescence, and collapse of the foam. In spite of this extensive body of work in the literature, a detailed mathematical description of the complex mass transfer process, which depends on the operational modes (continuous, semibatch, or static foam) and the physicochemical properties of the solution and surfactants, is very complex and, hence, has not been done to date in a relatively simple model (such as that of distillation).

Protein denaturation—the loss of biological activity owing to the change (usually unfolding) of molecular tertiary structure—when this biological molecule is exposed to a gas-liquid surface, can be minimized by choosing suitable operating conditions. A number of researchers have noted that foam separation can be used successfully for the purification of proteins and enzymes without necessarily adversely affecting the protein structure *(9)*.

If denaturation does not pose a problem, the performance of foam fractionation, as measured by the enrichment (the ratio of the protein concentration in the foamate to that of the original solution), will be determined by the interactions among the solution conditions (pH, ionic strength, and protein concentration) and the column operational parameters (gas flow rate, liquid pool height, foam column height, and bubble size) *(11)*. An ideal theoretical model to describe these interactions should be based on the physical dimensions and characteristics of the column, and the physicochemical properties of the components in the feed solution. The development of such a model will also depend on the understanding and careful describing of the foam drainage, coalescence hydrodynamics, and surface adsorption of the solutes including the desired proteins.

Grieves and Bhattacharya *(14)* developed an empirical model and fitted it to experimental foam volume data to describe surfactant behavior at various initial concentrations. Loha *(15)* correlated the relationship between the volumetric mass transfer coefficient and various physical parameters (viscosity, density, and surface tension) as well as the operational parameter (superficial gas velocity) in terms of dimensionless groups for a short foam layer (comparable to a single-stage semibatch foam fractionation column). He investigated the egg albumin and cellulase systems, respectively, and then predicted the desired performance using the determined volumetric mass transfer coefficients. Brown et al. *(16)* studied the foam fractionation of bovine serum albumin (BSA) and developed a single-stage continuous model incorporating drainage theory. The enrichment and recovery values of BSA, predicted by Brown et al.'s *(16)* model were greater than the experimental data values because complete equilibrium surface concentration was assumed. Allowing for only a fraction of equilibrium coverage to take place, Uraizee and Narsimhan *(17)* accounted for the effects of adsorption kinetics as well as coalescence in their continuous foam fractionation model, and got excellent results when compared with their data. However, they needed to introduce an empirical parameter, coalescence frequency, to the model, which was evaluated from the experimental bubble size distribution data. Bhattacharjee et al. *(18)* predicted the value of the separation factor for a single protein static foam column by combining a multilayer adsorption isotherm with the static foam drainage models of Ramani et al. *(19)* and Podual et al. *(20)*. The results were verified with data collected for casein and then for BSA. Lemlich *(4)* built simple models by analogy to the traditional counterflow distillation column with the simplifying assumption that the liquid streams (both the entrained upflow liquid and the descending liquid [a drainage term]) had constant volumetric flow rates in the column. And for simplicity, the surface concentration (Γ) was taken to be constant as a special case. He did not include the complex mechanism of foam drainage and coalescence in his model, and the effectiveness of the model has not been reported for protein foam fractionation.

The objective of the present study was to predict the enrichment of proteins in a foam fractionation column (semibatch or continuous) by a simple multistage model similar to that of Lemlich *(4)*. But, in this study, the flow rates of both liquid streams were not kept constant with respect to the column position. The flow rate of the entrained upstream was estimated using liquid holdup fraction data, whereas in Lemlich's *(4)* model, this flow rate was set equal to the overflow rate of the foamate, assuming negligible drainage. To determine the volumetric flow rates in the proposed model, experimental bubble size and liquid hold-up data were needed. The surface concentration (Γ) of the egg albumin or the BSA model system was assumed to be at equilibrium with the bulk protein concentration and described by a linear isotherm, which is also effective for multilayer adsorption at high protein concentrations ($\geq 10^{-3}$ wt%) *(21)*. The enrichments predicted for the single-stage process were compared with semibatch experimental data of egg albumin *(15)* and continuous foam separation data of BSA *(16)*. The results indicate that the proposed multistage model works well for the special case of one stage. Further work, however, needs to be done to extend the analysis to the multistage case.

Description of the Model

Figure 1 illustrates the phase changes occurring in a foam fractionation column. Gas is introduced into the bottom of a glass column through a fritted glass or other porous device, which partially determines the resulting bubble size. In the lower part of the column, protein molecules move and adsorb onto the rising bubble surface in the bulk liquid. If the formed bubble films are stable, the bubbles can sustain to leave the surface of the bulk solution to form a foam layer. The foam entrains proteins on both the foam cell surface and the interstitial bulk liquid in the formed films and plateau borders between the foam cells, all within the foam phase above the bulk solution surface, as shown in Fig. 1. The interstitial liquid drains as the foam rises because of plateau border suction and gravity, resulting in a thinning of the formed films. Finally, the bubbles in the foam coalesce into larger foam cells.

The foam phase, therefore, comprises an upflow liquid stream (U_n), which is entrained by the rising foam bubbles, and a downflow stream (L_n), which is owing to the drainage in the foam fractionation column. If U_n and L_n are known, or can be calculated from the physical parameters of the system at a given time and position, the column can be described by a multistage model, in which each stage is similar to a unit height of a packed distillation column (the so-called height of a transfer unit [HTU]). An important difference between a foam column and a distillation column is that the protein solute is not distributed between vapor and liquid phases but, rather, between the bubble surface and the bulk liquid.

The foam fractionation column is divided into N stages. The liquid pool (bulk liquid below the foam phase) is taken to be one stage and denoted

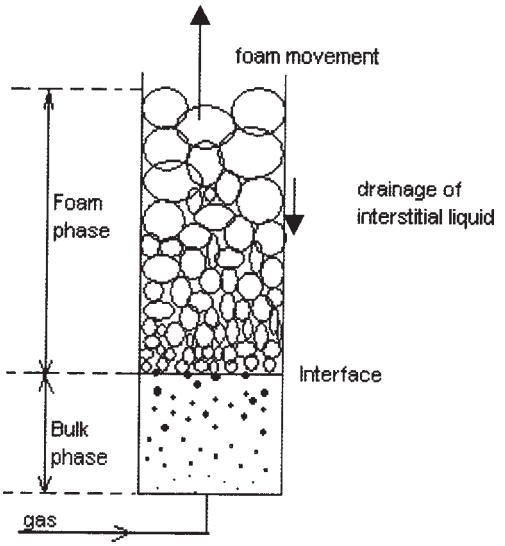

Fig. 1. Schematic diagram of the foam fractionation column.

as the zeroth stage here. The protein concentration in the liquid pool is assumed to be uniform for a well-mixed liquid pool when the height is short, as assumed in the work of Uraizee and Narsimhan *(17)* and Brown et al. *(16)*. This was also the case for the analysis of Loha's *(15)* experimental data on egg albumin. When the concentration is close to very well mixed, the pool is analogous to a reboiler in a distillation process. The theoretical stage number N is determined by the foam height and HTU, which depends on the properties of the solute and the operating conditions. The HTU can be as low as several centimeters under proper conditions *(4)*. Hastings found the minimum HTU was about 2.54 cm *(24)*. In our first implementation of the model proposed in this article, we choose N to be 1 since the foam height is below 5 cm for the data used to describe both the continuous protein foam fractionation column *(16)* and the semibatch protein foam column *(15)*.

The total liquid mass balance between stage n and the top of the column (as indicated by the dashed line of Fig. 2) is:

$$U_{n-1} - L_n - D = 0 \quad n = 1, 2, \ldots N \tag{1}$$

in which U_{n-1} is the upstream liquid flow rate entrained by the bubbles (the interstitial liquid) (cm^3/min); L_n is the downstream flow (drainage) rate (cm^3/min); and D is the overflow foamate flow rate (cm^3/min). The mass of liquid in the bubble boundary is omitted since the mass is negligible with respect to the mass of the interstitial liquid specified by U_n. Equation 1 does not include the respective density terms (g/cm^3) because the protein solution is dilute and the densities are nearly constant, approximating that of water. In Eq. 1, the quasi-steady state is assumed, which means at any time t, there is no change in liquid or protein accumulating at stage n in the foam.

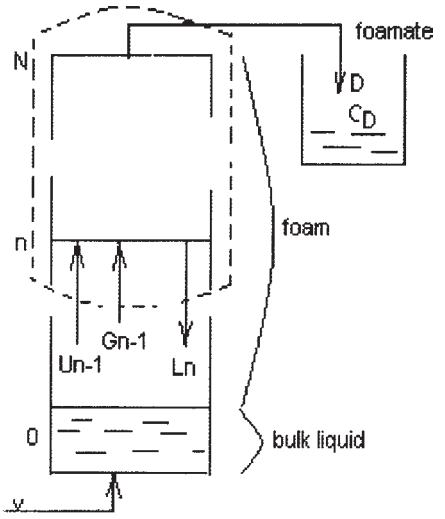

Fig. 2. Model of the foam fractionation column (semibatch).

Although this assumption may not be accurate for static foam drainage according to Bhakta and Ruckenstein *(13)*, it appears to be reasonably valid for semibatch or continuous operation, because the foam phase moves upward, approximating a plug flow.

The upstream flow term, U_n, is related to the liquid holdup, ε_n, and gas flow rate, G_n, by Eq. 2, which is derived from Eq. 3b:

$$U_n = G_n \varepsilon_n / (1 - \varepsilon_n) \quad n = 0, 1, \ldots N \tag{2}$$

$$\varepsilon_n = [(U_n + L_n)/(U_n + L_n + G_n)] \tag{3a}$$

Since $L_n << U_n$, ε_n becomes

$$\varepsilon_n = [U_n/(U_n + G_n)] \tag{3b}$$

in which G_n is assumed to be constant along the column (cm^3/min); and ε_n can be measured continuously by a photoelectric method *(22)*. Actually, ε_n in Eq. 3 is the flow liquid holdup, but we use it to approximate the static liquid holdup. ε_n may vary with both time and position, because the protein concentration and the bubble size may change with time. When U_n is known, the drainage term L_n can be obtained from Eq. 1. Hence, given the liquid holdup, ε_n, we can determine both L_n and U_n for $n = 1, 2, \ldots N$ in a multistage process. In this model, the complex mass transfer and hydrodynamic (the film and plateau border drainage and bubble coalescence) mechanisms between the foam cells are not elucidated. They are all included in the change of ε_n.

The protein balance around the foamate and stage n becomes:

$$U_{n-1} C_{n-1} + G_{n-1} S_{n-1} \Gamma_{n-1} - L_n C_n - D C_D = 0 \quad n = 1, 2, \ldots N \tag{4}$$

in which the second term on the left-hand side of Eq. 4 represents the surface protein mass at stage n; C_D is the foamate protein concentration (mg/cm^3); C_n is the liquid protein concentration of stage n (mg/cm^3); Γ_n is the surface protein concentration (mg/cm^2); and S_n is a measure of specific surface area of the foam bubble (cm^2/cm^3 gas), and is represented by Eq. 5:

$$S_n = (\text{surface area})/(\text{gas volume}) = 4\pi r^2/(4/3\pi r^3) = 6/d_n \quad (5)$$

in which d_n is the average bubble diameter at stage n (cm) (based on surface area). d_n becomes larger along the foam fractionation column as the height increases because of bubble coalescence. The degree of change of d_n depends on the degree of bubble coalescence.

The top stage, stage N, analogous to a total condenser in a distillation process, is described by Eq. 6:

$$C_D = (DC_N + G_N S_N \Gamma_N)/D \quad (6)$$

The surface concentration of protein Γ_n either is at equilibrium with the liquid concentration C_n or it can be related to C_n by nonequilibrium adsorption kinetics, depending on which is the rate-controlling step among adsorption, molecular diffusion, and convective mass transfer. It is assumed that the equilibrium relationship holds in this study, because the initial protein concentration used is high (>10^{-2} wt%). Therefore, both the transport rate by diffusion and convection and the adsorption rate are very rapid, resulting in the time for protein adsorption equilibrium to be much shorter than its residence time at stage n. The dynamics of mass transfer have to be considered when there is a nonequilibrium rate-controlling step. The general adsorption isotherm follows:

$$\Gamma_n = f(C_n) \quad (7)$$

For proteins, in general, adsorption on the air-liquid surface cannot be described simply by a Langmuir isotherm or a linear isotherm over the entire concentration range *(23)*. The study by Guzman et al. *(21)* on the adsorption isotherms of β-casein, BSA, and lysozyme indicates that at both lower (<10^{-5} –10^{-3} wt%) and higher (>10^{-2} wt%) protein concentrations, the isotherm may be represented by a linear relationship, with a much steeper slope at higher concentrations (multilayer adsorption) than at lower concentrations (monolayer adsorption).

Semibatch Column Analysis

For a semibatch operation (as shown in Fig. 1), a certain volume of feed solution is added initially to the column. Then gas is passed through that solution at a certain flow rate. The foam phase moves upward and overflows out of the top of the column. The overflow is collected and liquid (foamate) is recovered by collapsing the overflow foam. During this process, the liquid pool volume, V_b, and liquid pool protein concentration, C_b,

vary with time, as shown by Eqs. 8 and 9 when there is no accumulation of liquid and protein in the foam phase.

$$\frac{dV_b}{dt} = -D \tag{8}$$

$$\frac{d(V_b C_b)}{dt} = -DC_D \tag{9}$$

Eqs. 8 and 9 assume that the liquid pool is well mixed and acts like one stage. Eqs. 8 and 9 can be solved analytically when D is a constant; otherwise they can be solved numerically by differencing them into Eqs. 10 and 11, respectively:

$$V_{b,k} = V_{b,k-1} - D_k \Delta t \quad k = 1, 2, \ldots K \tag{10}$$

$$C_{b,k} = (V_{b,k-1} C_{b,k-1} - D_{k-1} C_{D,k-1} \Delta t)/V_{b,k} \quad k = 1, 2, \ldots K \tag{11}$$

in which Δt is the time interval; and k is an integer to indicate time at $t = k\Delta t$, $k = 1, 2, \ldots K$. The solution is quite accurate when the time step, Δt, is very small. For this semibatch column, D is not a constant, so the numerical method is used in our calculations.

The fractionation enrichment, or enrichment ratio, ER, at time $t = k\Delta t$ is defined as follows:

$$ER = C_{D,k}/C_{b0} \tag{12}$$

in which C_{b0} is the initial protein concentration of the feed solution.

It follows then that the cumulative average (in time) enrichment is

$$\overline{ER} = \sum_{k=1}^{K} D_k C_{D,k} / C_{b0} \sum_{k=1}^{K} D_k \tag{13}$$

Continuous Column Analysis

Equations can be developed for continuous operation. This is the process shown in Fig. 1, except a feed stream, F, is added to the liquid pool and a bottom residue stream, B, leaves from the liquid pool. At steady state, for the given feed flow rate F (cm^3/min) and concentration C_f (mg/cm^3), the residue output flow rate and concentration are given as B (cm^3/min) and C_B (mg/cm^3), respectively, so that the overall mass balance becomes

$$F = B + D \tag{14}$$

$$FC_f = BC_b + DC_D \tag{15}$$

Solving Eqs. 14, 15, and 1–5, the enrichment ratio can be obtained:

$$ER = C_D/C_f \tag{16}$$

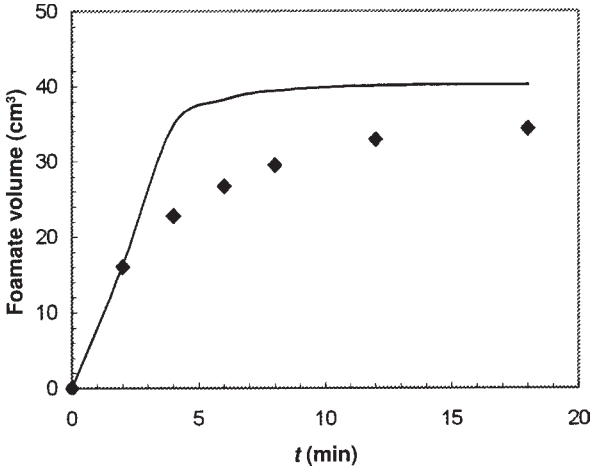

Fig. 3. The changes in cumulative foamate volume with time during semibatch foam fractionation of egg albumin. ◆, Experimental data of Loha *(15)*; curve depicts calculated values. pH = 4.0, initial concentration C_{b0} = 0.075 mg/mL, and superficial gas velocity v = 10 cm/min.

Application of the Model to the Semibatch Foam Fractionation of Egg Albumin

Loha's *(15)* experimental data for the semibatch foam fractionation of egg albumin were used to estimate the liquid holdup, ε_1, values for the foam phase (stage one), as follows:

$$\varepsilon_1 = 10^{-kt + 0.8371} \tag{17}$$

in which k is a constant in the range of 0.002–0.0035 (1/s), depending on the superficial gas velocity, v, and initial solution concentration; and t is time (s).

The bubble size of the foam phase change with time was also determined by Loha *(15)* as

$$d_1 = 0.2 + mt \tag{18}$$

in which the coefficient m is in the range of 0.001–0.002 (cm/s) and depends on the superficial gas velocity and the initial concentration; and d_1 is the average bubble diameter (cm).

The adsorption isotherm of egg albumin was assumed to be linear at higher concentrations (>10^{-2} wt%), based on the data of Guzman et al. *(21)*:

$$\Gamma = 1.2 \times 10^{-2}C - 3.0 \times 10^{-5} \tag{19}$$

in which Γ is in milligrams per square centimeter and C is in milligrams per cubic centimeter.

Figure 3 shows the comparison between the changes in the values of the calculated and experimental accumulative foamate volumes with time. Here, the model predicts values up to 40% higher than the values of the measured foamate volume after 5 min. A possible reason for this high pre-

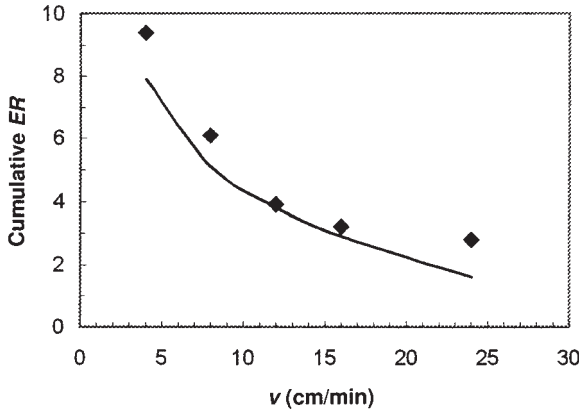

Fig. 4. Effect of superficial gas velocity on the cumulative ER for semibatch foam fractionation of egg albumin. ◆, Experimental data of Loha *(15)*; curve represents the calculations. Initial concentration C_{b0} = 0.075 mg/cm^3 and pH = 4. The time of each experiment was 15 min *(15)*.

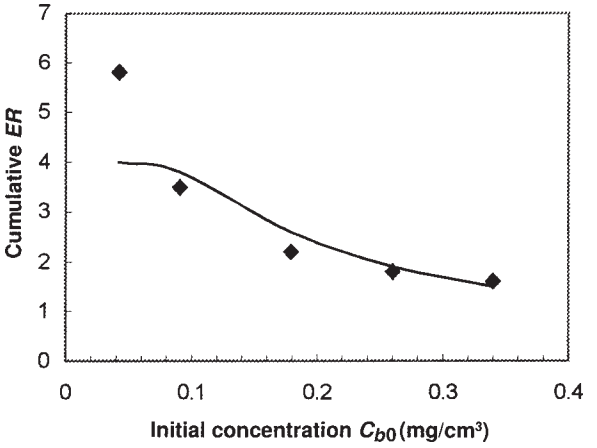

Fig. 5. The effect of initial concentration on enrichment for the semibatch foam fractionation of egg albumin. ◆, Experimental data *(15)*; curve shows the model prediction. pH = 4 and v = 10 cm/min.

diction may be that the liquid holdup assumed from Eq. 10 is larger than the actual value. If this is indeed the case, one way to sharpen the result of the predicted foamate volume would be to measure the liquid holdup directly.

Figure 4 indicates the calculated enrichment values for egg albumin compared with the experimental values. It appears that at both low and high superficial gas velocities, v, the predicted enrichments are about 20% lower than the experimental values, whereas at intermediate velocities, the prediction is quite accurate. These deviations may reflect the errors in the predicted foamate volumes at the two extreme gas velocities. When the gas velocity is either very low or very high, the predicted foamate

volume may be larger than the actual experimental data. This leads to a dilution of the protein solution, resulting in lower estimated foamate concentrations and lower enrichments.

Figure 5 depicts the effect of initial concentration on the enrichment in the egg albumin semibatch foam fractionation process. At very low initial concentrations, the model predictions are about 30% below the actual enrichment data. For initial concentrations higher than 0.1 mg/cm^3, the model fits the experimental data well.

Application of the Model to the Continuous Foam Fractionation of BSA

For BSA aqueous solution, at a concentration, C, within the range of 0.040–1.0 mg/cm^3, the adsorption isotherm can be approximated by a linear relationship based on experimental data *(16)*:

$$\Gamma = 1.13 \times 10^{-3}C + 3.21 \times 10^{-4} \tag{20}$$

in which Γ is in milligrams per square centimeter and C is in milligrams per cubic centimeter.

The model prediction results shown in Fig. 6 are developed using the average bubble size from Brown et al.'s *(16)* experimental data. It is observed that when the liquid holdup in the foam phase is determined between 0.1 and 0.5%, the predicted enrichments are very close to the experimental value. Brown et al.'s *(16)* study did not give liquid holdup data, but according to Loha's *(15)* data, it would be expected to be <3%. Our estimate for the liquid holdup is apparently close to the actual values, based on the results of Fig. 6. If we use actual experimental values in later calculations, the model would be evaluated with more accurate parameters. Figure 6 shows that with an increase in gas velocity, the enrichment decreases. Figure 5 shows that a higher feed concentration leads to a lower enrichment at a given gas velocity. Both are consistent with observations in the literature *(5,8,17,18)*.

The model evaluation was performed using a Mathematica 3.0 program, which we compiled. From the above calculations and comparisons with experimental data, it is observed that in order to predict accurately the performance by this simple single-stage model, the following parameters need to be measured or known more accurately:

1. Bubble size and liquid holdup, both of which may be functions of time and position. It may be appropriate, in certain cases dealing with new systems in which prediction is desired, to make these terms predictive in the simulation by studying the relationship between them and those key physicochemical properties of the solution (such as protein concentration, liquid viscosity, surface tension, and pH) and the operational conditions (such as gas velocity and column height).
2. The HTU, which leads to the stage number N.
3. The adsorption isotherm and/or adsorption kinetics relationship.

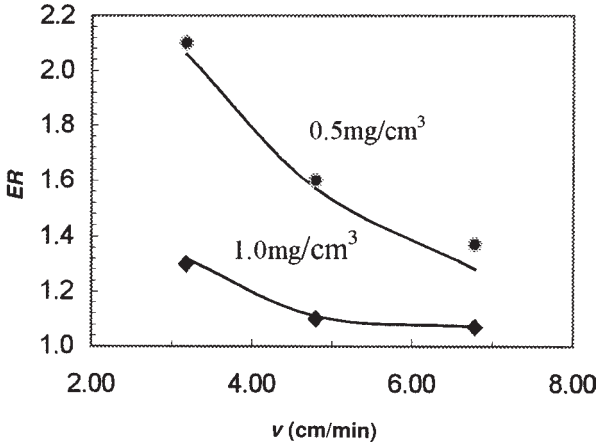

Fig. 6. The effect of superficial gas velocity on *ER*: comparison between results calculated by this model with that of Brown et al.'s data *(2)* for continuous foam fractionation of BSA. Here, the feed flow rate is $F = 0.02$ cm/s, pH = 7, and the foam height $z = 5$ cm. $C_f = 1$ mg/cm^3 (0.1 wt%) and 0.5 mg/mL (0.05 wt%), respectively; ● and ◆ are Brown et al.'s *(2)* data; curves are the calculated values.

Future work calls for the development of a photoelectric method in order to measure bubble size and liquid holdup for each system under different conditions. With these sharper measurements, more accurate theoretical correlations may then be developed between these two most significant parameters and the desired related properties. It is noted that this correlative model is much simpler than those previously developed using film drainage and coalescence theories *(16,17)*. Also, this single-protein correlative model is also easier to extend to mixtures present in typical protein foam fractionations. Evaluation of this model for a multistage (more than two stages) column can be made once the key bubble size and liquid holdup variables are measured at each stage.

Conclusion

The model developed in this article is characterized by the use of the quasi-steady-state assumption, which results in the relationship of upward and downward liquid flow rates. The upflow term is obtained from the liquid holdup and gas flow rate terms. The use of these latter two terms greatly simplifies the calculation since the detailed drainage and coalescence processes do not need to be considered. Finally, the model is able to predict the column performance accurately only when the bubble size and liquid holdup data are known, so obtaining such data either from an accurate correlation or by direct measurement is essential for predicting the performance of protein foam fractionation systems. It then becomes imperative to measure the bubble size and liquid holdup data to build up correlations for different systems and to predict the performance of new,

untested systems. The simulation examples of this model for the single-stage semibatch foam fractionation of egg albumin and the single-stage continuous foam fractionation of BSA we have evaluated indicate that the model is reasonably both quantitatively and qualitatively accurate for these two modes of operation. The model seems to be particularly accurate for predicting the behavior of continuous foam fractionation processes. The accurate prediction of the continuous column seems to validate the steady-state assumption invoked for continuous operation. When coupled with accurate data for both bubble size and liquid holdup, the model should be even more accurate for predicting the performance of steady- and unsteady-state foam fractionation processes.

Acknowledgments

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