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A Study on Protein Concentration by Foam Fractionation

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Abstract—In order to develop and model an efficient continuous separation process based on foam fractionation, bovine serum albumin (BSA) solution was experimentally investigated for the effect of operation parameters (feed concentration, foam sampling height, and superficial gas velocity) on separation ratio (SR). Then, an engineering approach was taken to describe this separation scheme, resulting in a design factor (ξ) which is strongly related to foam structure. A fairly good correlation between this design factor and the operating parameters, based on the nonlinear regression analysis with experimental data, was obtained.

Key Words : Foam separation, Foam fractionation, Empirical model, Protein, BSA

INTRODUCTION

Foam fractionation or separation is an adsorptive bubble separation technique in which soluble, surface-active substances can be removed from the solution by preferential adsorption at the gas-liquid interface. The foam fractionation technique has been widely used in several industrial fields including wastewater treatment (Jenkins et al., 1972), ore flotation (Fuerstenau and Healy, 1972), etc. Some biological substances, such as enzymes and proteins, contain surface-active groups that can be adsorbed at the gas-liquid interface generated by an ensemble of rising bubbles through the column. This amphipathic nature makes foam fractionation a viable technique for concentrating and enriching proteins and enzymes from very dilute solutions. Therefore, this technique has found its application in biochemical and food industries (Bhattacharya et al., 1991; Brown et al., 1990; Mohan et al., 1992; Montero et al., 1993). In addition to its low energy requirement, simple construction, and the ease for scale up, the cost efficiency in operation and maintenance makes foam fractionation an attractive technique.

Foam fractionation involves many complex mechanisms and phenomena such as interface absorption, bubble size distribution, mass transfer, bubble rupture, foam and/or bubble coalescence and fluid drainage between foams. Numerous studies have been reported using foam fractionation on separating or purifying bovine lactoferrin (Noel et al., 2002), wheat flour proteins (Keller et al., 1997), ovalbumin (egg white) and hemoglobin (Maruyama et al., 2000), lysozyme and β-casein (Hunter et al., 1991), BSA, cc-lactalbumin and Lactoferrin (Saleh and Hossain, 2001), lysozyme and albumin (Lockwood et al., 2000). Some researchers experimentally investigated the effect of several operating parameters on the separation ratio (SR), defined as the ratio of the protein concentration of the foam collected from the outlet of the foam column to that of the corresponding bulk protein solution in the bottom column. Some others also theoretically developed mathematical models for system liquid holdup and analyzed the related phenomena (Bhattacharjee et al., 1997, 2001; Brown et al., 1990; Desai and Kumar, 1982, 1983; Hartland and Barber, 1974; Lalchev et al., 1982; Lockwood et al., 2000; Narsimhan and Ruckenstein, 1986a, 1986b; Webb et al., 2002).

Although the results of the developed models were able to account for the trend of the experimental data (Brown et al., 1990; Desai and Kumar, 1982, 1983; Du et al., 2000; Hartland and Barber, 1974; Neely et al., 2001; Webb et al., 2002), the variable parameters, complex mathematics and fluid mechanics make them difficult in estimating and applying to practical engineering perspective. An attempt was made in the present work to establish a simple semi-empirical model and demonstrate its usefulness in correlating experimental data of the continuous foam fractionation of bovine serum albumin (BSA).

EXPERIMENTAL

As shown in Fig. 1, the experimental setup for continuous foam fractionation was designed and

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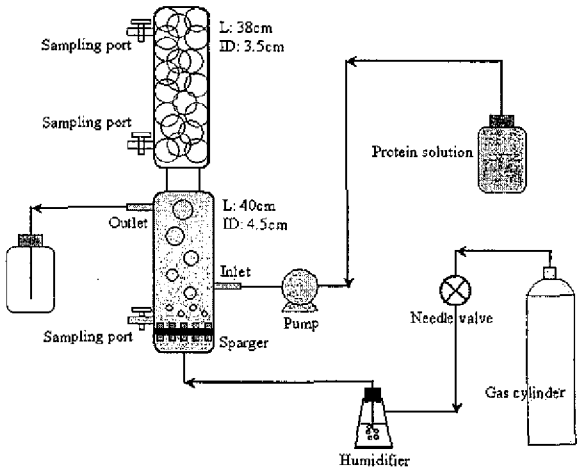

Fig. 1. Experimental setup for continuous foam fractionation.

constructed to produce gas-liquid dispersions under controlled conditions. It consisted of a 38 cm in height by 3.5 cm in diameter glass foam column on top of a 40 cm in height by 4.5 cm in diameter glass bottom column surrounded by a water jacket. BSA protein solution at desired concentration was filled into the bottom column to carry out the foaming experiments. Nitrogen gas, controlled by a rotameter, was passed through a humidifier, and then supplied into the bottom column through a sparger. Two peristaltic pumps were added to continuously supply and withdraw the protein solution into and out of the system. During the process of continuous operation, the height of the foam-liquid pool interface was observed visually and maintained by adjusting the liquid flow rate at the exit. Periodic sampling was taken to confirm that the steady state has been reached.

Protein foams collected from sampling ports at different heights were allowed to collapse in the collecting cups or beakers, and stored at 4°C. The protein concentration of the sample was determined by a UV spectrophotometer at wavelength of 280 nm and a pre-determined standard curve. Various concentrations of bovine serum albumin (Sigma, MO, U.S.A.) were prepared with 0.07 M phosphate buffer solution of pH 7.1.

RESULTS AND DISCUSSION

Experimental studies

Figures 2(a) and 2(b) show the effect of sampling height on the separation ratio (SR). Separation ratio is defined as the ratio between protein concentration in the foam and protein concentration in the feed. As can be seen in Figs. 2(a) and 2(b), the protein concentrations in the foam and the SR of BSA were greatly affected by the sampling height. The

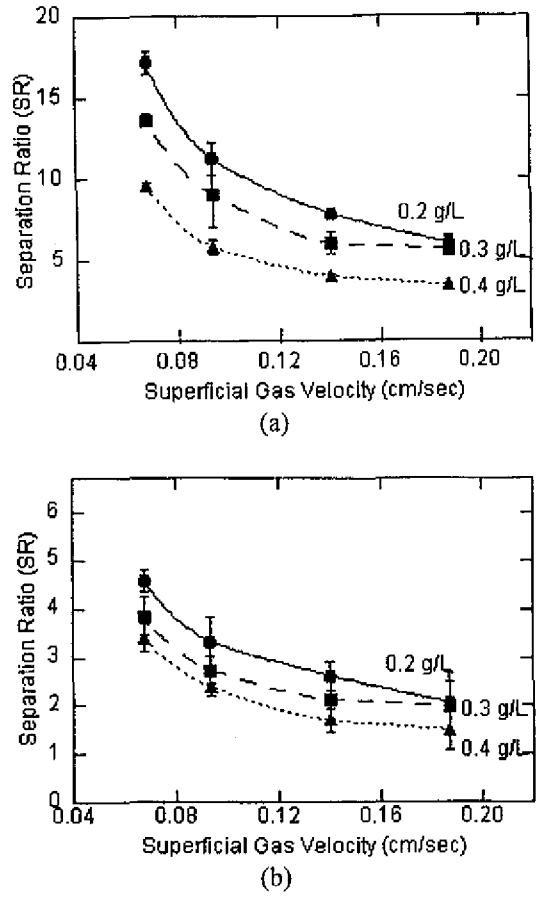

Fig. 2. Effects of nitrogen gas flow rate and feed protein concentration on separation ratio in the continuous foam fractionation. (a) All the operations were performed at the sampling height of 20 cm, temperature of 30°C, and pH 7.1. The operations were performed at feed protein concentration of 0.2 g/L (●), 0.3 g/L (■), and 0.4 g/L (▲). (b) All the operations were performed at the sampling height of 1 cm, temperature of 30°C, and pH 7.1. The operations were performed at feed protein concentration of 0.2 g/L (●), 0.3 g/L (■), and 0.4 g/L (▲).

sampling height is defined as the height of sampling port above protein liquid/solution surface. At the sampling port of 1 cm above liquid level, the SR roughly ranges from 2 to 5, showing that the protein concentration difference between the foam and bulk liquid phase is small. However, the protein concentration obtained at higher position (20 cm above the liquid level) in the foam column is much larger than that in the bulk liquid phase. Hence, the SR increases with increasing sampling height. For example, at feed protein concentration of 0.2 g/L and gas flow velocity of 0.65 cm^3/s (0.067 cm/s), the SR of lower sampling port (1 cm above the liquid surface) and higher sampling port (20 cm above the liquid surface) were 4.5 and 17.2, respectively. The variation is significant. Similar trend was observed in the other case

studies (Bhattacharya et al., 1991; Liu et al., 1995). Such improvement in the SR is believed to result from the effect of the sampling height. As the foam moved up along the foam column, the entrained liquid within the bubbles was drained and moved downward according to the action of gravity. Since the entrained solution, having similar protein concentration to the bulk solution, was left out from the foam, only the concentrated BSA solution in the thin films moved up due to preferential absorption of this surface-active protein at the gas-liquid interface. Higher concentration of bovine serum albumin was, therefore, obtained at the higher port of the top foam column. Similar result was also observed for batch-wise foam fractionation (Liu et al., 1995).

The effect of feed protein concentration on the SR is also shown in Figs. 2(a) and 2(b). It is indicated that, for a given superficial gas velocity or gas flow rate, SR tends to decrease with an increase of feed protein concentration. According to the characteristic of the adsorption isotherm for BSA reported by Graham and Phillips (1979), even though the surface concentration decreases along with the decrease in the concentration of bulk solution, the rate of decreasing become smaller as the concentration of bulk solution decreases. Therefore, the lower the feed protein concentration is, the larger the value of separation ratio would be.

In addition, Figs. 2(a) and 2(b) illustrate the experimentally found variation of SR with the superficial gas velocity in the continuous operation. It is evident that, for all feed concentrations, the SR decreases with an increase in gas velocity. Initially, at lower gas flow rates, there is a rapid decrease in the SR with a small increase in the gas flow rate. With increasing gas flow rate, however, the SR becomes less sensitive to the gas flow rate and levels off. This is due to the fact that, as the superficial gas velocity increases, more liquid would be carried (or entrained) to the foam column and results in a dilution effect caused by an increase in the amount of bulk solution in the foam column. As a result, the averaged protein foam concentration is decreased with increasing gas flow rate.

The SR was determined as a function of feed flow rate at constant gas flow velocity. In Fig. 3, the SR decreases with increasing feed flow rate. Results imply that the contact time of fluid element, which can be defined as the duration of foam traveling from liquid/foam interface to the outlet, affect the separation performance. This can be explained by the fact that the higher the feed flow rate was, the shorter contact time of foam could be expected. In the case at the sampling height of 20 cm, the SR decreased with increasing feed flow rate as the contact time between the fluid element and liquid in the pool became shorter.

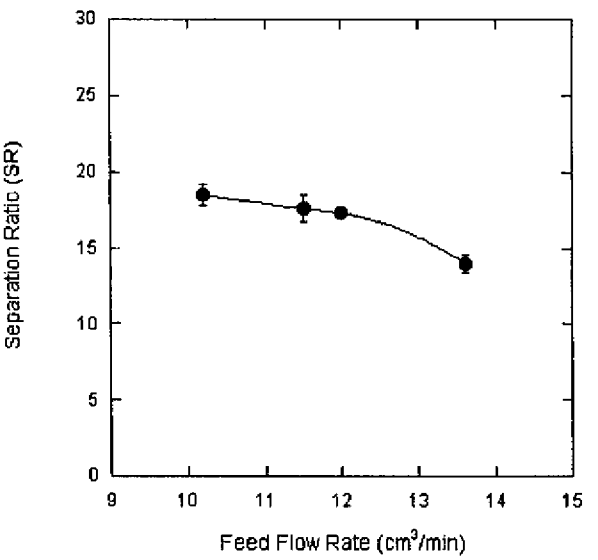

Fig. 3. Effect of feed flow rate on separation ratio in the continuous foam fractionation. The operations were performed at feed protein concentration of 0.2 g/L, temperature of 30°C, pH 7.1 and sampling height of 20 cm.

As seen from the previous results, the separation performance of the continuous operation was determined by the gas flow rate and feed flow rate. We examined the combined effect of these two variables (G/F, gas to feed flow rate ratio) on the SR. As shown in Fig. 4, the SR level drops with increasing G/F ratio up to a certain critical level. Also, it is evident that the SR decreases as protein concentration in the feed increases. Considering the separation efficiency (SE), defined as the product the flow rate of foam removal and protein concentration in the foamate divided by the product of the flow rate of effluent and protein concentration in the effluent, we examined the role of G/F ratio on the SE. It is known that an increase in G/F ratio results in an enhancement in the amount of protein molecules transferred from liquid pool to foam phase. Once a critical G/F value is reached, the possibility of foam breaking inside the foam column will increase. Similar phenomenon called flooding operation was also seen in the two-phase mass transfer operation (Bhattacharya et al., 1991).

Theoretical consideration

Description of the physical system

In the continuous foam fractionation operation under the steady state condition, foams are generated at the gas-liquid pool interface and removed from the top of the foam column. Thin liquid films and plateau borders surround cellular foams of protein. As foams move to the top of the column, the liquid in the foams is carried upward and the entrained liquid

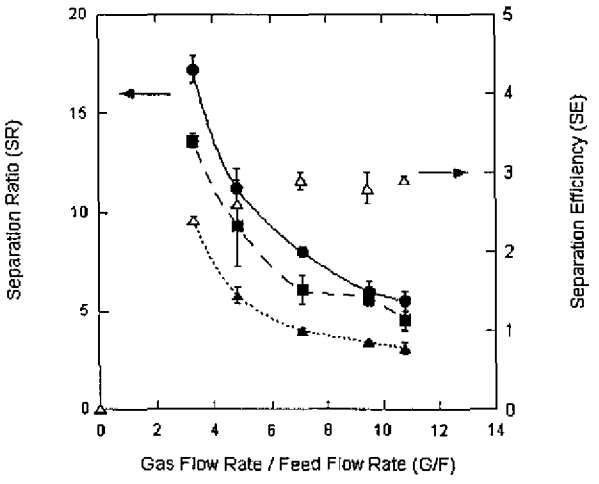

Fig. 4. Effect of feed flow rate to gas flow rate ratio on separation ratio and separation efficiency in the continuous foam fractionation. The operations were performed at feed protein concentration of 0.2 g/L (●), 0.3 g/L (■), 0.4 g/L (▲), temperature of 30°C, pH 7.1, and sampling height of 20 cm.

is distributed between the plateau borders and the thin films. The liquid in the films drains into the neighboring plateau borders under the action of plateau border suction and disjoining pressure, meanwhile, draining downwards through a network of plateau borders due to gravity pulling. As a result, the amount of liquid entrained in the foam decreases with height.

Simplification and modeling

The following assumptions were employed in the establishment of the simplified model.

1. The average size of foam bubbles in the foam column may be represented by some characterized diameter, d.
2. The movement of foam bed is approximated as the plug flow.
3. The surface concentration of BSA is the equilibrium concentration and follows the relationship presented by Graham and Phillips (1979).
4. The protein concentration of inter-bubble plateau borders is equal to that of the bulk solution.
5. The coalescence effect of foam maybe absorbed into the characterized diameter and therefore neglected.

The foam structure is usually assumed to be dodecahedron in shape by other investigators (Brown et al., 1990; Desai and Kumar, 1982, 1983; Hartland and Barber, 1974; Narsimhan and Ruckenstein, 1986a, 1986b). Because of the complexity of the dodecahedral shape, size distribution as well as coalescence of foam, the characterized spherical configuration of the bubble was assumed in this study to simplify the model formulation.

In order to determine the contribution of the protein absorbed onto the surface, the total surface area per volume of liquid must be determined. Since most of the surface area on the foam bubble comes from the thin films, the contribution of the surface area from the plateau borders may be neglected. Let d denote the characterized diameter of foam bubble and the volume of a single bubble equals $\pi d^3/6$. The number of foam bubbles per unit volume of foam bed is $6(1-\varepsilon)/\pi d^3$, where ε is the fraction of the liquid or liquid holdup of the foam bed. It should be noticed that d takes several factors, such as nonspherical shape of bubbles and size distribution of bubbles, into consideration, *i.e.* it is a characteristic diameter of an assemble of foam bubbles. The amount of protein absorbed per unit surface area, defined as surface concentration, is Γ, and the total amount of protein absorbed on the surface per unit volume of foam bed would be $6(1-\varepsilon)\Gamma/d$. If the flow rate of BSA in the top product contributed from the surface is denoted as F_{film} and the liquid flow rate of foam at the top is Q, the protein contributed from thin films surrounding the spherical foam bubbles can therefore be represented as Eq. (1):

$$F_{\text{film}} = \frac{6(1-\varepsilon)\Gamma Q}{\varepsilon d}. \tag{1}$$

Another protein contribution that comes from the bulk liquid in the plateau borders, F_{PB}, can be written as:

$$F_{PB} = c_{\text{bulk}}\, Q. \tag{2}$$

From material balance, the total flow rate of BSA protein on the top can be obtained by the combination of Eqs. (1) and (2):

$$c_{\text{foam}}\, Q = \frac{6(1-\varepsilon)\Gamma Q}{\varepsilon d} + c_{\text{bulk}}\, Q, \tag{3}$$

where c_{foam} is the average protein concentration in the foam. If Q is divided on both sides, c_{foam} will be obtained in the following formulation:

$$c_{\text{foam}} = \frac{6(1-\varepsilon)\Gamma}{\varepsilon d} + c_{\text{bulk}}. \tag{4}$$

Therefore, by definition, the SR can be written as:

$$\text{SR} = \frac{c_{\text{foam}}}{c_{\text{bulk}}} = \frac{6(1-\varepsilon)}{\varepsilon d\, c_{\text{bulk}}} + 1. \tag{5}$$

The protein adsorption isotherm can be expressed as a linear equation of protein concentration. The adsorption isotherm for BSA is provided by Graham and Phillips (1979); Brown et al. (1990):

$$\Gamma = 1.13\times10^{-5} c + 3.21\times10^{-7}, \tag{6}$$

where Γ and c are in g/cm^2 and weight percent and coefficients are valid for the protein concentration ranging from 0.004 to 0.1 wt% (Brown et al., 1990,

Graham and Phillips, 1979).

By rearranging Eq. (5) and defining a new parameter ξ, called the design factor, we can obtain Eq. (7):

$$SR - 1 = 6\,\xi(1.13\times10^{-3} + 3.21\times10^{-7}/c_{bulk}), \quad (7)$$

where $\xi = \dfrac{1-\varepsilon}{\varepsilon\, d}$.

In addition, the relationship between concentration of the bulk liquid c_{bulk} and concentration of the feed c_{feed} can be expressed by Eq. (8):

$$c_{bulk} = \{F / [F + (SR - 1)\, Q]\}\, c_{feed}. \quad (8)$$

By investigating several operating variables such as foam height, protein feed concentration, superficial nitrogen gas velocity, volumetric flow rates of feed, the dependence of these variables on the design factor can be represented as a simple correlation (shown in Eq. (9)) with one proportional coefficient, k and four exponents, a_1, a_2, a_3 and a_4.

$$\xi = k\, c_{feed}^{\,a_1} v^{a_2} h^{a_3} F^{a_4}. \quad (9)$$

Based on the experimental data along with the ANOVA statistical analysis, we concluded that the four variables, feed protein concentration c_{feed}, foam height h, superficial gas velocity v and volumetric flow rate of feed F mainly determined the corresponding value of design factor ξ. The correlation and values of coefficient and exponents were determined by the nonlinear regression analysis. The result is presented in Eq. (10).

$$\xi = 0.4326\, c_{feed}^{-0.4138}\, v^{-1.2049}\, h^{0.4824}\, F^{0.2831}. \quad (10)$$

The values of design factor ξ based on the experimental data were calculated from Eqs. (7) and (8) for all cases studied. The calculated design factors from Eq. (9) were compared to those obtained from experimental results in Fig. 5. As can be seen, the proposed semi-empirical correlation fits the experimental data in a fairly decent manner. The deviation is within ± 20% under most experimental conditions.

It is worth noticing that coefficients/constants used the equilibrium isotherm may vary with the type of protein, which leads to the dependency of the value of SR on the type of protein. Apparently, based on the calculation algorithm stated above, the numerical values of coefficients and exponents used in the designing correlation are fairly protein-dependent. However, the methodology employed in this study demonstrates, from engineering aspect, a decent attempt/approach for a complex bioseparation operation.

CONCLUSION

The following conclusions may be drawn from this work:

(1) Separation ratio, defined as the ratio between protein concentration in the foam and protein concentration in the feed, increases with increasing sampling height, while decreases with increasing nitrogen gas flow rate (or gas superficial velocity).

(2) Separation ratio decreases with elevated feed concentration or increasing feed flow rate.

(3) Separation ratio tends to decrease as the ratio between gas flow rate and feed flow rate increases.

(4) A design factor relating key operating variables and a simple semi-empirical correlation describing foam fractionation operation on protein concentration system are proposed in this study.

In summary, this work demonstrates the utility of a simple semi-empirical correlation along with experimental observations in examining foam fractionation on protein concentration. We believe that the methodology in this study can be further applied to other complex chemical and/or biochemical separation or fractionation operation systems.

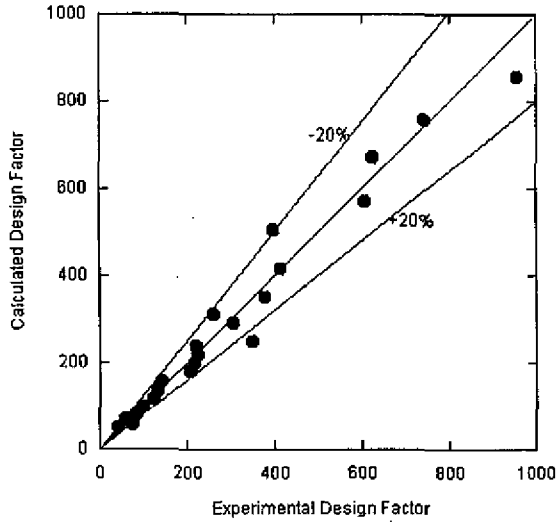

Fig. 5. Relationship between calculated and experimental design factors. Calculated design factors were obtained by the Eq. (9). Proportional coefficient and exponents in Eq. (9) were determined from experimental data along with nonlinear regression analysis.

NOMENCLATURE

a_i	exponents given in Eq. (9), i=1, 2, 3, 4
c_{bulk}	protein concentration of the bulk liquid, g/cm^3
c_{feed}	protein concentration of the feed, g/cm^3
c_{foam}	protein concentration of the foam, g/cm^3
d	equivalent diameter of the foam bubble, cm

F volumetric flowrate of the feed, cm^3/s
F_{film} mass flowrate of BSA in the top product contributed from the film, g/s
F_{PB} mass flowrate of BSA in the top product contributed from the plateau border, g/s
G volumetric flowrate of the nitrogen gas, cm^3/s
k proportional coefficient given in Eq. (9)
Q liquid flow rate of foam at the top, g/s
SE separation efficiency (defined as ratio between the amount of protein removal in the foamate and that in the effluent)
SR separation ratio (defined as the ratio between protein concentration in the foam and protein concentration in the feed)
v superficial gas velocity, cm/s

Greek symbols

Γ surface protein concentration, g/cm^2
ε liquid holdup
ξ design factor

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以泡沫分離技術濃縮蛋白質研究

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摘　要

本文研究以泡沫分離技術濃縮牛血清蛋白及各項操作變因（如進料濃度、泡沫管高度、氣體流速）對濃縮比之影響，同時以工程觀點推導一簡單模式，迴歸實驗中諸多操作變因對濃縮比之影響，結果顯示此一簡單模式可有效迴歸各操作變因。