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Protocols

# Role of foam drainage in producing protein aggregates in foam fractionation

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ABSTRACT

It is essential to obtain a clear understanding of the foam-induced protein aggregation to reduce the loss of protein functionality in foam fractionation. The major effort of this work is to explore the roles of foam drainage in protein aggregation in the entire process of foam fractionation with bovine serum albumin (BSA) as a model protein. The results show that enhancing foam drainage increased the desorption of BSA molecules from the gas-liquid interface and the local concentration of desorbed molecules in foam. Therefore, it intensified the aggregation of BSA in foam fractionation. Simultaneously, it also accelerated the flow of BSA aggregates from rising foam into the residual solution along with the drained liquid. Because enhancing foam drainage increased the relative content of BSA molecules adsorbed at the gas-liquid interface, it also intensified the aggregation of BSA during both the defoaming process and the storage of the foamate. Furthermore, enhancing foam drainage more readily resulted in the formation of insoluble BSA aggregates. The results are highly important for a better understanding of foam-induced protein aggregation in foam fractionation.



## 1. Introduction

Aggregation is an important issue in various pharmaceutical protein processes, such as renaturation, purification, shipping, and storage, because it often reduces protein efficacy and even causes harmful immune responses [1,2]. As a promising alternative for industrial protein separation, foam fractionation is hampered by protein aggregation. Protein molecules suffer structural unfolding that makes their hydrophobic groups exposed towards the gas phase as they are adsorbed at the gas-liquid interface [3]. Owing to bubble coalescence and defoaming, the adsorbed molecules are desorbed from the interface and, then, some of them readily aggregate via exposed hydrophobic groups [4–6]. In foam fractionation, most of the protein molecules must tolerate the adsorption-desorption process, so many aggregates will be produced and, hence, the separation performance will be poor [7]. At present, many studies on foam fractionation of proteins have been reported [8,9], but few have paid attention to protein aggregation.

The key to reducing the loss of protein efficacy in foam fractionation is to have a clear understanding of foam-induced protein aggregation. Several researchers have recently investigated the mechanisms of protein denaturation induced by foam [3,10–12]. However, their efforts were aimed at analyzing the relationship between the activity loss and the foam-induced structural change of a protein, but not at studying the foam-induced protein aggregation. Maa and Hsu [4] have confirmed that the presence of the air-liquid interface and shear force was able to enhance protein aggregation. Furthermore, Wiesbauer et al. [13] reported that the change of the air-water interfacial area played a critical role in protein aggregation, confirming the results of Bee et al. [14]. This conclusion is highly important for fundamental understanding of foam-induced protein aggregation. However, researchers have not yet considered the role of foam drainage, an intrinsic phenomenon of foam. Hence, further research is required to fully understand foam-induced protein aggregation.

Foam drainage is a critical step for protein enrichment in foam fractionation [15]. As foam drainage is enhanced, the amount of

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protein molecules adsorbed at the gas-liquid interface becomes much higher than that of protein molecules in the interstitial liquid between bubbles. The increase in the relative content of the adsorbed protein molecules in the foam may then intensify protein aggregation due to their unfolded structures [6]. Foam drainage also enhances bubble coarsening and coalescence to decrease the gas-liquid interfacial area [16]. With the increase in bubble size, the protein surface excess and the protein–protein interactions at the gas-liquid interface may increase to enhance protein aggregation [10,14]. Thus, foam drainage has significant effects on foam-induced protein aggregation.

To better understand protein aggregation in foam fractionation, the role of foam drainage in the foam-induced protein aggregation was an object of the present study. Bovine serum albumin (BSA) is a typical globular protein that has many applications in drug delivery, biochemical studies, and cell culturing [17–19]. Thus, BSA was chosen as a model process. In this work, the different levels of foam drainage were obtained by changing the foam height in the foam fractionation column. The effects of foam drainage on the following four areas of interest were then studied: (1) adsorption of BSA at the gas-liquid interface and bubble size in the rising foam, (2) production rates and partition coefficients of BSA aggregates, (3) mass fluxes and relative contents in the foamate of BSA aggregates, and (4) variations of the relative contents in the foamate of BSA aggregates with time.

## 2. Materials and methods

### 2.1. Reagents

Bovine serum albumin (BSA, purity > 99%) was purchased from Tianjin Unite Stars Biotechnology Co. Ltd., China. It was dissolved in a concentration of 0.30 g/L in a disodium hydrogen phosphate (0.165 M)–citric acid (0.018 M) buffer solution with pH 7.0. The specific composition of the BSA solution is presented in Table 1. Tween 20 (analytical grade) was purchased from Tianjin Yingdaxigui Co. Ltd., China. Ultrapure water (electrical resistance 18.25 MΩ) prepared in a UPR-II-10T water purification system (Chengdu Ultrapure Technology Co. Ltd., China) was used in all the experiments.

### 2.2. Equipment

A schematic of the continuous foam fractionation of BSA followed by defoaming is presented in Fig. 1. The foam fractionation column was constructed with two transparent polymethyl methacrylate tubes of 50 mm i. d. The heights of the two tubes were $H_l$ = 600 mm and $H_f$. The values of $H_f$ were 200, 400, 600, 800, and 1000 mm. The tubes were used to obtain different foam drainage levels. A gas distributor with an average pore diameter of 250 ± 20 μm was installed at the bottom of the foam fractionation column, through which air was pumped into the column to generate bubbles. The BSA feed solution was injected near the foam-liquid interface by a peristaltic pump (YW03, Changzhou Yuanwang Fluid Co. Ltd., China). The foam breaker was made of a transparent, 500-mm-high polymethyl methacrylate tube of 50 mm i. d. and four 450-mm-high, 15-mm-diam cylinders composed of synthetic sponge. The cylinders were fixed inside the tube by stainless-steel wires. The foam fractionation experiments were carried out at a volumetric feed flow rate of 10 mL/min, a volumetric air flow rate of 200 mL/min and at room temperature, 25.0 ± 1.0 °C. The foam flowed out of the foam fractionation column into the foam breaker, and then was completely collapsed to obtain the foamate. When the concentration and composition of BSA in the foamate became constant, the entire system was considered to have reached a steady state. The foamate and the residual solution were then sampled to obtain the required data.

**Table 1**
Specific composition of the feed solution of BSA.

| | Monomer | Dimer | Trimer |
|---|---|---|---|
| Molecular weight (kDa) | 66.4 | 132.8 ± 0.2 | 201.0 ± 5.2 |
| Relative content (%) | 91.5 ± 0.7[a] | 8.0 ± 0.1[b] | 0.5 ± 0.1[c] |

a, b and c were used to characterize the different levels in relative contents of monomer, dimer and trimer.

### 2.3. Measurements of the adsorption of BSA at the gas-liquid interface and bubble radius in the rising foam

The adsorption of BSA at the gas-liquid interface was characterized by three parameters: surface excess ($\Gamma_{BSA}$), and the relative content ($\eta$) and mass flux ($Q_m$) of BSA molecules adsorbed at the gas-liquid interface in a rising foam. $\Gamma_{BSA}$ and $Q_m$ were calculated by Eqs. (1) and (2), respectively, and were measured using the methods detailed by Li et al. [6]; $\eta$ is defined as Eq. (3):

$$\Gamma_{BSA} = \frac{(C_f - C_b)\, r_{32} Q_f}{3Q_g} \tag{1}$$

$$Q_m = Q_f(C_f - C_b), \tag{2}$$

$$\eta = \frac{\frac{3Q_g}{r_{32}}\Gamma_{BSA}}{C_f Q_f} = 1 - \frac{C_b}{C_f}, \tag{3}$$

where $Q_g$ and $Q_f$ are volumetric air flow rate and volumetric foamate flow rate, respectively, and $C_f$ and $C_b$ are the total BSA concentrations in the foamate and in the entrained liquid in the rising foam, respectively. $C_b$ is considered to equal the total BSA concentration in the bulk solution in the foam fractionation column. $r_{32}$ is the Sauter mean bubble radius and is calculated with Eq. (4) [6]:

$$r_{32} = \frac{1}{2}\frac{\sum_{i=1}^{n} {d_i}^3}{\sum_{i=1}^{n} {d_i}^2}, \tag{4}$$

where $d_i$ is the bubble diameter and $n$ the number of bubbles, $n$ > 300. Note that the photographs for the bubble radius measurement were taken near the top of the foam fractionation column.

### 2.4. Evaluation of production of BSA aggregates in foam fractionation

The production rate ($r_{agg}$), distribution coefficient ($K_{agg}$), and mass flux ($Q_{aggf}$) and relative content ($\xi_{agg}$) in the foamate of BSA aggregates were used to evaluate the production of BSA aggregates in foam fractionation. Specifically, $r_{agg}$ was used to characterize the mass of BSA aggregates produced per unit of time in the entire foam fractionation process. $K_{agg}$ was used to characterize the distribution of BSA aggregates in the foamate and in the residual solution. $Q_{aggf}$ was used to characterize the mass of BSA aggregates obtained in the foamate per unit of time. $\xi_{agg}$ was used to characterize the relative content of BSA aggregates in all the recovered BSA molecules in the foamate. The four parameters are defined as Eqs. (5)–(8), respectively:

$$r_{agg(i)} = Q_f C_f(i) + Q_r C_{r(i)} - Q_o C_{o(i)} \tag{5}$$

$$K_{agg(i)} = \frac{Q_f C_{f(i)}}{Q_r C_{r(i)}} \tag{6}$$

$$Q_{aggf(i)} = Q_f C_{f(i)} \tag{7}$$