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Zhonghe Xu[a]; Zhaoliang Wu[a]; Yanli Zhao[a]
[a] Department of Bioengineering, Hebei University of Technology, Tianjin, China

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Foam Fractionation of Protein with the Presence of Antifoam Agent

Zhonghe Xu, Zhaoliang Wu, and Yanli Zhao
Department of Bioengineering, Hebei University of Technology, Tianjin, China

Foam fractionation is a promising technology for protein concentration or purification. However, the presence of an antifoam agent in fermentation broth restricted direct application of the technology. A preliminary approach of the surfactant-assisted foam process was conducted with a simulated system consisting of targeted protein bovine serum albumin (BSA), and a mixed antifoam agent (AF520, silicon oil/PGE mixture). The effects of all three classes of surfactants (anionic SDBS, cationic CTAB, and non-ionic Tween-20) on BSA foam fractionation were examined respectively. Also, the influences of solution pH, PGE, BSA, and NaCl were taken into account. The results revealed that all three classes of surfactants could stabilize foam film, so that the foam process could be operated, while the ionic surfactant exhibited excellent performance on condition that it was allowed to firmly interact with BSA to form a more hydrophobic complex, especially for cationic CTAB. When solution pH was adjusted to 7.5 and CTAB was 20 mg · mL^{-1}, 90% of BSA could be extracted from a previous non-foaming system containing 100 mg · mL^{-1} BSA and 4 mg · mL^{-1} AFA, and the enrichment reached 7.42. A higher enrichment of BSA could be obtained with increasing addition of AFA but at the expense of the recovery. On the contrary, increasing BSA concentration gave rise to an opposite performance. The experiments also showed that the foam stability of the system was substantially enhanced by NaCl, significantly lowering the enrichment.

Keywords antifoam agent; foam fractionation; protein

INTRODUCTION

Foam fractionation is an adsorptive bubble separation technique, which bases on the fact that solutes with a high surface activity will preferentially adsorb to a bubble surface in rising foam; by collecting the foam, a solution enriched in the surface-active solute can be obtained (1). Foam fractionation to be a simple and effective method for surface-active substances separation, is a technology worthy of development. Investigation on the foam process and its application in recovery and enrichment of biological molecules from the production broths has been attempted for many decades. Previous work in this field has shown its potentiality for concentrating protein of interest from an ideal single dilute solution or mixture, including undesirable proteins, DNA, lipid, polysaccharide, et al. (2–4). It is currently unknown, however, whether it is feasible to employ the technology of the foam process to recover protein from a system with the presence of an antifoam agent. Further investigation on this system thus is essential to apply this technology in a large-scale fermentation production process.

Adding an antifoam agent is one of most available way to control foam in the fermentation process (5–6). In general, antifoam agents are surface active substances which can be broadly divided into two types, depending on whether they are based on insoluble oils or soluble oils (7–8):

The first type of antifoam agent is based on insoluble oils such as polydimethyl siloxane or oil. They act as a hydrophobic drop which first enters one of the foam film surfaces and form a lens. Then the lens has the option of spreading along the surface or forming a bridge. As the lens spreads rapidly on the surface of the film, the underlying liquid is dragged away from the surface, causing localized film to thinning and rupture. When a hydrophobic lens contacts with the opposite film surface, a bridge is formed. The capillary force from the bridge would dewet the film and then cause the film to rupture.

The second type is often based on nonionic surfactant containing polyethyleneoxyde or polypropylene oxide moieties, e.g., polyoxypropylene polyoxyethylene glylerin ether (PGE), having lower Gibbs free energy of adsorption than protein. Therefore, they can displace the adsorbed protein to form a low viscosity film, which is much weaker than the proteins gel-like film because of lower intermolecular interactions.

All of these effects will lead to film thinning and collapse, and eventually prevent metastable foam formation. Several studies dealing with the effect of antifoam agent on foam decay have been reported that the mixed-type antifoams exhibit usually excellent defoaming performance; thus, they are more widely applied than a single type in many industries (9–10). Here a mixture of silicon oil and PGE was chosen as an investigated antifoam agent for the simulated system.

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Address correspondence to Zhaoliang Wu, Department of Bioengineering, Hebei University of Technology, Tianjin City 300130, China. E-mail: zhaoliang.wu@163.com

Previous studies revealed that stable foam could be obtained even in the presence of an antifoam agent (usually are oil), assuming that a sufficient foaming booster (surfactant) was introduced into a previously non-foaming system; the main roles of the additive as a foam booster was to increase energy barriers to antifoam agent entry, which leads to suppressed activity of the oil as an antifoam (11).

On the other hand, because small molecule surfactants are very rapid at diffusing and adsorbing as compared with macromolecule proteins, the surfactant will tend to replace the adsorbed protein from adsorption layers; even hydrophilize protein via hydrophobic interaction when the molar ratio of surfactants/proteins exceed at certain critical value, which will accelerate desorption of proteins from the surface (12). As is known to all, the key to the foam process is the adsorption of the target. Just for this reason, it is our concern to deal with in this paper, whether it is practicable to accumulate proteins from an aerated system containing an antifoam agent by the foam process, when enough surfactants are required to be introduced into this system to eliminate the influence of the antifoam agent, so that the foam process can be run regularly.

Additionally, it has been proved that the surfactant type has a strong correlation with protein adsorption properties in the mixture (13). Therefore, three typical classes of surfactants (anionic SDBS, cationic CTAB, and non-ionic Tween-20) would be taken, for example, to elucidate the mechanisms of different surfactants stabilizing foam for a simulated system consisting of the antifoam agent and protein, at varying pH. In order to better understand the performance of protein bubble adsorption in this system, the influence of protein and antifoam agent concentration, and ionic strength were also taken into account. These results are practically important to the applications of foam fractionation in biological product process, especially for the protein and antifoam agent, in respect that the yield of protein and dosage of the antifoam agent are actually allowed to fluctuate, depending on the fermentation process.

MATERIALS

BSA (NW 69000) was purchased from Tianjin Ustar biotechnology Co Ltd., bicinchoninic acid (BCA) assay kit from Galen Biopharm International Co Ltd (Beijing)., CH_3COOH, CH_3COONa, NaCl, and CTAB (NW 364.45), SDBS (NW 348.47), Tween-20 (NW 1226.48) all analytical grade, were purchased from Tianjin Yingdaxigui Chemical Agents Co Ltd., AF520 crude product (containing 20% silicon oil and 80% PGE) was a gift from Tianjin Kangyi Bioengineering Co Ltd (China), used without further purification.

A custom-built foaming fractionation column (see in Fig. 1) was utilized for experiments. The plexiglass column

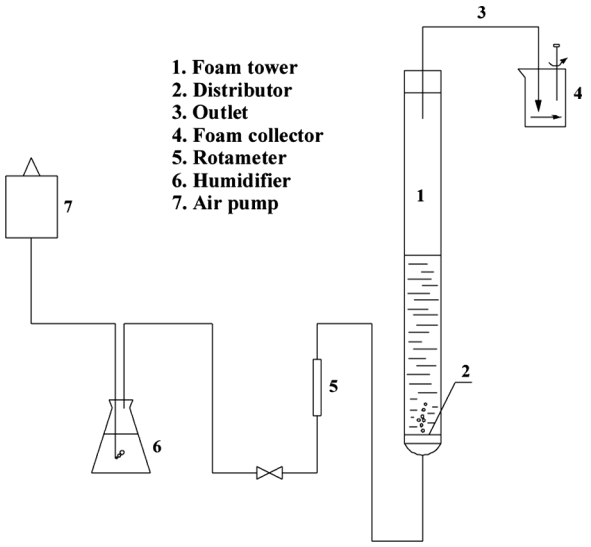

FIG. 1. Schematic diagram of the foam fractionation system.

was 90 cm high with an inner diameter of 3 cm. Sintered glass filter, which had pores of 60–100 μm in mean diameter, was installed as a gas distributor at the bottom of the column. A U-shaped glass tube (3.5 cm in height and 0.8 cm in inside diameter) was inserted through the rubber cork equipped at the top of the column for diverting the foam stream to the collector. Compressed air was produced by an air pump (Hailea, China). A rotameter (Wuhuan, China) was used to control the air flow rate, linking the pump with the column.

752 N spectrophotometer was from Shanghai Precision & Scientific Instrument Co Ltd (China)., and the Orion SA-520 pH meter was from Thermo Corp (US).

FOAM TEST METHODS

The investigations of foamability and foam stability as a function of PGE were conducted by a method of "pneumatic foam column" at ambient temperature. A measure of pH 4.6 acetic acid buffer (0.2 mol/L) was used as a solvent in the foaming experiment herein BSA concentration was 100 mg/L, and PGE was added before aeration. The rubber cork with an U-tube was removed prior to trial and the height of the freeboard in the column was 56 cm, enough to ensure that the rising foam did not overflow from the column in this given experimental range. Air was dispersed into the foam column containing 250 mL test solution at a constant speed of 100 mL/min for 2.5 min. Then, the height of the foam (H_f) above the liquid phase was momentarily measured as foam capability by a scale along the outside wall of the column. The foam was left to collapse. When the foam height had dropped to one half of the initial foam height for each test, the time ($t_{1/2}$) was registered as foam stability.

FOAM FRACTIONATION PROCEDURE

Batch foam process experiments were carried out in the foam column. A test solution was loaded in the column. The solution pH was adjusted by adding HCl/NaOH dilute aqueous. The initial solution volume of the foam column and the gas flow rate were kept constant (see in above). Air continued to flow into the column until no more foam was generated. Water loss in the effluent air stream was minimized by humidifying the air before it entered the column. The produced foam through the top outlet was allowed to collapse in the foam collector. All experiments including foaming test were replicated $3 \sim 5$ times for statistical purpose. Protein measurements of foam and the residual liquid left in the column were obtained using BCA Assay (14).

CALCULATION

E is the ratio of the concentration protein in foamate, compared to the concentration in the initial solution, and R is the percentage of proteins by mass recovered in the foam from the initial. In this study, the volume of foamate and concentration of residual protein left in the column was also taken as a performance indicator.

RESULTS AND DISCUSSION

Foam Test

As mentioned above, after oil drop entry in the protein film, the subsequent oil spreading or bridging would give rise to elimination of the stabilizing surface tension gradient and the surface elasticity. It is also known that a soluble antifoam agent PGE can destabilize foam by the displacement mechanism. However, the displacement of the adsorbed protein from the interface clearly does not occur simply by exchange of individual protein molecules by the more surface active molecules. The process of displacement can be described by the proposed "orogenic mechanism" in detail (15): Due to heterogeneity in the protein film, the PGE appeared to adsorb into localized defects in the protein network. These areas extend as PGE adsorbed into them, compressing the protein network until it fails. Then the interface was dominated by a sea of PGE containing islands of protein. Thus a loose packing interface would facilitate oil drop entry, causing a rapid rupture of the foam film. As a result of this synergistic effect, the mixed oil-solution formulations typically have much higher efficiency in defoaming than the individual components (oil or PGE).

Figure 2 shows foam decay with increasing AFA concentration. Foam was subjected to destabilization processes like drainage, coalescence, and rupture under the impact of AFA. As can be seen, the addition of AFA required to inhibit foam formation was very low. Even metastable foam above the interfacial area was not observed visually when the AFA content of the system reached 4 mg/L. It also has been found that such state of "zero foam" was entirely independent upon aeration time at this given gas flow rate. Thus the following experiments would be conducted to evaluate the effects of solution constituents on separation performance at fixed AFA concentration of 4 mg/L, which would certainly vary when the antifoam additive was taken as an investigated factor in the test.

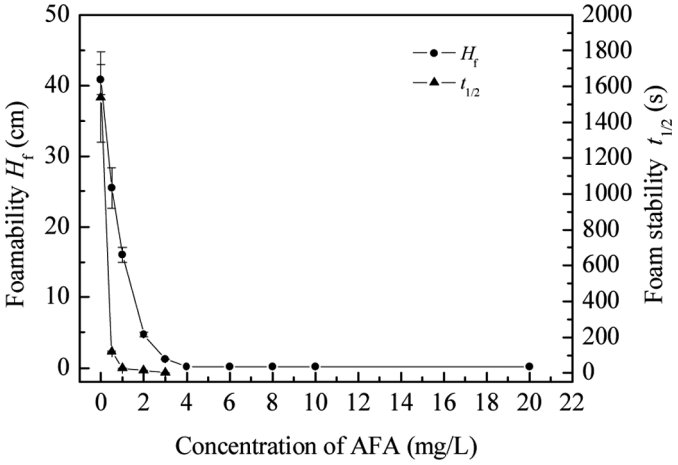

FIG. 2. Foamability and foam stability as function of AFA concentration.

Effect of pH on Foam Separation

The curves of BSA recovery for different foam boosters (20 mg/L CTAB; 20 mg/L SDBS; 200 mg/L Tween-20) as function of pH at a constant BSA level of 100 mg/L, were plotted in Fig. 3, respectively. It is well known that proteins are amphoteric electrolyte and their surface charge is varied with the pH environment. When solution pH deviated from the isoelectric point of BSA, resulting in a sharp

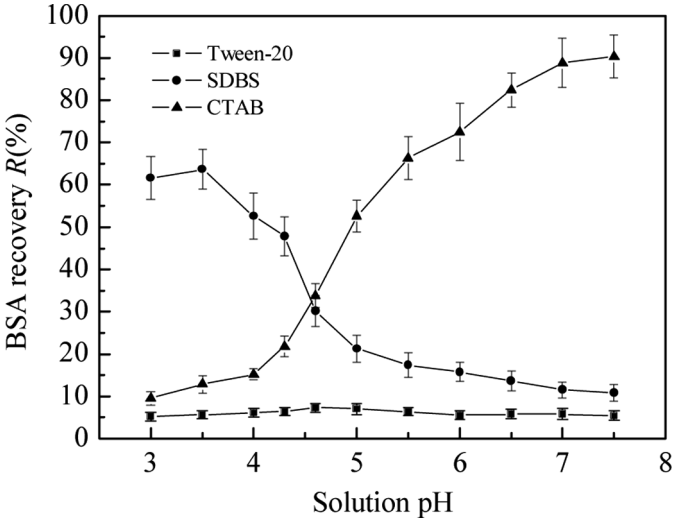

FIG. 3. BSA recovery as function of solution pH.

increase of the net charges on BSA surface, it is expected that the corresponding ionic surfactants stably interact with now oppositely charged BSA and form a more hydrophobic BSA-surfactant complex to compete against PGE for the interface (13,16). And this hydrophobized BSA was prone to aggregate to form a micelle, which consequently dissolved the silicon oil. It has been claimed that oil solubilized in micelle can cause a weaker defoaming action, because they cannot reenter the surface (10). On the other hand, this binding with high affinity also resulted in an enhancement of hydrophobic interaction and a reduction of electrostatic repulsion among neighboring BSA molecules in the interface, contributing directly to the formation of film. As compared to the former film, this strengthened film was found to be much harder for the adsorption of AFA, which would be favorable for forming a stable film during foaming. This date well accorded with the classic view that ionic surfactant binding with protein can strengthen the protein network effectively so that the failure of the network was found to occur at higher surface pressures than that required for non-ionic surfactants (17). According to this mechanism, for the simulated system using the ionic surfactant as foam booster, the BSA recovery was thus supposed to rise with improving the electrostatic binding capacity of the target with the foam booster by the means of varying solution pH to increase the net charge of the target. In contrast to the ionic surfactant, as a result of the absence of charge on the foam booster, Tween-20-assisted foam system appeared to have no significant change in protein recovery which was held at low level all the time with varying solution pH.

Besides the charge of the protein, the conformational isomerization with changes in pH should not be neglected, since the protein conformation also played an important role in its adsorption (2,18). It has been clearly demonstrated that the BSA conformation undergoes reversible N-F transition when the pH values are below 4.3; The F-form is characterized by unfolding of domain III and a significant loss in helix, resulting in a larger hydrodynamic size and hence slower diffusion (18–19), which would perhaps have an impact on its efficiency of competition with AFA BSA for interface. Despite offering more electrostatic binding sites for SDBS, the F-form protein was not capable of adsorbing as compactly as N-form (at pH 4.3–8) on the bubble surface. Maruyama and his coworker argued that maximal extent of protein adsorption on the interface was normally obtained at its most condensed state (20). Furthermore, this loose packaging film also created more defects for adsorbing of AFA, accelerating the foam collapse. Maybe this disparity to a large extent contributed to SDBS less efficiency in BSA separation than that employing CTAB, even though there are good reasons to believe that the former exhibits a higher tendency to interact with protein (13).

Effect of Surfactants Dosage on Foam Separation

The addition of ionic surfactant makes it possible to concentrate BSA from a mixture containing AFA using foam fractionation at an appropriate condition where electrostatic binding of the surfactant to BSA occurs. The following experiment would be thus conducted at its optimal solution pH (pH 7.5 for CTAB, pH 3.5 for SDBS and pH 4.6 for Tween-20), respectively. As discussed in the above section, an increase of binding to BSA with adding surfactant would result in more BSA adsorbed on the surface, but an unexpected low concentration of BSA in foamate in response to less drainage in film. As the available charges in the BSA molecule were saturated by the surfactant ions, with further increasing surfactant, the competition for the interface from the unbound surfactant became more and more significant, decreasing the mean density of BSA on the surface and further reducing the enrichment. However, it did not mean that the decline in density brought about by competition should be disadvantageous for recovery as well as for enrichment. In fact, the excess surfactant would produce more foam to entrain BSA, overcompensating for the loss of BSA adsorption density. More important, the complex remained high surface active, permitting the complex to adsorb on the surface despite posterior to surfactant. Similar results could be observed in literature of Gerken et al. even though their explanation differed from ours in detail (21).

This muted growth of recovery would proceed until the accumulation of the surfactant in bulk was sufficient to induce the hydrophobic binding of the surfactant with the complex. Under this extreme condition, the ionic surfactant turned to hydrophibilize the protein and step by step increased the hydrodynamic radius of protein, and eventually made much less affinity to the surface than the raw protein, preventing the protein from adsorbing on the bubble (12). The recovery went into decline in spite of increasing foamate, not to mention enrichment. It was conceivable that the loose packaging proteins should be much easier to involve cooperative binding with the surfactants (22). In this case, the hydrophilization of the complex would be found to occur at a lower threshold concentration for SDBS than that required for CTAB under its respective condition employed in the present study. In good agreement with this hypothesis, as obviously shown in Fig. 4, the system using SDBS as foam booster appeared to reduce the BSA recovery prior to that using CTAB in terms of concentration of the foam booster. Moreover, such a loose folding structure also was thought to be the main reason for the lower efficiency in foaming than that expected for dense structure because there was no doubt that this loose molecule would expose more hydrophobic binding sites for the surfactant, leading to a higher depletion of surfactants which were incorporated into a less foaming complex

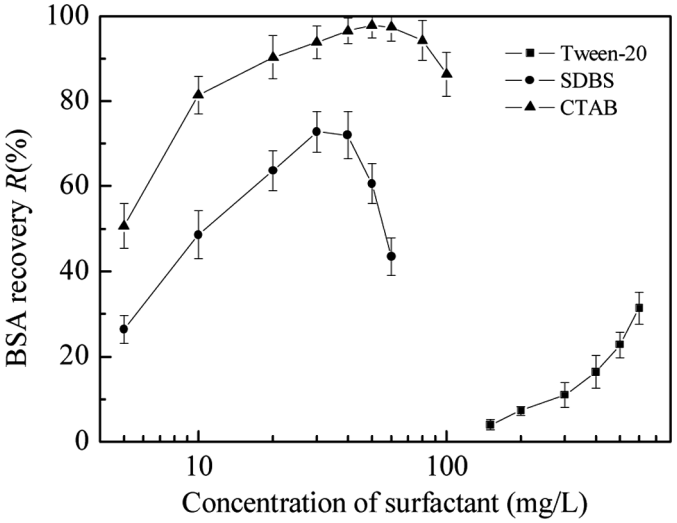

FIG. 4. BSA recovery as function of foam booster concentration.

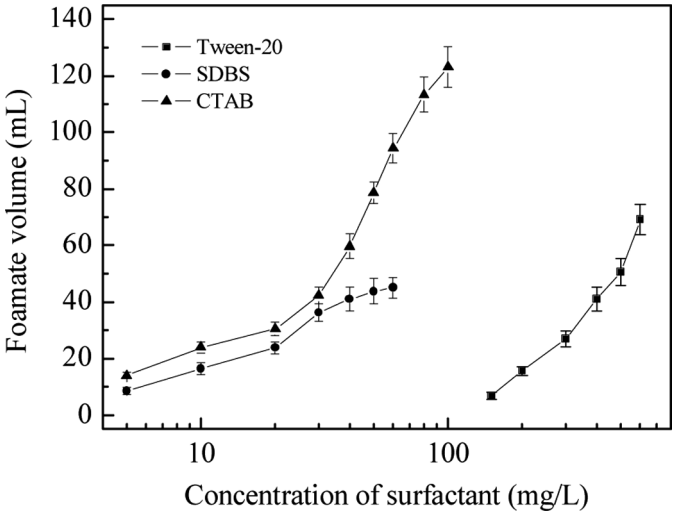

FIG. 6. Foamate as function of foam booster concentration.

(more hydrophilic) instead of adsorbing on air/liquid interface to boost foam. As presented in Fig. 6, the foamate generation rate in the tested system with SDBS was visibly lower than that with CTAB.

As discussed above, due to the absence of binding of Tween-20 with protein, a small addition of Tween-20 could not be capable to make the film strong enough to resist destabilization arising from AFA, even it got involved in thermodynamically unfavorable conditions for the formation of protein film, principally attributed to incompatibility of foam stabilizing mechanisms between the protein and the surfactant (23): Profiting from strong interaction among neighboring molecules, proteins form viscoelastic layers, in which the molecules are essentially immobile. In contrast, surfactants rely on a high degree of surface mobility to counter deformation, generally referred to as the Gibbs-Marangoni mechanism. It means that the mixed film would become more unstable, because the existence of surfactants in the interface could weaken the neighboring proteins cross-linking and on the other hand the macromolecule protein in turn could also result in huge steric hindrance for the mobility of surfactant. To generate foam, the mixed system should require sufficient addition of Tween-20 to displace BSA and PGE from the surface to form a relatively stable film dominated by individual species, contradicting the purpose of protein enriching. In such a case, the amount of target adsorbing on the bubble surface would be a little fraction of the total entrained in foam.

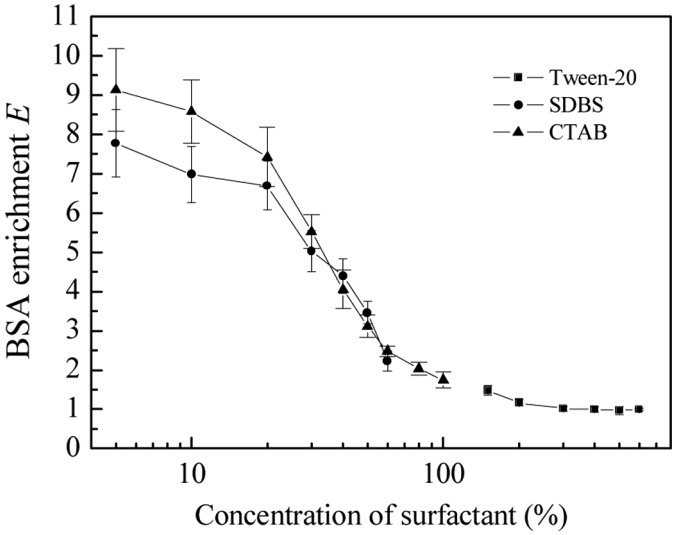

FIG. 5. BSA enrichment as function of foam booster concentration.

In good agreement with the analysis just presented, as shown in Fig. 5, the concentration of the target in the fomate was found to approach the value obtained in the initial solution, while the recovery appeared to rise in leaner relation with the foamate. However, this proposed mechanism based on in compatibility could not satisfactorily explain why Tween-20 is still less efficient in foaming when the amount of Tween-20 in bulk exceeded its folds of critical micelle concentration 208 mg/L (24), at which the adsorption layer is generally thought to be formed predominantly by surfactant molecules. This situation may be due to the fact that the polar group of Tween-20 molecule was composed of multi-substituted polyethylene chains, which is characterized by a loose packing structure at the interface (25): In other words, Tween-20 is not a good foam agent in spite of possessing high surface active. When it comes to the binding of a nonionic surfactant with protein many experimental studies have demonstrated that the hydrophobic force driving for this type of binding is so weak that its effect on the protein surface activity is neglected in normal cases (26–27). Even with a high addition of the

surfactant, it would be a more thermodynamic favorable process for a nonionic surfactant to self-aggregate to form a micelle than to bind with protein in the bulk solution (28). In contrast to the nonionic surfactant, the micelle formation of the ionic surfactant is generally found to occur at higher free concentration (mole concentration), far enough to induce this low affinity binding to protein because of the repulsion of the charged surfactant in bulk solution (28).

Apparently, adding nonionic surfactant Tween-20 could not bring about a valid separation performance for BSA, similar to foam fractionation of other proteins (29–30). Among three classes of typical surfactants tested in this article, cationic surfactant CTAB turned out to be a most appropriate foam booster for separating BSA. Hereby, the following experiments would all be carried out in the presence of 20 mg/L CTAB.

Effect of BSA and AFA Initial Concentration on Foam Separation

As BSA was added to the solution, more and more silicon oil was entrapped in the micelle formed by BSA (complex), then the oil entry action became less and less probable; and PGE originally adsorbed on the surface was also gradually released into the bulk under the pressure of BSA competition, leading to a suppression of the destabilizing effect of the antifoam agent on foam. In this way, no remarkable liquid drainage and bubbles coalescence were visually observed along with the foam height as clearly shown in Fig. 7, and a more "wet" and spherical foam was made to entrain more targets but also substantially diluted the targets with water. Under this extreme condition, this highly liquefied foam necessitates taking conventional measures of drainage promotion to reach a higher enrichment, such as lowering the gas flow rate or lengthening the foam column etc. In practice this retarding effect of protein on film drainage would be easily reversed by the antifoam agent. In the following tests, the value of BSA concentration was still identical to the previous (100 mg/L).

As illustrated above, adding AFA would give rise to an increase in liquid drainage and bubble coalescence, hence in internal reflux, which also facilitates the enrichment process of target by theory (1). As presented in Fig. 8, the enrichment of BSA was significantly enhanced with the increasing addition of AFA as expected, whereas the recovery only appeared to be in a slight decline, when AFA concentration was lower than 5 mg/L. With further increase the AFA, PGE would occupy more interfaces in place of BSA molecules. At the same time the silicon oil would exceed the solubility limit in micelle and attempt to enter the film. With the synergistic effects of PGE and oil, this heterogeneous film showed more sensitivity to perturbation, and then the rupture of film was visually found to occur more frequently than that with a lower addition of AFA, leaving more liquid in the column. As a result of a rapid foam collapse, the recovery decreased dramatically as the addition of AFA arose from 5 mg/L to 10 mg/L, while the enrichment still kept its upward trend. Therefore, whether the increased antifoam agent is beneficial or not depends on the goals of the separation.

Effect of NaCl on Foam Separation

At a low NaCl concentration, the counterion, Cl^- would shield the electric repulsion of positively charged beads of the unbound surfactant (at pH 7.5 solution condition, a mole of BSA can capture $16 \sim 18$ mole of CTAB via electrostatic interaction (18), in this case the binding CTAB at most take up one half of the total CTAB employed in this system), assuring the free surfactant of a higher adsorption rate and a more close packaging at interface even a favorable condition for dissolving silicon oil by forming a micelle, accompanied by an increase of surface

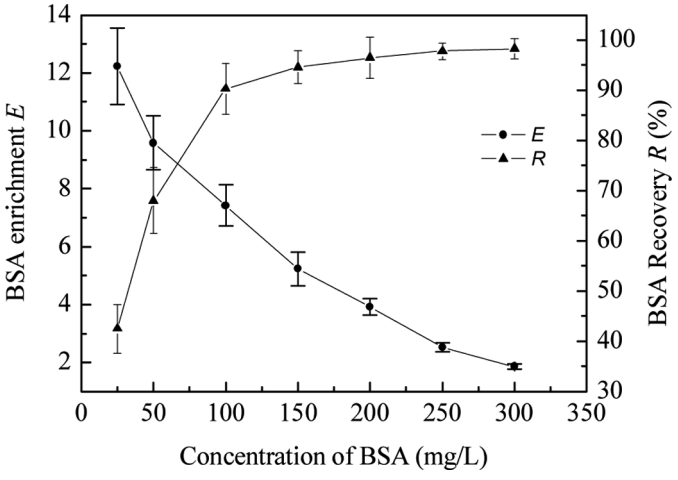

FIG. 7. BSA enrichment and recovery as function of initial BSA concentration.

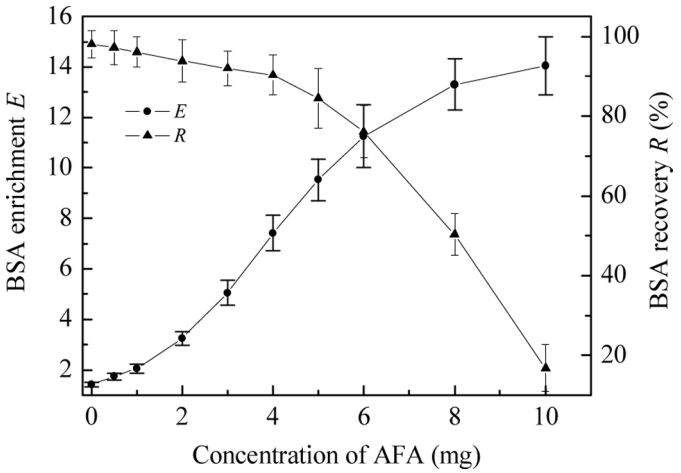

FIG. 8. BSA enrichment and recovery as function of AFA concentration.

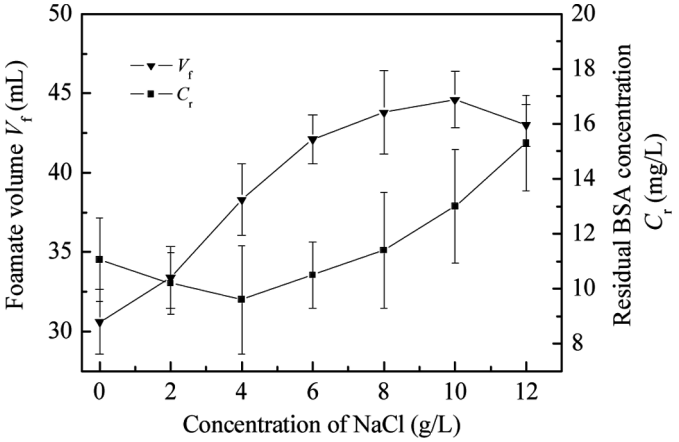

FIG. 9. Foamate volume and residual BSA concentration as function of antifoam agent concentration.

viscosity. Then this rigid foam appeared much wetter in response to the decreased rate of film drainage and the increased thickness of the lamellae. As clearly shown in Fig. 9, the foamate was found to sharply rise with increasing addition of NaCl at the initial phase.

Above a certain critical concentration, however, elevated ionic strength may also have an adverse effect on foam stability by shielding repulsion of opposing interfaces that resist the thinning of film of the adjacent foam when the film was stabilized by a charged element (31), which was presumably responsible for the subsequent fall of the foamate. A significant difference with a simple ionic surfactant, the net charges (acidic residues) in the protein surface are relatively low for such a macromolecule, and moreover most of them would be screened by electric binding of CTAB under this solution condition. Hence the adsorption capacity of the bound protein on the bubble would be impossible to be improved as much as expected for the surfactant by the electrolyte, reasonable for a slight decline in residual BSA at low concentration of NaCl. On the other hand, elevated ions would usually tend to weaken the electric interaction of the surfactant with BSA (32), which is a foundation for BSA separation in this system, leading to a low degree of BSA hydrophobilization, hence an increase in concentration of residual BSA.

CONCLUSIONS

The foam stabilizing mechanisms of three typical classes of surfactant (anionic SDBS, cationic CTAB and non-ionic Tween-20) for a simulated system with the presence of an antifoam agent were investigated. The ionic surfactants served as amphiphilic ligands for hydrophobilizing the protein by assembly on the protein electrostatic binding sites, assuring the bound protein of a rapid adsorption rate and a stronger crosslink intermolecules at the interface to resist the antifoam agent. In contrast to the ionic surfactant, the nonionic surfactant that lacks an electric binding site appeared to be a major building block of the film during foaming, required to act as competitor to fully replace the antifoam agent and protein from the interface, which would lessen the destabilization from the antifoam agent, and to a certain extent avoid the problem of hydrodynamic incompatibility of mixing the interface composed of protein and the surfactant, respectively. A similar transition from the BSA and AFA coadsorbed layer to surfactant dominan layer also has been observed at a larger addition of ionic surfactant; however, significantly different from the nonionic surfactant, this displaced process also involved a hydrophilization of protein as surfactant was the up to a certain concentration, freeing the protein which then desorbs from the interface. Probably due to synergism of the surfactant possessing high surface active and protein characterized by viscoelastic properties at the interfaces, the complex showed more tolerance to the antifoam agent than the simple surfactant or free protein, facilitating BSA fractionation from the mixture.

The obtained experimental results clearly revealed that separation of proteins is strongly dependent on solution pH, which had a direct impact on its net charge and conformation. It is proposed that the expansion of BSA structure with decreasing pH perhaps led to the reduction in its adsorption rate and packing density on the interface, accounting for SDBS being less efficient in BSA separation, which also may be valid to explain a similar result found in the mixing system of SDS/laccase (30). Looking at the above studies, we may give a sensible proposal for the choice of foam booster for enzyme separation: an alternative ionic surfactant has to specifically bind with the target only in a limiting pH range where now this oppositely charged enzyme can maintain its maximum activity. An increase of recovery but at a cost of enrichment corresponding to rise in initial BSA concentration, could be explained by the strengthening effect of protein on foam, similar to those employing a single protein. In contrast to BSA, adding an antifoam agent obtained an opposite result. It has been demonstrated that the existence of an electrolyte has a reciprocal effect on foam formation and target adsorption, which make the prediction of separation performance more difficult when using this foam process.

Although, the present approach seems difficult to give even an approximate estimate for the dynamics of protein adsorption on the interface occurring in foam fractionation for such a complex mixture consisting of protein and antifoam agent and foam agent, this tentative investigation expands the potential for the application of foam fractionation in biological product process and also paves the way for a low-cost operation of biomacromolecules purification. Further studies examining the intrinsic interaction of the target with a different component in interface and target stability in separation process are under way.

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REFERENCES

1. Lemlich, R. (1972) Principles of foam fractionation and drainage. In: *Adsorptive Bubble Separation Techniques*; Lemlich, R., ed.; Academic Press: NY, 33–51.
2. Lockwood, C.E.; Jay, M.; Bummer, P.M. (2000) Foam fractionation of binary mixtures of lysozyme and albumin. *Journal of Pharmaceutical Sciences*, 89 (6): 693–704.
3. Saleh, Z.S.; Hossain M.M. (2001) A study of the separation of proteins from multicomponent mixtures by a semi-batch foaming process. *Chemical Engineering and Processing*, 40 (3): 371–378.
4. Brown, A. K.; Kaul, A.; Varley, J. (1999) Continuous foaming for protein recovery. I. recovery of beta-casein. *Biotechnology and Bioengineering*, 62 (3): 278–290.
5. Al-Masry, W.A. (1999) Effects of antifoam and scale-up on operation of bioreactors. *Chemical Engineering and Processing*, 38 (3): 197–201.
6. Joshia, K.S.; Jeelania, S.A.K.; Blickenstorferb, C.; Naegelib, I.; Windhaba, E.J. (2005) Influence of fatty alcohol antifoam suspensions on foam stability. *Colloids and Surfaces A: Physicochem. Eng. Aspects*, 263 (1–3): 239–249.
7. Etoc, A.; Delvigne, F.; Lecomte, J.P.; Thonart, P. (2006) Foam control in fermentation bioprocess: from simple aeration tests to bioreactor. *Applied Biochemistry and Biotechnology*, 130 (1–3), 392–404.
8. Vardar-Sukan, F. (1998) Foaming: consequences, prevention and destruction. *Biotechnology Advances*, 16 (5–6): 913–948.
9. Tamura, T.; Kageyama, M.; Kaneko, Y.; Kishino, T.; Nikaido, M. (1999) Direct observation of foam film rupture by several types of antifoams using a scanning laser microscope. *Journal of Colloid and Interface Science*, 213 (1): 179–186.
10. Racz, G.; Koczo, K.; Wasan, D. (1996) Mechanisms of Antifoam Deactivation. *Journal of Colloid and Interface Science*, 181 (1): 124–135.
11. Basheva, E.S.; Denkov, N.D.; Kasuga, K.; Satoh, N.; Tsujii, K.; Ganchev, D. (2000) Role of Betaine as Foam Booster in the Presence of Silicone Oil Drops. *Langumir*, 16 (3): 1000–1013.
12. Alahverdjieva, V.S.; Fainerman, V.B.; Aksenenko, E.V.; Leser, M.E.; Miller, R. (2008) Adsorption of hen egg-white lysozyme at the air-water interface in presence of sodium dodecyl sulphate. *Colloids and Surfaces A: Physicochem. Eng. Aspects*, 317 (1–3): 610–617.
13. Otzen, D.E.; Sehgal, P.; Westh, P. (2009) α-Lactalbumin is unfolded by all classes of surfactants but by different mechanisms. *Journal of Colloid and Interface Science*, 329 (2): 273–283.
14. Smith, P.K.; Krohn, R I.; Hermanson, G.; Mallia A.K.; Gartner, F.H.; Provenzano, M.D.; Fujimoto, E.K.; Goeke, N.M.; Olson, B.J.; Klenk, D.C. (1985) Measurement of protein using bicinchoninic acid. *Analytical Biochemistry*, 150 (1): 76–85.
15. Mackie, A.R.; Gunning, A.P.; Wilde, P.J.; Morris, V.J. (1999) Orogenic displacement of protein from the air/water interface by competitive adsorption. *Journal of Colloid and Interface Science*, 210 (1): 157–166.
16. Burapatana, V.; Booth, E.A.; Prokop, A.; Tanner R.D. (2005) Effect of Buffer and pH on Detergent-Assisted Foam Fractionation of Cellulase. *Industrial and Engineering Chemistry Research*, 44 (14): 4968–4972.
17. Gunning, P.A.; Mackie, A.R.; Gunning, A.P.; Woodward, N.C.; Wilde, P.J.; Morris, V.J. (2004) The effect of surfactant type on surfactant-protein interactions at the air-water interface. *Biomacromolecules*, 5 (3): 984–991.
18. Bohme, U.; Scheler, U. (2007) Effective charge of bovine serum albumin determined by electrophoresis NMR. *Chemical Physics Letters*. 435 (4–6): 342–345.
19. Khan, M.Y. (1986) Direct evidence for the involvement of domain III in the N-F transition of bovine serum albumin. *Biochemical Journal*, 236: 307–310.
20. Maruyama, H.; Seki, H.; Suzuki, A.; Inoue, N. (2006) Variation of saturated surface density of ovalbumin on bubble surface in continuous foam separation. *Journal of Colloid and Interface Science*, 299 (1): 416–420.
21. Gerken, B.M.; Nicolai, A.; Linke, D.; Zorn, H.; Berger R.G.; Parlar, H. (2006) Effective enrichment and recovery of laccase C using continuous foam fractionation. *Separation and Purification Technology*, 49 (3): 291–294.
22. Turro, N.J.; Lei, X.G.; Ananthapadmanabhan, K.P.; Aronson, M. (1995) Spectroscopic probe analysis of protein-surfactant interactions: the BSA/SDS system. *Langmuir*, 11 (7): 2525–2533.
23. Clark, D.C.; Wilde, P.J.; Wilson, D.R. (1991) Destabilization of α-lactalbumin foams by competitive adsorption of the surfactant Tween-20. *Colloids and Surfaces*, 59: 209–223.
24. Chen, J.S.; Dickinson, E. (1993) Time-dependent competitive adsorption of milk-proteins and surfactants in oil-in-water emulsions. *Journal of the Science of Food and Agriculture*, 62 (3): 283–289.
25. Calik, P.; Ileri N.; Erdinc, B.I.; Aydogan, N.; Argun, M. (2005) Novel antifoam for fermentation processes: fluorocarbon-hydrocarbon hybrid unsymmetrical bolaform surfactant. *Langmuir*, 21 (19): 8613–8619.
26. Dickinson, E. (1999) Adsorbed protein layers at fluid interfaces: interactions, structure and surface rheology. *Colloids and Surfaces B: Biointerfaces*, 15 (2): 161–176.
27. Kragel, J.; Wustneck, R.; Clark, D.; Wilde, P.; Miller, R. (1995) Dynamic surface tension and surface shear rheology studies of mixed β-lactoglobulin/Tween 20 systems. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 98 (1–2): 127–135.
28. Moore, P.N.; Puvvada, S.; Blankschtein, D. (2003) Role of the surfactant polar head structure in protein-surfactant complexation: zein protein solubilization by SDS and by SDS/$C_{12}E_n$ surfactant solutions. *Langmuir*, 19 (4): 1009–1016.
29. Burapatana, V.; Prokop, A.; Tanner, R.D. (2005) A comparison of the activity reduction occurring in two detergent-assisted protein (cellulase and lysozyme) foam fractionation processes. *Separation Science and Technology*, 40 (12): 2445–2461,
30. Linke, D.; Zorn, H.; Gerken, B.; Parlar, H.; Berger, R.G. (2007) Laccase isolation by foam fractionation-new prospects of an old process. *Enzyme and Microbial Technology*, 40 (2): 273–277.
31. Mita, T.; Nikai, K.; Hiraoka, T.; Matsuo, S.; Matsumoto, H. (1977) Physicochemical studies on wheat protein foams. *Journal of Colloid and Interface Science*, 59 (1): 172–178.
32. Hashim, M.A.; Chu, K.H.; Tan, P.S. (1995) Effects of ionic strength and pH on the adsorption equilibria of lysozyme on ion exchangers. *Journal of Chemical Technology and Biotechnology*, 62 (3): 253–260.