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ABSTRACT

The effect of pH on the bubble size distribution, void fraction, and enrichment ratio of a continuous foam fractionation column containing ovalbumin was investigated. The bubble size and void fraction were measured using a photoelectric capillary probe for different solution pHs (3.5, 4.5, 6.5, and 9.7). The bubble diameters for pH 3.5 and 4.5 were the largest of the four pHs studied. At these two pHs, the foam was less stable and formed aggregates, leading to lower enrichment and mass recovery. For the nearly neutral pH 6.5 or the more basic pH 9.7, the bubble size was smaller and the foam was more stable, resulting in both high enrichment and high mass recovery. The void fraction was smallest for pH 6.5, but the effect of pH on void fraction was not significant. In the lower foam phase, the calculated specific area increased as the pH increased from 3.5 to 9.7, which may partially contribute to the higher enrichments at pH 6.5 and 9.7.

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Key Words: pH; Bubble size; Void fraction; Foam fractionation; Ovalbumin.

INTRODUCTION

Foam fractionation is a type of bubble-adsorptive separation based on the surface activity of solutes. The pH of the feed/initial solution is an important variable that affects the performance of the foam fractionation of proteins. Other significant independent variables for the continuous operation are the feed concentration, superficial gas velocity, and feed flow rate.[1–9] Some researchers found that the effect of pH on enrichment (a measure of protein foam fractionation performance) is small[2,4,5] (overall change in enrichment is less than one) in the pH ranges studied, while others found that the effect of pH is significant.[1,3,6–8] The isoelectric point (pI) of a protein has been shown to be a critical point in influencing the enrichment ratio. For example, the maximum enrichment was obtained at a pI of 4.7 for BSA in the pH range of 3 to 10.[1] Some other protein foam fractionation experiments showed similar results, such as cellulase at a pI of 5.0,[3] gelatin at a pI of 4.9,[5] soybean protein at a pI of 4.5,[6] and egg albumin at a pI of 4.0.[10] However, an exception to the pI being the pH point where the maximum enrichment is reached was observed by Brown et al.[2] and Uraizee and Narsimhan.[4] Both groups found for BSA that at the pI, minimum enrichment was obtained. Generally, it is expected that at the pI of a protein, the surface adsorption is enhanced as a result of both the decreased electric repulsive forces and the minimum solubility of the protein in solution. It is noted that the surface tensions for both cellulase and egg albumin protein solutions at their respective pI are at a local minimum.[3,10] Generally, foam stability was observed to be maximized at the pI.[1–8] The conflicting enrichment results regarding the pI may result from the interplay between the pH and the other factors such as the bubble size, feed concentration, and foam column characteristics. For example, Brown et al.[2] and Uraizee and Narsimhan[4] found that the minimum enrichment at the pI of BSA (when the pH equaled 4.8) occurred because the bubble size was the smallest at that pH.

The effect of pH on foam fractionation often causes foam protein property changes at the gas–liquid interface. All protein molecules are comprised of the same type of linear polymers, built of various combinations of the same 20 amino acids.[11] Proteins differ only in the sequence in which the amino acids are assembled into polymeric chains. The primary bonding in proteins is the peptide bond between the carboxyl group and the amino group of the respective two amino acids. The most common types of crosslinking

(used to develop a 3-dimensional molecule with folding) are covalent disulfide bridges with bond strengths of 50 kcal/mol and weaker hydrogen bonds of about 6 kcal/mol. The ionizable amino and carboxyl groups of a protein molecule make its charges markedly affected by the solution pH. At lower pHs, the ionized NH_3^+ causes the protein to be positively charged, and at higher pHs, the dissolution of the carboxyl groups dominates and the protein has a net negative charge. At a certain pH, the net charge of the protein is zero due to the balance of the two types of ionization. This pH is called the *isoelectric point* (pI), and its value depends on the type of the protein. The two ionizable ion groups cause the protein molecules to be hydrophilic. The nonpolar part of the protein molecules (hydrocarbon groups in the amino acids), on the other hand, causes the molecules to be hydrophobic. Protein molecules tend to pack at the air–water interface (by adsorption), with the hydrophobic ends sticking outward to the air and the hydrophilic ends turning inward to the solution. Moreover, this packing is more concentrated than that originally in the bulk solution. The packing configuration in the monolayer and/or multilayer at the air–water interface is such as to reduce the internal energy of packing, while retaining the stability. This packing process (adsorption) may be slow due to the large size and the slow configurational changes of the protein molecules at the interface. The packing configuration and the density at the interface determine the surface properties such as the surface modulus or viscoelasticity and the surface deformation, and, thus, the foamability and stability of the foam.[12]

Hammershøj et al.[12] studied the influence of pH on the surface properties of aqueous egg albumen solutions regarding the bubble size distribution and drainage within the formed foam. It was found that egg albumen could slow surface expansion and lower the dynamic surface tension. At a pH of 4.8, the surface was more rigid than at higher pHs. The surface modulus increased over time at pH 4.8, but at higher pHs it was constant. Foam stability against drainage was best at pH 7.0 just after 30 min, but on a longer time scale, foam at pH 4.8 was most resistant to drainage. The smallest bubble was found when the pH was 4.8 and the largest one when the pH was 7.0. Hammershøj et al. concluded their study with a qualitative relationship between the foaming behavior of egg albumen and the dynamic surface properties at pH 4.8: The more rigid behavior of the surface at this pH favors a smaller bubble size (more stable liquid film, thus less coalescence) and a slower drainage of liquid from the foam.

The effect of pH on the continuous foam fractionation of ovalbumin is reported here. A photoelectric capillary probe is used to measure the bubble size distribution in the continuous foam fractionation column at several different pHs. The experimentally measured results of the bubble size and void fraction at different pHs are presented and correlated with the performance of the foam fractionation system, as determined by the enrichment ratio. A little

understanding of the foam fractionation process may follow from the development of this correlation.

METHODS AND MATERIALS

The continuous foam fractionation and bubble size measurement experimental setup and procedure have been previously described.[15] Ovalbumin (Grade II, cat#A5253) was purchased from the Sigma Chemical Company (USA). An aqueous solution of ovalbumin was used in the foam fractionation experiments. The solution was prepared by dissolving a given amount of ovalbumin solid into distilled water to give the desired concentration. The pH of the solution was adjusted by adding 1.0 N NaOH or HCl as needed. A pH meter (Cole Parmer, Chemcaded pH meter) was used to measure the solution pH.

During the experiments described here, the fresh feed solution was fed to the column at two different flow rates (24 and 45 ml/min). The liquid pool height was kept constant at 28 cm with the bulk liquid output near the bottom of the column.

The ovalbumin concentration was determined using the Bradford Coomassie blue dye technique, as previously described.[13] The Bradford Coomassie method, a total protein concentration method, applies here since the ovalbumin is the only protein present. The methodology for obtaining the bubble size distribution and the void fraction measurements at different positions (Fig. 1) of the foam fractionation column (the so-called "point" values) using a capillary probe are described in the literature.[14]

While the bubble size distribution and the void fraction are measured online, the effluent gas–liquid dispersion is collected simultaneously as

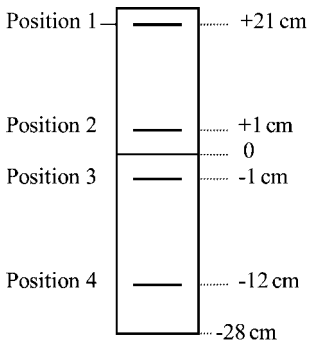

Figure 1. Measurement points along the column. The 0 mark represents the interface between the bulk liquid phase (at the bottom) and the foam phase (at the top).[15]

a liquid (foamate) in a tube, and its protein (ovalbumin) concentration is subsequently analyzed offline using the Bradford Commassie blue method.[13] In addition, the top foamate (collapsed foam) is collected continuously over time, and its concentration is analyzed at the end of the experiment.[15] Therefore, both the local and the overall enrichments are obtained.

EXPERIMENTAL VARIABLES

The main investigated independent variable in this study is the ovalbumin solution pH. Other variables include the superficial air velocity and the feed flow rate. Four different pHs were investigated. Here the feed solution pH is the same as the pH of the initial bulk solution in the column. We chose 3.5 as the lowest acidic pH value and 9.7 as the highest basic value, since at these bounds, the molecular properties of ovalbumin change and, thus, foaming properties were expected to change.[12] The isoelectric point (pI) of ovalbumin of 4.5[12] was selected as one of the pHs, since it has been shown in many studies that the pI is a critical point in a foam fractionation process.[1,5,6,10] The pH of the freshly prepared ovalbumin solution without pH adjustment was 6.5. This pH was denoted as the unadjusted pH.

The ovalbumin concentration studied was in the range of 30 to 100 mg/l. The operating superficial gas velocity varied between 0.05 and 0.2 cm/s and the feed flow rate between 24 and 60 ml/min.

DEFINITIONS

$$\text{Sauter mean diameter}: d_{32} = \frac{\sum_{i=1}^{N} d_i^3}{\sum_{i=1}^{N} d_i^2} \tag{1}$$

Specific area[15] (bubble surface area per unit volume of column):

$$a = \frac{6\varepsilon_g}{d_{32}} \tag{2}$$

$$\text{Local enrichment}: ER_l = \frac{C_{fl}}{C_0} \tag{3}$$

$$\text{Overall enrichment}: \quad ER_o = \frac{C_{fo}}{C_0} \tag{4}$$

The variables d_i, and thus d_{32} and ε_g, are obtained from the online bubble size measurement directly. The foamate concentration, C_{fl} (where subscript *fl* means foamate local) and C_{fo} (where subscript *fo* means foamate overall) can be obtained experimentally (as discussed in the Methods and Materials section). C_0 is the ovalbumin feed/initial concentration.

RESULTS AND DISCUSSION

pH Effect on Bubble Size Distribution

Figures 2–3 exhibit bubble size distribution variations with time during the startup of a continuous ovalbumin foam fractionation process for the lower bulk liquid pool under common process conditions listed in the figure captions. The bubble size distribution patterns at pH 4.5 in Fig. 2 (similar to pH 3.5, figure not given) are fitted better by a log normal distribution [$f(d) = \frac{1}{d\sqrt{2\pi}\sigma} e^{-\frac{(\ln d - \mu)^2}{2\sigma^2}}$, where *f(d)* is the number fraction of bubbles with

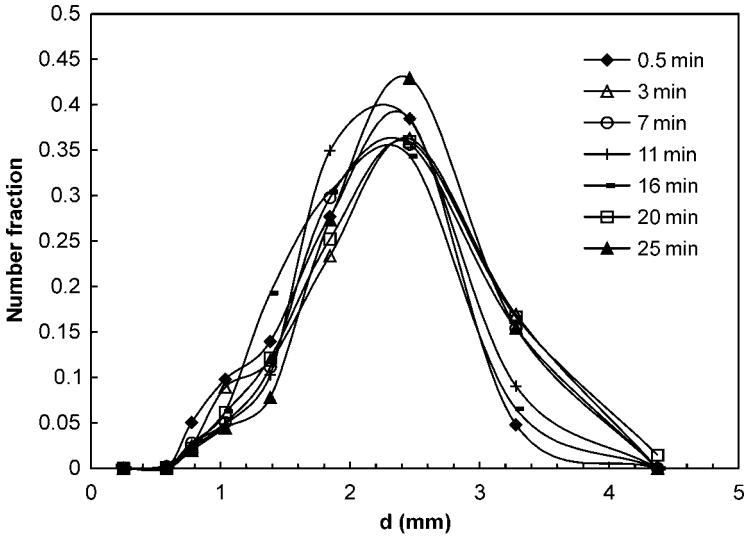

Figure 2. Bubble size distribution with time at −12 cm, pH 4.5, superficial gas velocity, 0.2 cm/s; feed flow rate, 24 ml/min; feed concentration, 40 mg/l. Startup of a continuous ovalbumin foam fractionation column.

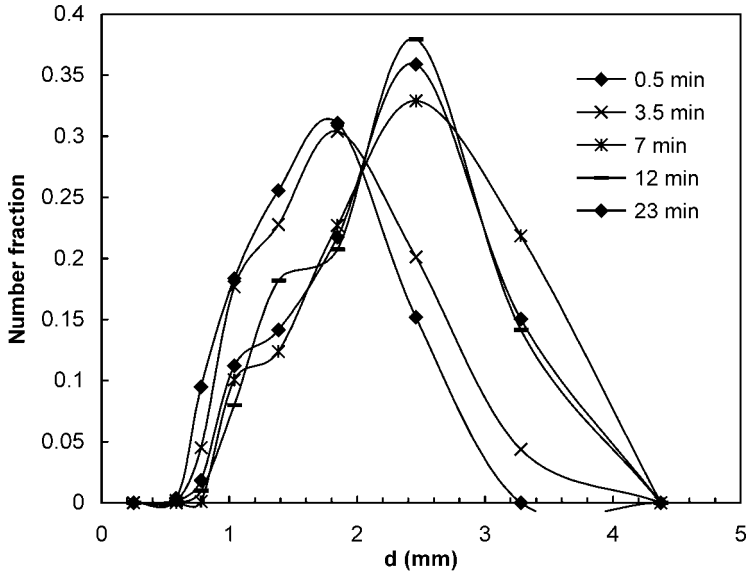

Figure 3. Bubble size distribution with time at −12 cm, pH 9.7, superficial gas velocity, 0.2 cm/s; feed flow rate, 24 ml/min; feed concentration, 40 mg/l. Startup of a continuous ovalbumin foam fractionation column.

diameter d] than a normal distribution [$f(d) = \frac{1}{\sqrt{2\pi}\sigma} e^{-\frac{(d-\mu)^2}{2\sigma^2}}$].[15] Generally, in a gas–liquid dispersion (for example, a reactor), the bubble size distribution is assumed to be a normal distribution. Our finding of the log normal distribution best fit may change this conventional assumption. For pH 9.7, the bubble size distribution is still closer to a log normal distribution than a normal distribution, with a small shoulder in the region spanning the 1.0 mm to 1.5 mm bubble diameter range.[15] For pH 3.5 and 4.5, such shoulders do not appear to be as significant. This seems to indicate that bubble coalescence is significant in the lower bulk liquid pool for pH 9.7. The first 3.5-min bubble size distributions differ significantly from those after this time for pH 9.7, as seen in Fig. 3, unlike the pH 3.5 and 4.5 cases, where the differences appear to be scatter in the data. At present, the theory underpinning the pH effect on bubble size in a foam fractionation process has not been developed.

The bubble size distributions in the lower foam phase (+1 cm) for different pHs are similar to Fig. 2. However, the bubble diameter range extended, as reflected in the following average bubble diameter.

pH Effect on d_{32}

It is shown in Fig. 4 that in the bulk liquid pool (−12 cm and −1 cm), d_{32} (time averaged) is not significantly influenced by pH (change is less than 0.3 mm or 20% for either position). But for the lower foam phase, d_{32} becomes up to 40% larger for the pH 3.5 and 4.5 cases than for the pH 6.5 and 9.7 cases. The exchange of thiol and disulphide bonds of ovalbumin at high pHs at the bubble surface may have caused enhanced stability of the bubble films and, hence, the d_{32} became smaller at pH 6.5 and 9.7.[12] It is noted that the charge of the protein molecule is determined by the solution pH. The change of charge with pH has an effect on protein–protein interactions, and, thus, conformational states and adsorption at an air–liquid interface. At the isoelectric point of a given protein, the net charge of a protein molecule is zero, and this point takes an additional significance here. The large negative charges of ovalbumin at pH 6.5 and 9.7 (above *p*I of 4.5) may enhance the repulsive forces between proteins in a bubble surface film and thus enhance the film stability. The mechanism underlying the pH effect on the foam film and the resulting bubble size is not clarified by the work in the literature. Since Figure 4 shows that the d_{32} variation with pH in the bulk liquid pool

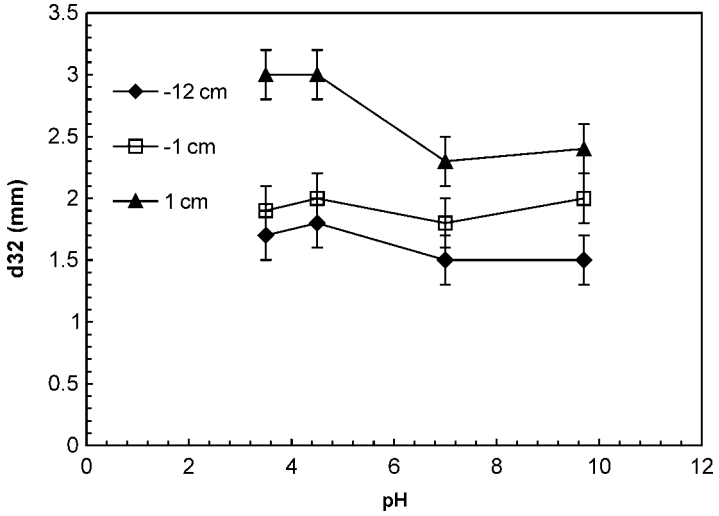

Figure 4. Effect of pH on d_{32} at different column positions under the following conditions: superficial gas velocity, 0.1 cm/s; feed flow rate, 24 ml/min; feed concentration, 40 mg/l.

(lower two curves) is not as significant as that in the lower foam phase, this may indicate that interfacial films in general are more likely to collapse and coalesce into larger bubbles in the foam phase than in the bulk liquid phase (since the foam consists of bubbles separated by very thin liquid films). Thus, in the bulk liquid pool, interactions between bubbles are much less than in the foam phase.

An interesting result occurred when we measured the surface tension of ovalbumin solution at different pHs. In Fig. 5, the surface tensions of ovalbumin solutions in the pH range of 2.4 to 10.4 (ovalbumin concentration of 100 mg/l) are displayed. At the *p*I of ovalbumin (ca. pH 4.5), the surface tension is at a local minimum value, and at pH 9.7, the surface tension is at a local maximum value. This indicates that at the *p*I, the ovalbumin has a stronger ability to lower the surface tension. It would be expected that because Marangoni flow (resulting from either a surface tension gradient or a surface concentration gradient on the bubble surface) is favored, a more uniform distribution of ovalbumin at the bubble surface leads to a more stable bubble film and, thus, a smaller bubble size at the *p*I. However, d_{32} measurements at different pHs do not support this expectation since a larger d_{32} was attained at pH 4.5 (*p*I) than at pH 9.7 (the local maximum surface tension) in the lower foam phase.

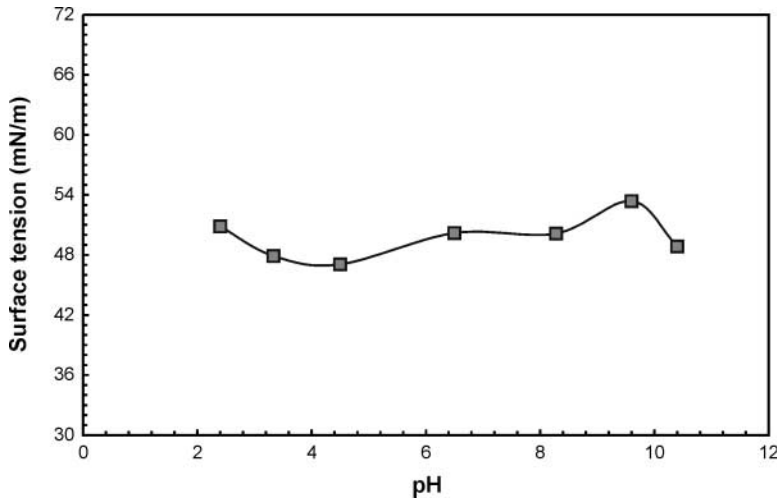

Figure 5. Surface tension variation with pH for the ovalbumin solution concentration of 100 mg/l concentration.

pH Effect on Void Fraction

Fig. 6 shows how the void fraction changes with pH. The lowest void fraction is at pH 6.5 for the two positions near the liquid pool and foam interface. The higher void fractions in the lower foam phase location for pH 3.5 and 4.5 result from large drainage flow rates, which lead to thin (less stable) bubble films. At pH 6.5, more stable bubble films result in smaller bubble sizes (less coalescence), as seen in Fig. 4, and slower drainage in the lower foam phase, which in turn contributes to a lower void fraction, as seen in Fig. 6.

pH Effect on Specific Area

Fig. 7 shows the specific area calculated using Eq. (2) with the measured ε_g and d_{32} at different pHs and column positions. In the lower foam phase, the specific area at pH 9.7 is the highest. It is interesting to note that, in the upper position (−1 cm) of the liquid pool, the specific surface area for different pHs tends to converge to the lower foam phase values as the pH approaches 10.

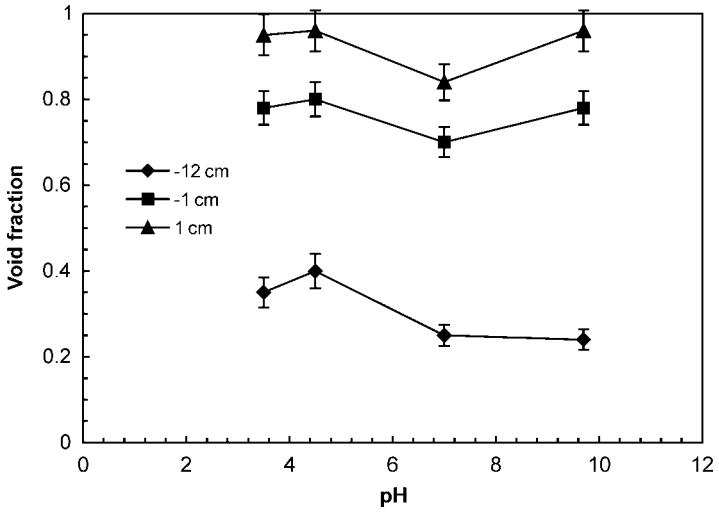

Figure 6. Effect of pH on the void fraction under the following conditions: superficial gas velocity, 0.1 cm/s; feed flow rate, 24 ml/min; ovalbumin feed concentration, 40 mg/l.

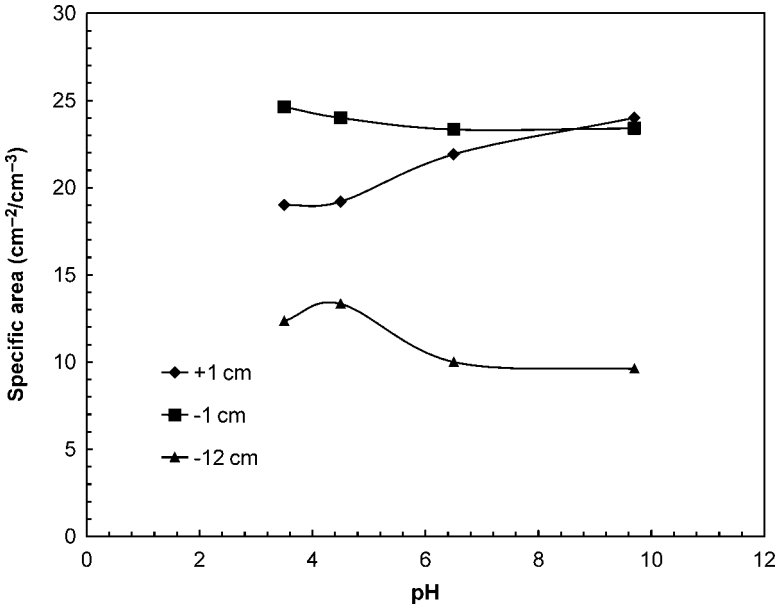

Figure 7. Effect of pH on the specific area under the following conditions: superficial gas velocity, 0.1 cm/s; feed flow rate, 24 ml/min; ovalbumin feed concentration, 40 mg/l.

pH and Local Enrichment

Fig. 8 displays the typical local enrichment results at the top of the foam phase (+21 cm) for different pHs. A strong pH effect on the foam fractionation performance was observed. The best pH for enriching the top foam position is 9.7. For pH 9.7, the local enrichment value reached 51 at the end of experiment (25 min from startup of the continuous foaming experiments), which was much higher than that for pH 3.5 (only 15.0). The next-best pH for enhancing the enrichment is pH 6.5, which was the original solution pH without any adjustment with HCl or NaOH. The top foam local enrichments at pH 4.5 and 3.5 were very low. It is observed in Figs. 9 and 11 that at pH 4.5 (pI) and pH 3.5, both the d_{32} and void fraction were larger than at other pHs for the lower foam phase. In general, a larger void fraction corresponds to drier foam and, thus, higher enrichment. It would, therefore, be expected that the top foam enrichment would be higher at pH 4.5 and 3.5. But it is shown in Fig. 7 that the specific area for pH 9.7 in the lower foam phase is the largest, which may contribute to the higher local enrichment. It is

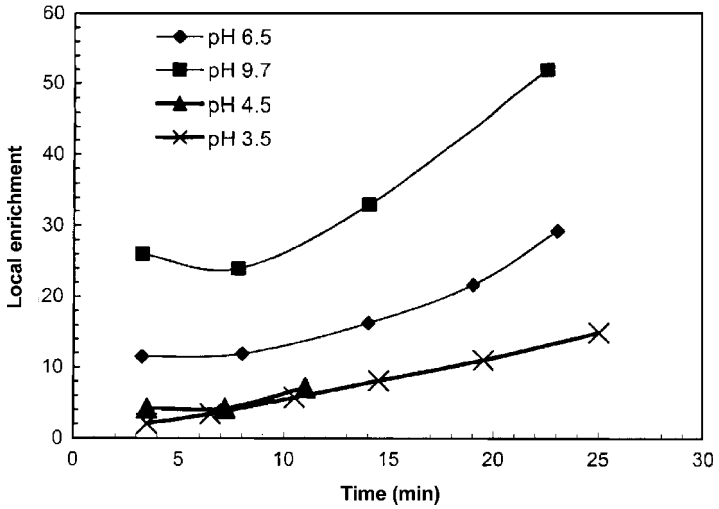

Figure 8. Local enrichment time profiles of the top foam (+21 cm) at different pHs for a superficial gas velocity of 0.1 cm/s, a feed flow rate of 45 ml/min, and a feed concentration of 36 mg/l. Transient (startup) in a continuous operation.

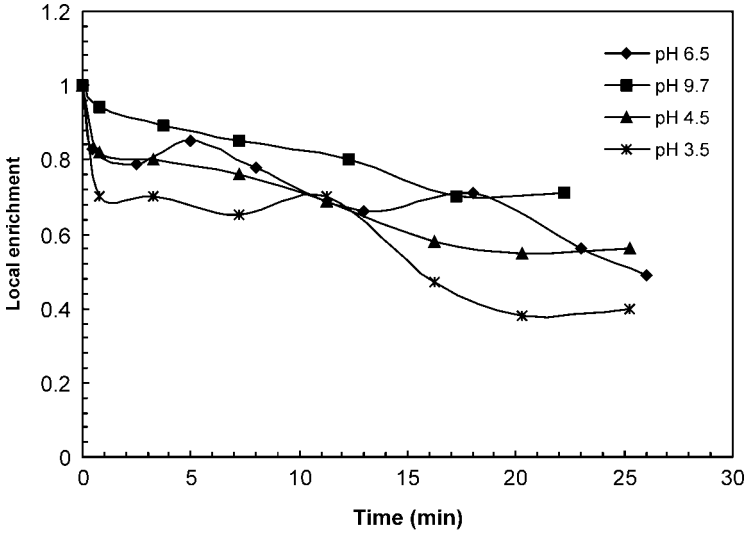

Figure 9. Effect of pH on the lower bulk liquid local enrichment for the following conditions: superficial gas velocity, 0.1 cm/s; feed flow rate, 24 ml/min; ovalbumin feed concentration, 36 mg/l. Dynamic startup of ovalbumin foam fractionation column.

interesting to note that, in our experiments, we observed that an aggregate or precipitate (white solid) formed and adhered to the wall of the column at both pH 4.5 and 3.5. This is consistent with the fact that when the pH equals the *p*I, the protein solubility is at a minimum because without the protein charge between the protein and water, the protein tends to separate from the water solution. At acidic pHs, ovalbumin may be denatured and coagulation might occur.[12] Therefore, the ovalbumin aggregation in the foam at these pHs (4.5 and 3.5) may also contribute to the lower enrichments. It may be possible, on the other hand, that pH 4.5 is suitable for the foam fractionation of ovalbumin when the ovalbumin is part of a mixture to be separated (when the other proteins are more hydrophilic).

Local enrichments in the lower bulk liquid pool at the measured four pH values are depicted in Fig. 9. These enrichments reflect the depletion of protein from the bulk liquid pool with time. The trajectories in Fig. 9 for pH 3.5 and pH 9.7 show that more protein is taken out of the bulk liquid pool at pH 3.5 than at pH 9.7. This is contrary to the fact that in the top foam phase, the local enrichments are least at pH 3.5. However, when the white protein aggregate (that appeared in the pH 3.5 and 4.5 foam phases) is taken into account, this apparent discrepancy is reconciled. Therefore, the presence of a foam phase strongly affects the performance of a foam fractionation process compared with only a bubble column with just a bulk liquid pool.

pH and Overall Enrichments

For the lowest feed concentration of 20 mg/l, the overall enrichment approached 70 for both pH 6.5 and pH 9.7 in our experiments. As the feed concentration increased to 100 mg/l, the overall enrichment decreased to about 10 for pH 6.5 and 15 for pH 9.7. For pH 4.5 and 3.5, the overall enrichments were always lower (less than 10) under all conditions. Therefore, both pH 9.7 and 6.5 are favorable for foam fractionation of ovalbumin at low feed concentrations (<50 mg/l), and, in particular, pH 9.7 is the best. This is in agreement with our local enrichment observations.

Figure 10 clearly shows the effect of pH on the overall enrichment for the feed concentration of 30–40 mg/l. It shows again that pH 6.5 and pH 9.7 are better for obtaining high overall enrichments than pH 4.5 and pH 3.5. On the other hand, Schnepf et al.,[1] Montero et al.,[3] Wang et al.,[5] Xie et al.,[6] and Loha[10] found that maximum enrichments were obtained at the pH that corresponded to the respective *p*I of BSA, cellulase, gelatin, soybean, and egg albumin. Our observation is in agreement with Brown et al.[9] and Uraizee et al.'s[4] observations, that the minimum enrichment was found at the *p*I of

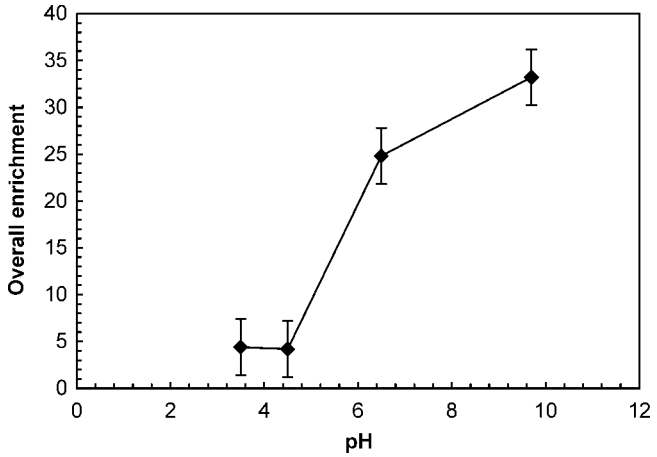

Figure 10. Effect of pH on overall enrichment for the feed flow rate, 24 ml/min; the superficial gas velocity, 0.1 cm/s; and the ovalbumin feed concentration, 30–40 mg/l.

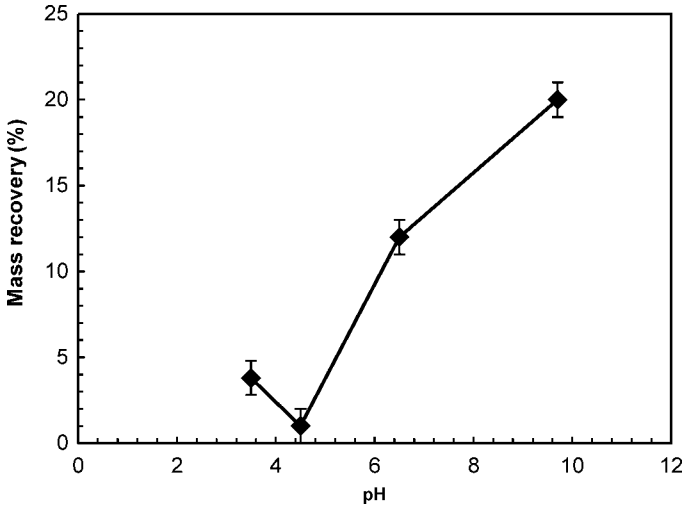

Figure 11. Effect of pH on the mass recovery of ovalbumin in the foamate for a feed flow rate of 24 ml/min, a superficial gas velocity of 0.1 cm/s, and a feed concentration of 30–40 mg/l.

β-casein[9] and BSA,[4] respectively, in a continuous foam fractionation process. It was postulated in these references that the minimum enrichment occurs at the *p*I as a result of small bubbles rather than aggregation. Further studies are needed to clarify these inconsistencies, particularly for egg albumin, which is largely made up of ovalbumin.

pH and Mass Recovery

Figure 11 shows the mass recovery of ovalbumin as a function of pH under the same operating conditions. The mass recoveries for pH 4.5 (*p*I) and pH 3.5 are very low because the low overall enrichments and small foamate volumes collected at the top of the foam for these pHs. The unmeasured formed protein aggregate, which adhered to the wall of the foam column, was not included in calculating the mass recovery of ovalbumin.

CONCLUSIONS

In summary, the effect of pH on foam fractionation is complex. When the pH equaled the *p*I of ovalbumin (4.5) or acidic pH 3.5, d_{32} was large, foam was less stable, and aggregate formed, leading to low enrichments and low mass recoveries. For the neutral pH 6.5 or the more basic pH 9.7, d_{32} was small and foam was more stable, resulting in both high enrichments and mass recoveries.

The pH had a significant effect on the bubble size distribution. For pH 9.7, a short "shoulder" appeared in the bubble size distribution curve, perhaps resulting from bubble coalescence. The measured d_{32} results for pH 4.5 and 3.5 were larger in both the bulk liquid pool and the lower foam phase than those for pH 9.7 and pH 6.5. Surface tension results showed that for pH 9.7 the surface tension was the highest, but the estimated saturated surface concentration using the Gibbs equation was the smallest at this pH.[15] It appears that the exchange of thiol and disulphide bonds strengthens the stability of the bubble films at higher pHs. In the lower foam phase, the specific area increased as the pH increased from 3.5 to 9.7, which may partially contribute to the higher local enrichments at pH 6.5 and 9.7. The local and overall enrichment results along with the mass recovery were maximized at pH 9.7. This indicates that this pH may be close to the best pH for operating the continuous foam fractionation of ovalbumin. The appearance of protein aggregation/precipitation at pH 4.5 and 3.5 led to the lower enrichments and mass recoveries at these lower pHs.

NOMENCLATURE

a	Specific area, cm^2 interfacial area/cm^3 column volume
C_0	Feed ovalbumin concentration, mg/l
C_{fl}	Local foamate (collected in vacuum jar) concentration, mg/l
C_{fo}	Top foamate (collected in top foamate collector) concentration, mg/l
d_i	Individual bubble diameter, mm
d_{32}	Sauter mean diameter, mm
ER_l	Local enrichment ratio
ER_o	Overall enrichment ratio
N	Total number of bubbles sampled
ε_g	Void fraction

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REFERENCES

1. Schnepf, R.W.; Gaden, G.E.L., Jr. Foam fractionation of proteins: concentration of aqueous solutions of bovine serum albumin. J. Biochem. Microbiol. Technol. Eng. **1959**, *1*, 1–8.
2. Brown, L.; Narsimhan, G.; Wankat, P.C. Foam fractionation of globular proteins. Biotechnol. Bioeng. **1990**, *36*, 947–959.
3. Montero, G.A.; Kirschner, T.F.; Tanner, R.D. Bubble and foam concentration of cellulase. Appl. Biochem. Biotechnol. **1993**, *39/40*, 467–475.
4. Uraizee, F.; Narsimhan, G. Effects of kinetics of adsorption and coalescence on continuous foam concentration of proteins: comparison of experimental results with model predictions. Biotechnol. Bioeng. **1996**, *51*, 384–398.
5. Wang, L.; Shi, H.; Ouyang, F. Continuous foam separation of protein. Eng. Chem. Metal. (Huagong Yejin) **1996**, *17* (4), 343–347.

6. Xie, J.; Cheng, X.; Deng, X. Study of separation of soybean protein by foam fractionation technique, I. Operation conditions. J. East China Univ. Sci. Technol. (Huadong Ligong Daxue Xuebiao) **1997**, *23* (3), 270–274.
7. Liu, Z.; Liu, Z.; Ding, F.; Yuan, N. Concentration and separation of proteins by foam fractionation (II). J. Tsinghua Univ. (Sci. Tech.) (Qinghua Daxue Xuebao, ziran kexue ban) **1998**, *38*, 20–22.
8. Hossain, M.M.; Fenton, G. Concentration of proteins from single component solution using a semi-batch foaming process. Sep. Sci. Technol. **1998**, *33* (11), 1703–1721.
9. Brown, A.K.; Kaul, A.; Varley, J. Continuous foaming for protein Recovery: part I. Recovery of β-casein. Biotechnol. Bioeng. **1999**, *62* (3), 278–290.
10. Loha, V. Optimizing Protein Separation in a Foam Fractionation Process. Ph.D. Dissertation, Vanderbilt University, Nashville, TN, USA, 1997.
11. Sober, H.A. *Handbook of Biochemistry*; Chemical Rubber Co.: Cleveland, Ohio, 1968.
12. Hammershøj, M.; Prints, A.; Qvist, K.B. Influence of pH on surface properties of aqueous egg albumen solutions in relation to foaming behavior. J. Sci. Food Agric. **1999**, *79*, 859–868.
13. Du, L.; Prokop, A.; Tanner, R.D. Effect of bubble size on foam fractionation of ovalbumin. 23rd Biotechnology Symposium for fuels and Chemicals, Breckenridge, CO, May 6–9, 2001.
14. Du, L.; Ding, Y.; Prokop, A.; Tanner, R.D. Measurement of bubble size distribution in a protein foam fractionation column using a capillary probe with photoelectric sensors. Appl. Biotechnol. Biotechnol. **2001**, *91–93*, 387–404.
15. Du, L. Measuring Bubble Size Distribution in a Foam Fractionation Column Using a Photoelectric Capillary Probe. Ph.D Dissertation, Vanderbilt University, Nashville, TN, USA, 2001.

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