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Evaluation of Foam Fractionation Column Scale-Up for Recovering Bovine Serum Albumin

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EVALUATION OF FOAM FRACTIONATION COLUMN SCALE-UP FOR RECOVERING BOVINE SERUM ALBUMIN

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ABSTRACT. *Foam fractionation is an adsorptive-bubble separation method that, according to researchers, is a feasible technique for the separation and/or concentration of proteins. The foam fractionation of bovine serum albumin (BSA) in laboratory-scale foam fractionation columns (750 and 1250 mL) and the relationship between the two laboratory-scale columns and a pilot-scale column (5000 mL) were investigated. Recovery, enrichment, and performance factor values were experimentally determined with three different column volumes with varying pore sizes, gas superficial velocities, and, in the case of the 750 mL column, foam column height. As the pore size decreased, the amount of protein recovered from the dilute protein solution increased and the enrichment decreased. As the flow rate of the gas increased, the effect of the pore size decreased. For the three column volumes, the optimal column conditions were achieved with the largest pore size (145-174 μm) and an intermediate superficial gas velocity (7 mm/s). Increasing the foam column height increased the enrichment without sacrificing the recovery of the target protein. In the case of the largest pore size, the linear relationships between the recovery and the ratio of gas volume to initial liquid volume are parallel, such that the recovery in a pilot-scale column (5000 mL) can be predicted with the recovery found with a laboratory-scale column (750 or 1250 mL).*

Keywords. *Downstream processing, Foam fractionation, Protein recovery, Scale-up.*

Foam fractionation is an adsorptive-bubble separation method that is a feasible technique for the separation and/or concentration of proteins (Uraizee and Narsimhan, 1996), including recombinant pharmaceutical proteins (Crofcheck et al., 2003; Lockwood et al., 1997) and enzymes such as cellulase (Loha et al., 1999). Standard separation and recovery techniques can be expensive, especially when the purity requirement of the protein or enzyme is high. In fact, the cost of protein recovery and purification may be the determining factor in whether a product is economically viable (Kusnadi et al., 1997). Hence, targeting better protein recovery and purification techniques will result in a significant reduction in processing costs.

Foam fractionation takes advantage of the surface activity (hydrophobic/hydrophilic nature) of the protein of interest (Uraizee and Narsimhan, 1996). Separation is achieved by bubbling gas through a dilute protein solution. The surface-active proteins adsorb to the gas-liquid interface of the bubbles; the bubbles rise to the top of the liquid and form a protein-rich foam layer. In the foam layer, liquid drains between the bubbles back into the liquid, further concentrating the foam layer. Finally, the foam is collected and collapsed (Brown and Varley, 1999), resulting in a protein-rich solution (foamate). The performance of a foam fractionator for a particular protein depends on column operating parameters (including gas flow rate, feed flow rate, feed solution height, foam layer height, and bubble size) and feed solution conditions (including pH, ionic strength, and protein concentration) (Lockwood et al., 1997).

Foam fractionation can be utilized in a semi-batch (Chai et al., 1998; Ko et al., 1998; Lockwood et al., 2000; Loha et al., 1999; Varley and Ball) or continuous (Brown et al., 1999; Brown and Varley, 1999; Chen et al., 1994) manner. It involves low capital and operational costs, and offers great potential for scale-up (Chai et al., 1998; Uraizee and Narsimhan, 1990). There have been several proteins investigated for use with foam fractionation (Brown and Varley, 1999; Chai et al., 1998; Lockwood et al., 1997; Uraizee and Narsimhan, 1990), including bovine serum albumin (BSA) (Brown and Varley, 1999; Du et al., 2000; Lockwood et al., 2000) as discussed here. Foam fractionation conditions have been optimized for the recovery of a target protein from a dilute protein solution containing only the target protein (Brown and Varley, 1999; Chai et al., 1998; Ko et al., 1998; Varley and Ball, 1994) or from heterogeneous protein solutions (Brown et al., 1999; Lockwood et al., 2000; Loha et al., 1999). Since foam fractionation is well suited for concentration of dilute protein solutions, it may prove useful as a first concentration step to be followed by ultrafiltration (Brown et al., 1990) or as a replacement for a chromatography step (Lockwood et al., 2000).

For foam fractionation to be a viable option for separating/concentrating proteins on a commercial scale, critical scale-up parameters of the process need to be determined. To date, research efforts for separating/concentrating proteins have been investigated on a laboratory scale (typically <1000 mL columns). In this work, the foam fractionation of bovine serum albumin (BSA) in laboratory-scale foam fractionation columns (750 and 1250 mL) and the relation-

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ship between the two laboratory-scale columns with a pilot-scale column (5000 mL) were investigated empirically. The effect of foam column height on enrichment and recovery was also investigated. This parameter was selected because it is a potentially influential parameter that would be easy to control in an industrial foam fractionation column. The following specific objectives were addressed:

- Determine the conditions under which the laboratory-scale and pilot-scale columns result in optimal performance.
- Determine whether the resulting conditions scale with length to diameter ratio (L/D), column diameter, and/or another column or operating parameter.
- Determine the extent that foam height influences column performance.

The performance of a foam fractionation column depends on the enrichment and recovery. The *enrichment* (E) refers to the ratio of the protein concentration in the foamate to the protein concentration in the initial solution (as the protein concentration in the foamate increases, the enrichment increases). The *recovery* (R) is the percentage of protein from the initial solution that is found in the foamate (as the recovery increases, the mass of protein in the foamate increases). Typically, as recovery increases, enrichment decreases. To illustrate this, consider that the maximum recovery (R = 100%) can easily be achieved by foaming all of the initial solution, hence recovering all of the available protein, resulting in the minimum possible enrichment (E = 1). In this work, the conditions leading to the highest combined enrichment and recovery are defined as optimal. In practice, there may be instances where a high recovery with a smaller enrichment is desirable (e.g., when the protein is very valuable and losses of protein to the retentate need to be minimal). Some researchers have used the product of the recovery and enrichment, referred to as a process efficiency (Loha et al., 1999), as a means of determining column performance. However, simply multiplying these two values creates a bias towards the recovery, since enrichment values are typically 2 to 10 and recovery values vary from 10% to 100%. In this work, column performance was quantified using a performance factor, defined as the sum of the enrichment and recovery after being normalized over the range found experimentally:

$$\text{performance factor}_i = \frac{E_i}{E_{max} - E_{min}} + \frac{R_i}{R_{max} - R_{min}} \quad (1)$$

Research Methods

Figure 1 is a schematic of the semi-batch foam fractionation system utilized. All columns were constructed of clear polyvinyl chloride (PVC) pipe (McMaster-Carr, Atlanta, Ga.). The 750 and 1250 mL volumes were tested in columns with diameters of 5.08 cm (2 in.), while the 5000 mL volumes were tested in a column with a diameter of 10.16 cm (4 in.). Column dimensions are summarized in table 1. The bottom of each column allowed for the introduction of gas through interchangeable porous glass filter discs (7176-37, 7176-38, 7176-39, 7176-57, 7176-58, and 7176-59, ACE Glass, Vineland, N.J.) creating bubbles of various numbers and sizes

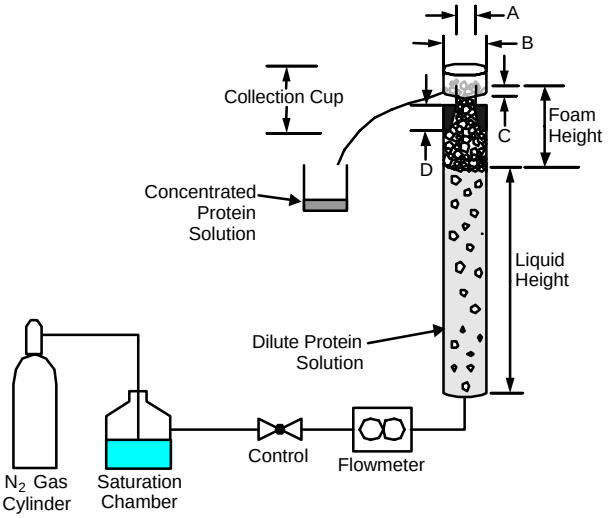

Figure 1. Schematic of the semi-batch foam fractionation setup (dimensions given in table 1).

Table 1. Foam fractionation column and collection cup dimensions.

	Initial volume (mL)		
Dimension	750	1250	5000
Liquid height (cm)	40.4	67.4	68.8
Inside column diameter (cm)	4.86	4.86	9.62
Length/diameter ratio (L/D)	8.32	13.87	7.16
Porous filter diameter (cm)	4	4	9
A in figure 1, ID (cm)	2.37	2.37	3.75
B in figure 1, ID (cm)	4.86	4.86	7.27
C in figure 1 (cm)	2.54	2.54	2.54
D in figure 1 (cm)	5.08	5.08	5.08

depending on the porosity of the filter disc. The filters were sized to cover as much of the bottom of the column as possible, hence different filters were used for the 5.08 and 10.16 cm columns. The top of the column was fitted with a foam collection cup, such that the foam would rise through the column, enter a narrowed pipe, and spill over the sides into an annular trough. In the 750 mL column, the collection cup was moved up and down in order to vary the foam height.

The recovery and enrichment for the semi-batch foam fractionation of BSA with three initial volumes (750, 1250, and 5000 mL), three pore sizes (A = 145 to 174 μm, B = 70 to 100 μm, and C = 25 to 50 μm), and four superficial gas velocities (5, 7, 9, and 11 mm/s) for a foaming time of 45 min were to be determined experimentally. Data were obtained for all conditions except the lowest flow rate (5 mm/s) with the 5000 mL volume; under these conditions, collectable foam was not produced. In addition, with the 750 mL volume, three different foam column heights were tested (13, 15, and 17 cm), where the intermediate foam height was equal to the foam height (15 cm) used in the 1250 mL column. The volumes were chosen such that the height was the same for the 1250 and 5000 mL columns and L/D was similar for the 750 and 5000 mL columns. The data from the 750 (at the intermediate foam height), 1250, and 5000 mL experiments were analyzed to determine the optimum pore size and superficial gas velocity based on the performance factor. Finally, using the experimentally determined optimum column conditions, foam fractionation was allowed to continue until the foam layer collapsed and was no longer

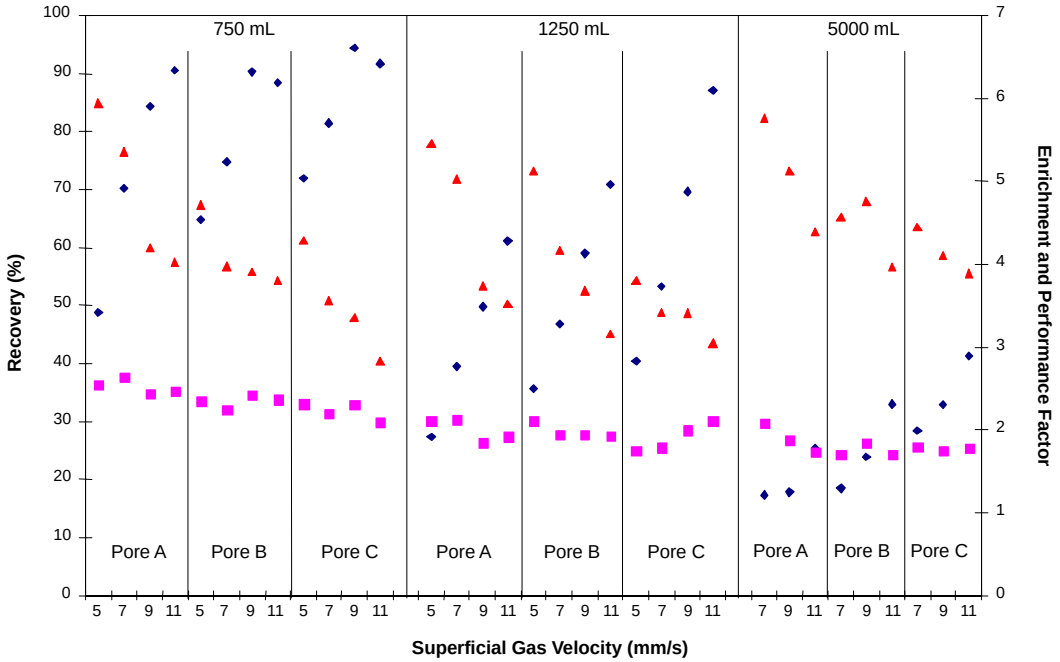

Figure 2. Recovery (%, diamond shapes), enrichment (triangles), and performance factor (squares) averages (n = 3) for three column sizes (750, 1250, and 5000 mL), three pore sizes (A, B, and C) and varying gas superficial velocities (5 to 11 mm/s) after 45 min of foaming.

collectable, providing recovery and enrichment values based on running the foam fractionation to completion.

For each column volume, the order in which the pore sizes were tested was completely randomized, as well as the order in which the superficial gas velocities were tested within each pore size (3×4 factorial for the 750 and 1250 mL columns, and 3×3 factorial for the 5000 mL column) with three replications. The same initial concentration of BSA was used for all experiments: 100 mg/L BSA in 10 mM Tris buffer adjusted to a pH of 7.5 (Lockwood et al., 2000). BSA (BP1600-100) and Tris buffer (2M, BP1759-500) were purchased from Fisher Scientific (Pittsburgh, Pa.) and used as received. Water was of research analytical grade, prepared by reverse osmosis of household tap water, followed by deionization and filtration through activated carbon (NANOpure Ultrapure Water System, Model 04741, Barnstead International, Dubuque, Iowa).

The same procedure was used for each foam fractionation test. Briefly, the initial BSA solution was charged to the column, and then nitrogen gas was introduced into the column and bubbled through the liquid pool, creating a stable foam. The foamate (collapsed foam) was collected for 45 min for the treatment combination experiments or until the foaming had reached completion for the experiments run at the resulting optimum conditions. The volumes of the foamate and retentate were recorded. Finally, the protein concentration of the foamate solution was determined using the Bradford method (Bradford, 1976). Bradford reagent was purchased from Sigma (B-6916, St. Louis, Mo.). For each test the enrichment, recovery, and performance factors were calculated. Columns were consistently washed with soap and rinsed several times, which was shown to ensure the column's inner surface area was consistent for each test.

Results and Discussion

The recoveries, enrichments, and performance factors for the 750 (at an intermediate foam height), 1250, and 5000 mL experiments are shown in figure 2. As the superficial gas velocity increases, recovery increases and enrichment decreases. As the pore size decreases (from A to C) recovery increases slightly, enrichment decreases slightly, and the performance factors decrease. In both cases, the recovery is increasing because the liquid hold-up in the foam layer is increasing, creating a "wetter" foam, and resulting in an increase in the volume and the amount of protein in the foamate and a decrease in the concentration of the protein in the foamate (Uraizee and Narsimhan, 1996).

For the 750 mL data, with the two smaller pore sizes (B and C) a superficial velocity of 9 mm/s is sufficient to recover as much protein as possible, such that the foaming is actually complete before 45 min. Hence, the increase in gas volume as the superficial velocity is increased to 11 mm/s does not result in an increase in the recovery. However, for the 1250 mL data at the same pore sizes and flow rates, the recoveries are still increasing linearly. This illustrates the importance of choosing a superficial gas velocity for a particular liquid pool height. For all three columns, the highest performance factor corresponded to the largest pore size (pore A) with a superficial velocity of 7 mm/s (table 2). When the foam fractionation was carried out to completion

Table 2. Summary of the optimum conditions based on maximizing the performance factor.

Volume (mL)	Pore Size	SGV[a] (mm/s)	45 min		Complete	
			E	R%	E	R%
750	A	7	5.36	70.30	6.50	91.5
1250	A	7	5.03	39.50	6.20	98.0
5000	A	7	5.43	17.29	6.11	96.0

[a] Superficial gas velocity.

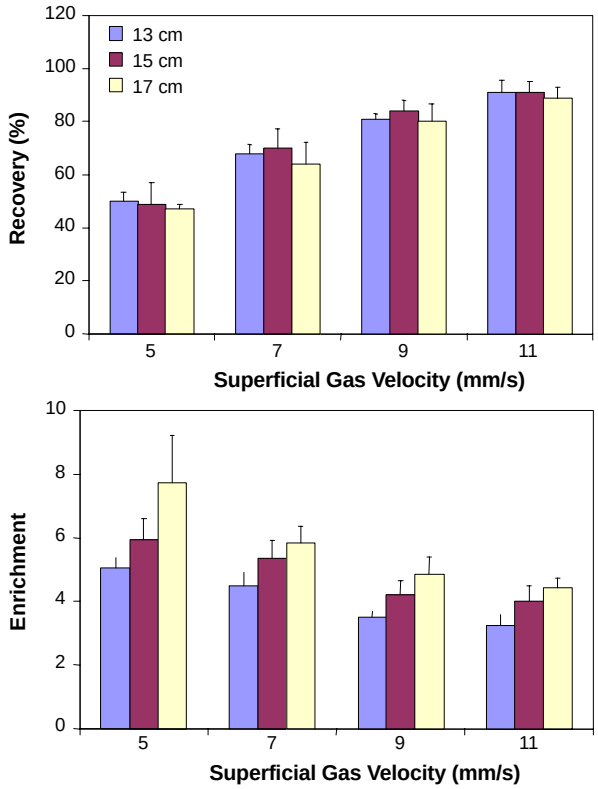

Figure 3. Recovery (%) and enrichment for the 750 mL foam fractionation column with a constant pore size (pore A) and varying foam height (13 to 17 cm). Error bars indicate standard errors (n = 3).

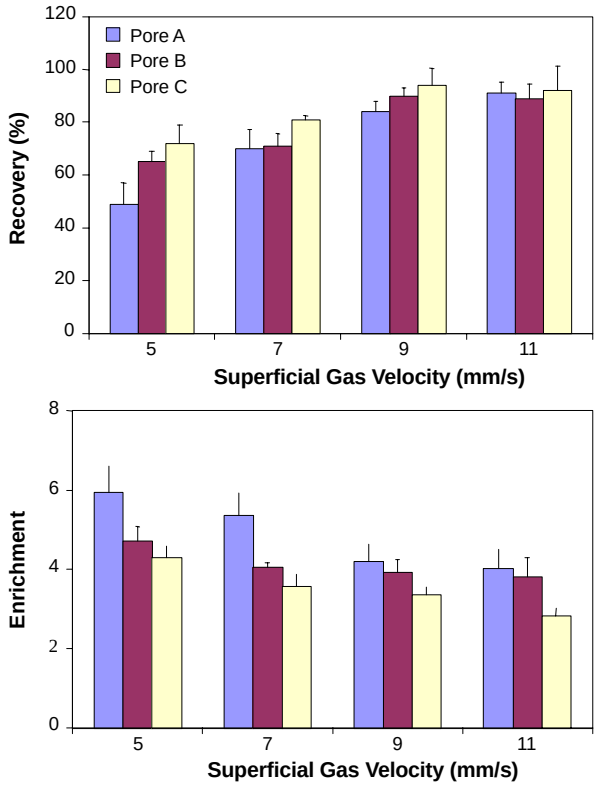

Figure 4. Recovery (%) and enrichment for the 750 mL foam fractionating column with a constant foam height (15 cm) and varying pore sizes (A, B, and C). Error bars indicate standard errors.

at these conditions (~1.5 h for 750 mL, ~2.5 h for 1250 mL, and ~5.5 h for 5000 mL), the recoveries increased and the enrichments remained relatively the same. From these empirical results, it appears that the most effective pore size and flow rate does not vary appreciably with changes in column volume, diameter, length, or length to diameter ratio.

However, there are still scaling issues that may need to be addressed, including foam height and column design. The recoveries and enrichments for the 750 mL experiments with the smallest pore size and varying foam height are shown in figure 3. The foam height has a greater effect on the enrichment compared to recovery. For this reason, increasing the foam height may be used to increase the enrichment without affecting recovery. However, with the larger column, the foam stability is not sufficient to support a foam layer similar to the foam layers generated in the smaller columns. In order to take advantage of the additional drainage in the foam column associated with a large (4 in.) column, the foam may need to be generated in a column with a smaller diameter (<2 in.).

The recoveries and enrichments for the intermediate foam height and varying pore size are shown in figure 4. As the gas flow rate increases, the effect of pore size on the recovery of BSA decreases. Hence, if a lower gas flow rate is selected, the choice of pore size becomes more important.

As the gas volume increases, the bubble volume and surface area increase and the recovery increases. As the initial liquid volume increases, the amount of protein available to recover increases, so that the recovery in the same amount of time for two different volumes will always be less for the larger volume. Hence, the individual recoveries and enrichments from the different volumes cannot be directly compared. In order to illustrate these two effects together, the recovery is plotted as a function of gas volume per initial liquid volume in figure 5. The recovery for the 750 mL experiments with the highest flow rate have been omitted, since the gas volume used was in excess of what was needed to remove all the protein from the initial solution. Parallelism was determined using Student's t-test, where the null hypothesis was that the two slopes being compared were equal (i.e., parallel, H_o: $m_1 = m_2$). Table 3 summarizes the results from the parallelism tests. Interestingly, the slopes for the three different column sizes using the largest pore size (pore A) are not statistically different (P = 0.67 for comparing 750 and 1250 mL, and P = 0.31 for comparing 1250 and 5000 mL). As the pore size is decreased, the slopes for the 750 mL column decrease and increase for the 1250 mL column; the slopes for two different volumes are statistically different (P = 0.01 for pore B, and P = 0.003 for pore C). However, the slopes are not statistically different for the 1250 and 5000 mL data for all three pore sizes (P = 0.31 for pore A, P = 0.23 for pore B, and P = 0.30 for pore C). These results indicate that with the larger pore size, the recovery scales proportionally from 750 to 1250 mL with respect to gas volume per initial liquid volume. It appears that recovery scales from 1250 to 5000 mL (constant height) and 750 to 5000 (similar L/D) with respect to gas volume per initial liquid volume.

CONCLUSION

Targeting lower-cost protein recovery techniques will result in a significant reduction in processing costs. The work

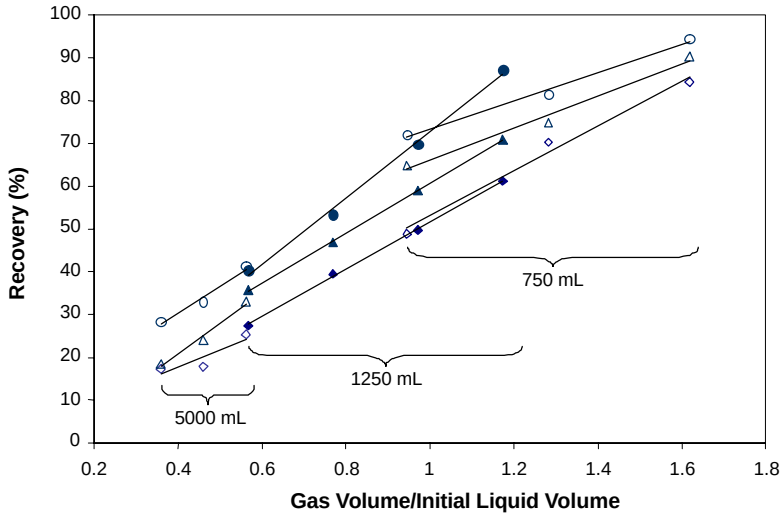

Figure 5. Recovery (%) versus the ratio of the gas volume to initial liquid volume for three column volumes and three pore sizes: A (diamond shapes), B (triangles), and C (circles).

Table 3. Statistics for parallelism tests with H_0: $m_1 = m_2$ and $\alpha = 0.05$.

Pore Size	750 vs. 1250 mL (t_{crit} = 3.18)			1250 vs. 5000 mL (t_{crit} = 3.18)			750 vs. 5000 mL (t_{crit} = 4.30)		
	t	*p*		*t*	*p*		*t*	*p*	
A	-0.046	0.67	Parallel	1.20	0.32	Parallel	0.60	0.64	Parallel
B	-5.14	0.014	Not parallel	-2.18	0.12	Parallel	-2.50	0.13	Parallel
C	-9.18	0.0027	Not parallel	1.24	0.30	Parallel	-2.81	0.11	Parallel

described here is a necessary step before foam fractionation, a less expensive separation choice, can be used on a commercial scale to recover proteins. The recoveries, enrichments, and performance factors for three different column sizes with varying superficial velocities and pore sizes were experimentally determined. For all three volumes, the largest pore size (A: 145-174 μm) and an intermediate flow rate (superficial velocity of 7 mm/s) resulted in the highest performance factor. It was also found that increasing the foam height might be used to increase the enrichment without affecting recovery. As the superficial gas velocity increased, the effect of pore size decreased. Finally, for foam fractionations with a larger pore size, the recovery scales proportionally from 750 to 1250 mL (constant D) with respect to gas volume per initial liquid volume, and from 1250 to 5000 mL (constant L/D ratio) for the entire range of pore sizes tested here. Hence, the recovery in a pilot-scale column (5000 mL) can be predicted with the recovery found with a laboratory-scale column (750 or 1250 mL).

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