0273-2289/03/105-108/0905/$20.00

Effect of Lidocaine on Ovalbumin and Egg Albumin Foam Stability

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Abstract

Foam fractionation is a simple separation process that can remove and concentrate hydrophobic molecules such as proteins, surfactants, and organic wastes from an aqueous solution. Bovine serum albumin and ovalbumin have been widely used as model proteins due to their strong foaming potential and low price. Here, we study the effect of lidocaine on albumin foam, since drugs like lidocaine are known to bind with albumin. We observed that lidocaine not only enhances the amount of foam produced but also the stability of that foam as well. The foam stability was evaluated as the decay rate constant of the foam, determined from a change in height (or volume) of the foam over a given time period.

Index Entries: Foam; egg albumin; ovalbumin; lidocaine; foam stability.

Introduction

Foam fractionation has been in use in some forms since the early 1960s. Its practical application was for the removal of surfactants in waste treatment plants. The underlying process is the removal of surfactant molecules. Another possible application for foam fractionation is to produce a stable foam for extinguishing fires while providing a medium for breathable oxygen. Du et al. *(1)* describe the process as follows:

> Foam is a gas-liquid dispersion system in which liquid is considered the continuous phase while gas bubbles are the noncontinuous phase. Foam fractionation is a separation technique based on the surface activity of solutes in a solution. Foam fractionation is carried out in a column that consists of two parts separated by a distinct interface during foaming.

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The lower part is the bulk liquid solution while the upper part is the foam phase.

There are two aspects that need further attention in order to develop a successful foam fractionation. The first is foam drainability and the second is foam stability. *Drainability* is defined as the ability of the bulk liquid residue to flow away from foam formation. Surface tension plays a major role in the drainage mechanism. *Foam stability* is defined as the resistance to drainage, without bubble rupture. The foam must be stable enough to be removed from the bulk solution.

Several factors influence foam stability, such as the initial solute concentration, pH, and ionic strength. Column conditions, such as gas velocity and bulk liquid height, also play significant roles. Ovalbumin, the predominant protein in egg albumin to be foamed in the present study, contains hydrophobic end groups and hydrophilic head groups, making it a surfactant. Surfactants can interact with water in a variety of ways, each of which disrupts or modifies the hydrogen-bonding network of water. When a high concentration of ovalbumin, the surfactant, is placed in water, the long, nonpolar hydrocarbon tails tend to aggregate because of favorable intermolecular interactions of hydrophobic ends. The surfactant molecules thereby organize themselves into three-dimensional spheres called *micelles* that have a hydrocarbon core and sulfate groups around the outer surface. Surfactants can also form other structures. Rather than form a sphere, some surfactants can coat the surface of the water to form a layer one molecule thick (a molecular monolayer) at low concentrations.

It is known that many drugs, such as lidocaine, can nonspecifically complex with proteins such as those in egg albumin *(2)*. In our previous experiments, lidocaine was found to increase albumin foam volume. It was hypothesized that lidocaine would stabilize the foam, or decrease the bubble size. Since bubbles are the carriers of proteins, bubble size is expected to have an influence on foam fractionation. By stabilizing the foam, the film thickness of each bubble increases and therefore will not rupture or coalesce with neighboring bubbles. Liquid flow or drainage and coalescence cause the foam to be unstable. Bubble size characteristics are outlined in ref. *3* as follows:

1. Bubble size increases as air flow rates increase in a column.
2. Increase in protein concentration leads to reduced bubble diameter.
3. Finer air stones produce finer bubbles, however the air stone pore size loses significance with increased air flow.

Smaller bubbles are effective in transferring proteins because of high surface area–to–volume ratio and slower rise in the column.

It was also found that the fractionating bubbles developed a "skin" as the proteins and surfactants were removed from the water *(3)*. These skins were formed by the dissolution of the hydrophobic groups into the bubble surface while the hydrophilic ends remain in the water. This leads to a stable foam that acquires these skins as the foam rises. The skins are pushed

up the column through accumulation and collected for removal. The surface layer also has a high viscosity *(3)*. According to Du et al. *(1)*,

> Foam fractionation has a drawback in that protein denaturation can occur (the loss of bioactivity of the protein). Clarkson et al. studied protein denaturation mechanism in foams and found that protein damage is mainly due to surface denaturation at the gas-liquid interface. Surface tension measurement and changes in protein structure occurring at the interface showed that conformational changes were induced but no fragmentation or dissociation of subunits occurred in these foams. Denatured proteins may alter their foaming properties during the foaming process because structural changes can change their adsorption properties and surface properties, thus, the foam stability and foam ability.

The specific objective in the study is to measure the foam decay time for various concentrations of lidocaine in ovalbumin aqueous solutions in order to measure the stability of the foam as a function of the lidocaine concentration.

Materials and Methods

Solutions and Equipment

Ovalbumin protein was purchased from Sigma (lot no. 19H7002; St. Louis, MO). Egg albumin was purchased from Matheson Coleman & Bell (lot no. CB 951). A 20 mg/mL lidocaine HCl solution from Abbot was used. The glass column used was 52 cm high with a 13-cm id. The apparatus was the same as previously used by Du et al. *(1)*. In addition, a 1-L polypropylene graduated cylinder with a 6-cm id was used as a foaming column.

Foam Decay

An ovalbumin solution of 60 mg/L was made by dissolving 240 mg of ovalbumin in 4000 mL of water. The ovalbumin solution was then added to the glass column. The stopcock at the bottom of the column was kept closed until air was fed to the system. Once the solution was added, the inlet airflow rate was set at 85 L/min and the stopcock was turned to the "open" position. For consistency, the foam was generated for 3 min. At 3 min, the height was measured with a ruler. With no aeration, height measurements were then taken every minute as the foam collapsed. When the foam reached approx 4 cm, the foam decay process was stopped. This procedure was repeated for various levels of lidocaine over the range of 0–1.6 mL from a 20 mg/mL stock solution over 0.125-mL increments for a constant ovalbumin concentration (60 mg/L).

Foam decay experiments were then conducted in a 1-L polypropylene graduated cylinder using egg albumin. The albumin solution of 60 mg/L was made by dissolving 240 mg of egg albumin in 4000 mL of water. To create a foam in the closed-bottom polypropylene cylinder, a sparger connected to the air hose was inserted from the top of the cylinder (like in a fish

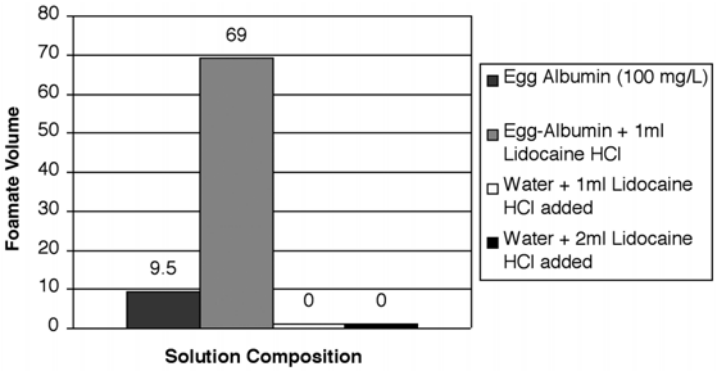

Fig. 1. The bar graphs show the effect of adding lidocaine to 100 mg/L of albumin solution before foam is formed.

tank). For each run, 200 mL of albumin solution was used. After foaming up to the 800-mL mark in the cylinder, the air was turned off. Foam height measurements were taken at approx 1 min intervals to monitor the foam decreases in height.

Measurement of Surface Tension

The surface tension of egg albumin was directly determined using a KSV Sigma 70 surface tension/contact angle meter. This device is fully computer controlled. The measurements made in this experimentation were based on the Wilhelmy plate method *(4)*.

Results and Discussion

When lidocaine was added, it caused an increase in the foam volume (from a 100 mg/L initial albumin solution) from 9.5 to 69 mL, as shown in Fig. 1. Figure 1 shows that lidocaine by itself did not foam. The great increase in foam volume from the addition of lidocaine seems to result from the interaction between lidocaine and egg albumin protein. The addition of lidocaine to ovalbumin, a major protein in egg albumin, reduced the surface tension of the solution (Fig. 2). A lower surface tension, in general, indicated a higher ability to foam. The foam decay rates for different increments of lidocaine are shown in Fig. 3. The data were fit to first-order height decay curves,

$$\frac{dH}{dt} = -kH$$

and the time constants were evaluated by fitting the data using the Excel computer program. The rate constant (k) fluctuated as the lidocaine levels decreased. Figure 4 displays the rate constants generated by Excel from Fig. 3 as a function of lidocaine volume added to the 60 mg/mL ovalbumin solution. The rate constants also characterize the stability of the foam. The

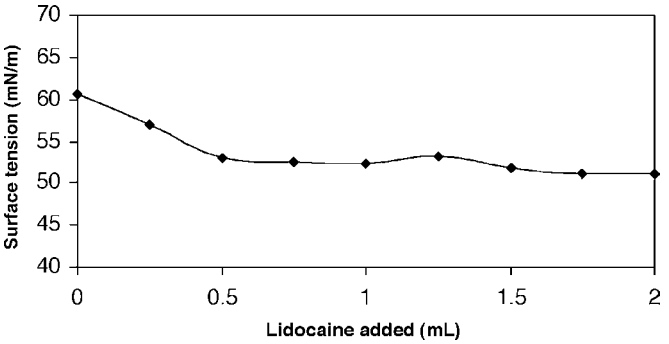

Fig. 2. Change in surface tension with added lidocaine. Initially, 60 mg/L of ovalbumin was present.

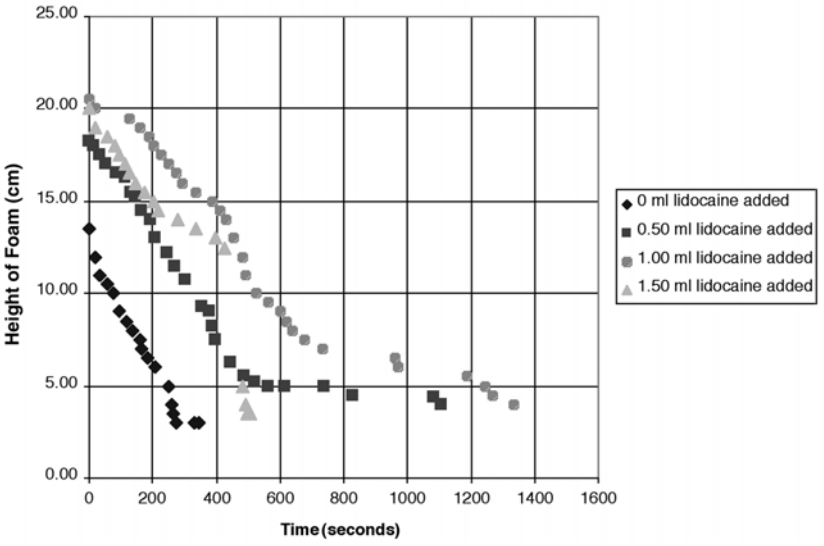

Fig. 3. Changes in height of foam at different lidocaine concentrations are shown. Ovalbumin was kept constant at 60 mg/L.

smaller the rate constant, the longer the foam remained in the column and, hence, the more stable the foam. The decay rate profile appears to have three characterizing intervals. The first interval is from 0 to 0.4 mL of lidocaine added. In this interval, the decay rate is constantly decreasing. The second interval is from 0.4 to 1.2 mL of lidocaine added. Here, the decay rate seems to stabilize and hold constant. In this interval, the solution seemed to be "saturated" with the amount of lidocaine added. A different effect is observed in the third interval (from 1.2 to 1.6 mL of lidocaine added). The foam is most stable when 0.375 mL of lidocaine is added. The decay rate was used instead of the leveling time because the leveling time

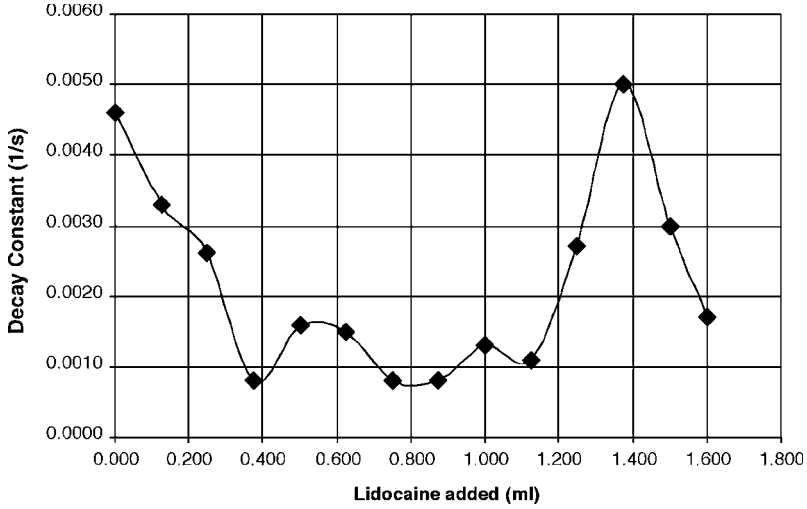

Fig. 4. Foam decay constants for increasing amounts of lidocaine added to a 60 mg/L ovalbumin solution. A lower decay constant represents a more stable foam. The foam was most stable when 0.375 mL of lidocaine was added to the 60 mg/L ovalbumin solution.

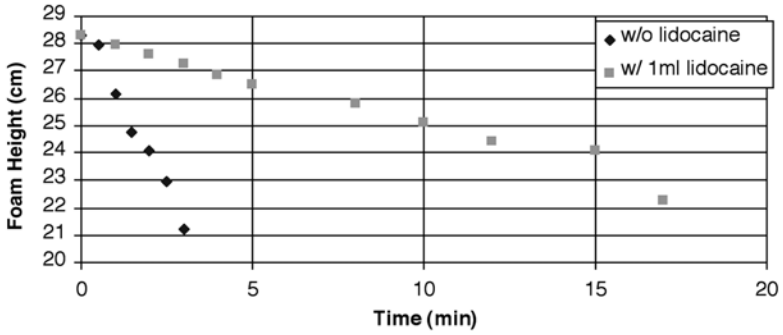

Fig. 5. Foam decay time in polypropylene column. The egg albumin concentration was at 60 mg/L. One milliliter of lidocaine was added. The decay rate constant for the foam without lidocaine addition was 0.0956/s, and for the foam with 1 mL of lidocaine added was 0.0125/s, indicating a 7.6-fold enhancement in foam stability.

was taken from only one data point. The leveling time is defined as the time it takes for the foam height to decrease by 63% ($1 - 1/e$). Because of the random collapsing pattern of the foam, one point would not be a good representation of the foam-decaying behavior. Figure 5 shows the effect of lidocaine on foam decay in a polypropylene column. The foam with lidocaine took about six times longer to reach the same height as the foam without lidocaine.

For the purposes of this experiment, the foam collapse rates were fit to first-order decay curves. Each set of data fit first-order decay curves with R^2 values >0.95. In addition, a new variable, τ, or foam-leveling time was defined. In the glass column part of the experiment, it was observed that after one duplicated run, the error in measuring foam decay time was 80%. In the plastic column, the data also had a lot of uncertainty. The decay rate varied about 30% from the mean value. The plastic column diameter was 2.7 times smaller than the glass column. In both columns, the foam-collapsing pattern was random. The level of the foam initially decreased at a constant rate. Then, the random pattern started to have a very strong effect. Although the height might not change as fast, the number of bubbles was decreasing. The liquid fraction of the foam was also an important factor. Wetness of the foam is a function of liquid fraction of the foam. From the experiment, the wet foam (high liquid fraction) tended to stick less to the wall. The dry foam (low liquid fraction) created many problems during the experiment because it would form a layer around the wall. The addition of lidocaine increased the foam decay time. The foam from 60 mg/L egg albumin solution usually took about 3 min to decrease 200 mL (out of 800 mL initial foam). The foam from 60 mg/L initial albumin solution with the addition of 1 mL of lidocaine took 18 min to decrease the volume by 200 mL. The addition of lidocaine decreased the surface tension of the solution; therefore, it was much easier to foam. At the same gas velocity, the foam with lidocaine had smaller bubbles. Smaller bubbles suggested that the foam contained more water. Wet foam was more stable than dry foam.

Conclusions

The addition of lidocaine to ovalbumin and egg albumin aqueous solutions not only created much more foam, but this wetter foam was more stable. There appears to be a peak in foam stability at a lidocaine concentration (0.375 mL of lidocaine added) where the foam decay rate constant reached a minimum. For both glass and polypropylene columns, the lidocaine generally stabilizes the foam by creating smaller bubbles at certain concentrations (0.375 mL of lidocaine added) of lidocaine while larger bubbles were seen at other concentrations.

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