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FOAM SEPARATION OF PROTEINS

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ABSTRACT

In downstream processing of biochemical products, the separation of a product component from the nutrient medium containing many residual substrates is a very important operation. Foam separation, an adsorptive bubble separation method, has emerged as an alternative to traditional separation techniques such as ion exchange, chromatography and precipitation. A study about the enrichment of water soluble binary proteins namely 'Bovine serum albumin' and 'Bovine hemoglobin' was carried out in a batch column. The effects of gas flow rate, liquid pool height, protein concentration, pH of the feed and foam height on the enrichment of proteins by foam separation was studied to optimize various parameters. The separation of binary proteins was found to highest at pH 5.5. An enrichment ratio of 1.42 was obtained at the optimum operating conditions of 0.2 Lpm of air flow rate, 20 cm of liquid pool height, feed concentration of 1.8 mg/mL of BSA and 1 mg/mL of hemoglobin, 5.5 pH of feed and 25 cm of foam height.

Key words: Foam separation, Enrichment ratio, Bovine serum albumin, Hemoglobin.

INTRODUCTION

The purification of proteins from recombinant sources is an important aspect for the biochemical processing of pharmaceutics, enzymes, antibodies etc. Foam separation, a novel separation technique, is attractive not only because of low energy consumption and operational costs but also due to its advantage in recovering substances from highly diluted solutions. The two characteristic features of foam, useful in separation, are its very large gas-liquid interfacial area and very low liquid holdup. Solutes to be removed or recovered are adsorbed on to the foam surface and concentrated in the collapsed foam liquid (foamate) phase.

A variety of applications have been reported such as protein fractionation[1], separation of solutes from mineral ore, hazardous metal ions from effluents and surfactants recovery[2]. Brown et al. [3], reported the continuous foam separation of three binary protein mixtures: Casein-Bovine Serum Albumin (BSA), BSA-Lysozyme and Casein-Lysozyme. They discussed the influence of different foamate rates for various combinations of protein concentrations. Their results showed that the degree of lysozyme enrichment in the presence of either casein or BSA is very small. But the addition of them enhances the stability of foam. Kinoshita et al.[4-6] studied the batch and

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continuous separation of Au (III) from dilute aqueous solutions with a non-ionic surfactant PONPE 20. Continuous recovery of surfactant SDS and Cd^{2+} from permeate in micellar enhanced ultrafiltration (MEUF) was carried out by Qu et al.,[7] to study the effects of various operating parameters on the separation characteristics in a continuous column. Wong et al.[8] examined the enrichment and recovery of bovine serum albumin in continuous column and reported that maximum protein recovery was found to be at the isoelectric point (pH 4.8) of the protein and at the air velocity in the range of 0.05-0.15 cm/s. Narsimhan and Ruckenstain[9] examined the semi batch foam fractionation of a non ionic surfactant on the basis of a model which accounts for (i) the gravity drainage from the plateau borders (ii) the thinning of the liquid lamellae (films) caused by capillary pressure, the plateau border suction and disjoining pressure and (iii) the vanderwaals mediated rupture of the thin films. The result indicated the existence of an optimum inlet bubble size and optimum inlet concentration of the surfactant for maximum enrichment. An increase in superficial gas velocity, viscosity or surface viscosity decreased the enrichment factor. Samita Bhattacharjee et al.[10,11] developed a model in concentrating protein solutions using batch foam columns. The model was verified with casein and BSA solutions and found that with increase in liquid pool height above the gas distributor and the time allowed for drainage resulted in a better separation. Neely et al.[12] developed a mathematical model of a batch foam fractionation of aqueous protein solution obtained from kudzu (Pueraria lobata) vine retting broth on analogies to a distillation model. Time varying trajectories of measured total protein levels were fitted to the model such that localized distribution coefficients were determined at each stage, at 15 minute intervals, for the six stage equilibrium model. Co adsorption studies on lysozyme-casein was first reported by Hunter et al.[13,14]. It was suggested that casein being a flexible coil like molecule, can quickly adsorb at the air water interface whereas lysozyme being an elliptical rigid molecule has adsorption rate less than that of casein.

EXPERIMENTAL

Materials and methods

Bovine Serum Albumin and Hemoglobin used in this study were purchased from Hi Media Chemicals. The properties of protein used are shown in Table 1. Distilled water was used and all the chemicals used were of analytical grade. The concentration of protein in the foamate was measured by Lowerytal method using Jasco UV Visible Spectrophotometer at a wave length of 740 nm. Quantitative estimation of individual components in a protein mixture was done using SDS-PAGE method.

Table 1: Properties of protein used

Protein	Structure	Isoelectric point
Bovine Serum Albumin	Globular	4.7
Hemoglobin	Globular	6.8

A sketch of the experimental set-up employed in the present work is shown in Fig. 1. A glass tube of inner diameter 2.03 cm is used as the batch foam column with a top hemispherical

bend to collect the foam. Filter cloth wound on a sponge is used for air distribution. This filter cloth is attached to a stainless steel nozzle, and its edge is sealed with a water insoluble glue to prevent the formation of bubbles at its outer periphery. The distributor assembly is then mounted on a close fitting rubber stopper which is inserted at the bottom of the glass column. The distributor is connected to a compressor via a two way valve and manometer. Between the manometer and the compressor, a valve and flow meter are connected to control and measure the air flow rate respectively.

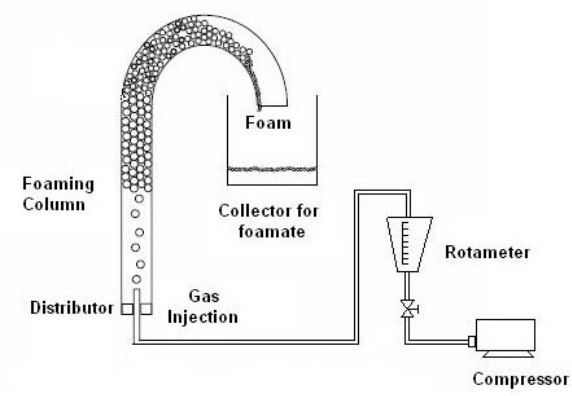

Fig. 1: Experimental set up for batch foam fractionation

A known volume of the protein mixture solution, having predetermined concentrations of Bovine Serum Albumin and Hemoglobin ions was taken into the foam column. The initial level of the liquid was noted in the column. Air was then passed through the nozzle into the feed solution at a controlled flow rate, till the foam attained a desired height. As soon as the foam reached the required height, the gas supply was bypassed through the two-way valve. Liquid, which was present in the foam, started draining down into the liquid pool. After allowing drainage for some specific time, the foam and the residual liquid were separated. The liquid obtained by breaking the foam and the residual pool liquid were then analyzed to determine the protein concentrations.

RESULTS AND DISCUSSION

Experimental studies in batch foam fractionation column for various feed concentrations at different pH of the solutions, liquid pool heights and flow rates of air were conducted using binary protein mixtures to determine the enrichment ratio and percentage recovery.The results obtained are discussed in detail. ‘Enrichment ratio’ or ‘Separation factor (E)’ is defined as the ratio of the concentration of metal ion in the foamate (C_P) to the concentration of metal ions in the feed liquid (C_f) from which the foam has been generated. ‘Percent removal (P.R %)’ is defined as the amount of metal ions recovered to the amount of metal ions in feed solution.

Enrichment ratio (E) = Concentration of metal ions in Foamate (C_p)

Concentration of metal ions in feed solution (C_f)

Percentage removal (P.R %) = Amount of metal ions recovered ($C_f - C_b$) x 100

Amount of metal ions in feed solution (C_f)

where C_b is the concentration of metal ion in residual solution.

Effect of air flow rate

Experiments were conducted by varying air flow rates at fixed other conditions of 20 cm liquid pool height, feed concentration of 1.8 mg/mL of Bovine Serum Albumin (BSA) and 1.0 mg/mL of Hemoglobin, 5.5 pH of feed, drainage time of 4 min. and 25 cm of foam height. The results obtained for the effect of air flow rate on enrichment ratio are shown in Fig. 2. It was found that as the air flow rate increased from 0.2 to 0.5 lpm, the separation factor or enrichment ratio decreased from 1.42 to 0.968. These observations agreed well with those of Brown et al. (1990).

As the air flow rate is increased initially from 0.1 to 0.2 lpm, there is an increase in both enrichment ratio and percentage removal. However, for further increase in air flow rate, both enrichment ratio and percentage removal decreased. This is due to the fact that initially, at low flow rates, the bubbles sizes are larger and therefore coalescence and drainage are more. Hence enrichment ratio and percentage removal increased initially. Subsequently on further increase in air flow rate, the foam bubble size decreased and coalescence as well as drainage decreased. This leads to decrease in both enrichment ratio and percentage removal. The results are in agreement with the reported literatures by Wong et al. (2001) and Yun-Huan Qu et al. (2007).

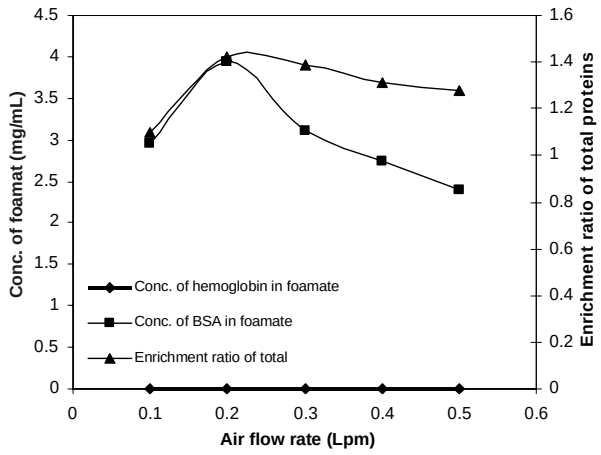

Fig. 2: Effect of air flow rate on foamate concentration and enrichment ratio

[Liquid pool height = 20 cm, Feed concentration = 1.8 mg/mL of BSA and 1 mg/mL of hemoglobin, pH of the feed 5.5, Drainage time = 4 min, Foam height = 25 cm]

Effect of liquid pool height

The effect of liquid pool height on foamate concentration and enrichment ratio of

proteins at fixed other conditions of 0.2 Lpm of air flow rate, feed concentration of 1.8 mg/mL of Bovine Serum Albumin (BSA) and 1.0 mg/mL of Hemoglobin, 5.5 pH of feed, drainage time of 4 min and 25 cm of foam height are shown in Fig. 3. From the figure, it is observed that as the height of the liquid pool is increased from 5 to 25 cm, the enrichment ratio of metal ions increased from 1.24 to 1.42. The residence time of bubbles in the liquid pool is high when the height of the liquid pool is more. This leads to a higher enrichment of metal ions on the bubble surface and it could reach an equilibrium beyond which enrichment may not increase. In the present study this equilibrium is reached at a pool height of 20 cm.

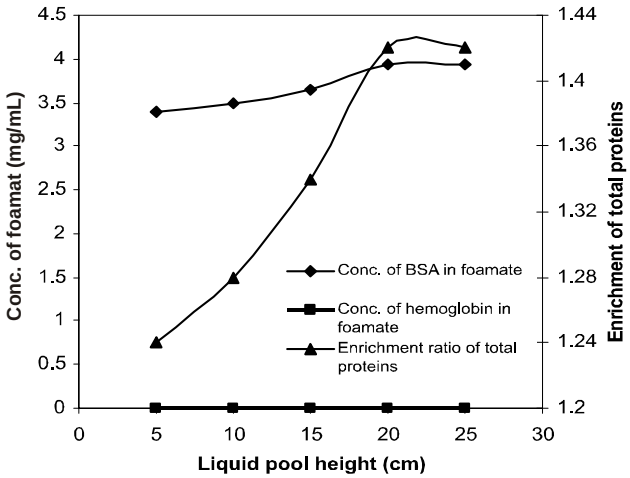

Fig. 3: Effect of liquid pool height on foamate concentration and enrichment ratio

[Air flow rate = 0.2 lpm, Feed concentration = 1.8 mg/mL of BSA and 1 mg/mL of hemoglobin, pH of the feed 5.5, Drainage time = 4 min, Foam height = 25 cm]

Effect of feed concentration

The effects of concentrations of hemoglobin and BSA in feed on foamate concentration and enrichment ratio are shown in Fig. 4 and Fig. 5. By keeping the feed concentration of BSA at 2 mg/L, the concentration of hemoglobin was increased from 0.2 to 1.0 mg/L and it was found that as the concentration of Hemoglobin was raised in the feed solution its adsorption decreases but there is an increase in the adsorption of BSA. This is due to the fact that the presence of Hemoglobin in the bulk solution helps in the adsorption of BSA on the bubble surface. From Fig. 4, it is clear that the BSA concentration in the foam is raised in the presence of Hemoglobin. When the feed concentration of hemoglobin reaches 1 mg/mL, it completely stops adsorbing on the bubble surface.

With increase in feed concentration of BSA from 1.8 to 2.6 mg/mL at fixed other conditions of 1 mg/mL of hemoglobin in feed, 0.2 lpm of air flow rate, 20 cm of liquid pool height, 5.5 pH of feed, drainage time of 4 min. and 25 cm of foam height, the enrichment ratio and the foamate concentration of BSA decreases as shown in Fig. 5. This may be due to the fact that as the feed concentration increases, the surface tension decreases. This results in the

formation of quite stable bubbles with lesser coalescence leading to decrease in drainage. Hence, the wetness of foam is higher which decreased the enrichment ratio and percentage removal.

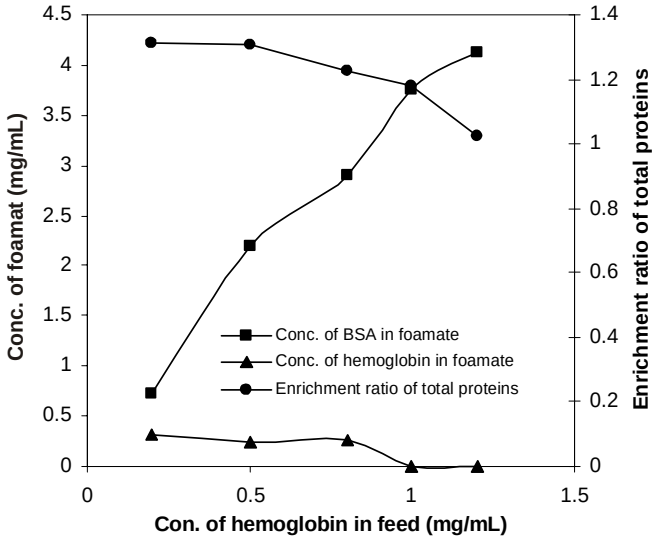

Fig. 4: Effect of concentration of hemoglobin in feed on foamate concentration and enrichment ratio

[Air flow rate = 0.2 Lpm, Liquid pool height = 20 cm, pH of the feed 5.5, Drainage time = 4 min, Foam height = 25 cm]

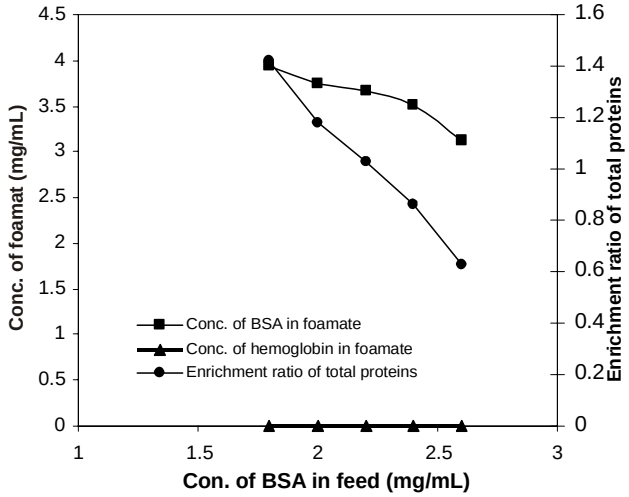

Fig. 5: Effect of concentration of BSA in feed on foamate concentration and enrichment ratio

[Air flow rate = 0.2 Lpm, Liquid pool height = 20 cm, pH of the feed 5.5, Drainage time = 4 min, Foam height = 25 cm]

Effect of pH of feed

The effect of pH of the feed on foamate concentration and Enrichment ratio of proteins is shown in Fig. 6. From the figure, it is seen that the maximum enrichment ratio of 1.199 is obtained at a pH of 5.5 (isoelectric point).

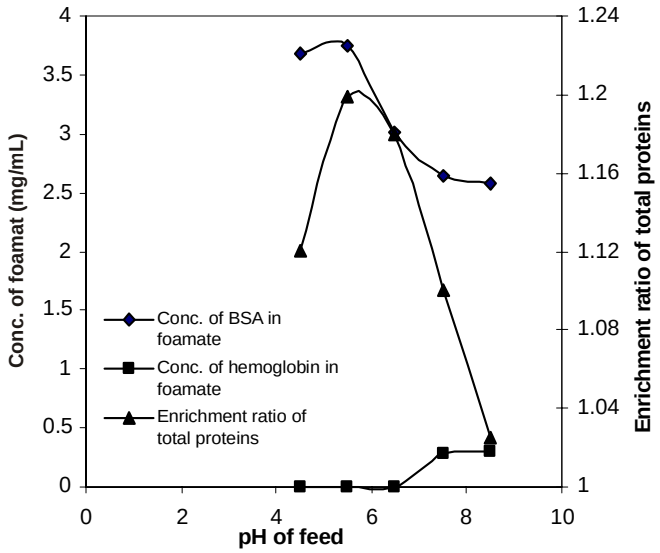

Fig. 6: Effect of pH of feed on foamate concentration and enrichment ratio of total proteins

[Air flow rate = 0.2 Lpm, Liquid pool height = 20 cm, Feed concentration = 2 mg/mL of BSA and 1 mg/mL of hemoglobin, Drainage time = 4 min, Foam height = 25 cm]

This is due to the increased hydrophobicity of protein at its isoelectric point. An electrostatic repulsive force and the vanderwaals attractive force act between proteins adsorbed on the air-liquid interface. The surface charge on the proteins molecule originates from the dissociation of amino acid residues. The electrostatic repulsion between protein molecules adsorbed on the bubble surface is considered to be weakest at the isoelectric point and therefore the proteins are expected to be adsorbed more compactly on the bubble surface at the isoelectric point.

Effect of foam height

The experimental results obtained for the effect of foam height on foamate concentration and enrichment ratio is shown in Fig. 7. As the foam height was increased from 20 to 35 cm, a longer foam residence time results, which allowed for more drainage of the liquid in the films. This leads to a drier foam and higher enrichment ratio. Near the liquid- foam interface the bubble size was observed to be larger and hence the drainage is more. As the foam height is increased, the dry foams are present at the top where maximum drainage has already occurred. Hence, beyond certain foam height, no significant change in enrichment ratio was observed.

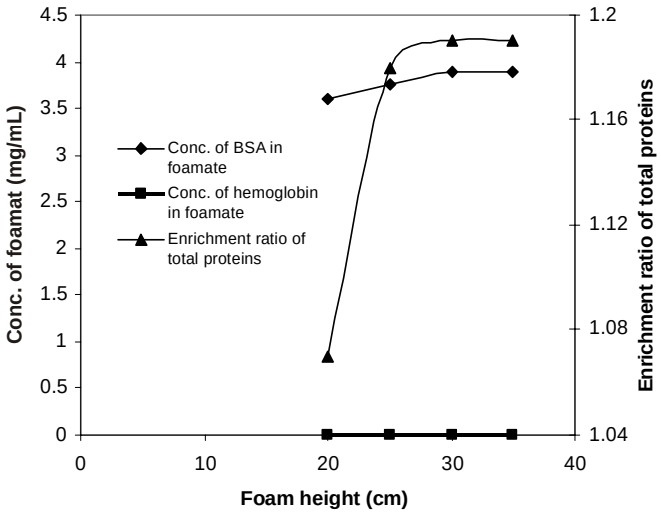

Fig. 6: Effect of foam height on foamate concentration and enrichment ratio of total proteins

[Air flow rate = 0.2 Lpm, Liquid pool height = 20 cm, Feed concentration = 2 mg/mL of BSA and 1 mg/mL of hemoglobin, pH of the feed = 5.5, Drainage time = 4 min]

Summary

Experimental studies were conducted on the batch foam separation of binary proteins namely Bovine serum albumin (BSA) and hemoglobin and the effects of parameters like air flow rate, liquid pool height, feed concentration, pH of the feed and foam height on the foamate concentration and enrichment ratio was studied. The optimum pH for maximum separation was found to be 5.5 which may be due to the increased hydrophobicity of proteins at its isoelectric point. With increase in feed concentration of hemoglobin, the amount of BSA adsorbed on the foam increases. This is due to the fact that the presence of Hemoglobin in the bulk solution helps in the adsorption of BSA on the bubble surface. An enrichment ratio of 1.42 was obtained at the optimum operating conditions of 0.2 Lpm of air flow rate, 20 cm of liquid pool height, feed concentration of 1.8 mg/ml of BSA and 1 mg/mL of hemoglobin, 5.5 pH of feed and 25 cm of foam height. Therefore the effective separation of binary proteins BSA and hemoglobin can be done by the foam separation technique with pure BSA concentrated on the foamate.

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