Studies on improvisation of Protein purification by foam fractionation

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Abstract

In bioprocess industries, the separation of desired components from the residual mixture is a very important operation. Foam separation is one such downstream process which has wide application in varied industrial fields from ore floatation to industrial effluent treatment. This work concerns the study of separation of protein from protein-containing mixtures by selective foam fractionation, including the mechanism of the enrichment of water-soluble protein in batch foam separation. The fractionations of binary proteins used in this study were 'Bovine serum albumin (BSA)' and 'Hemoglobin (HG)'. The gas flow rate, pH of the feed solution, and the protein concentration in the bulk solution were varied to study the effect of 'enrichment factor' in foam fractionation and to optimize the operating parameters in this present investigation. It has been found that an increase in the gas flow rate and the allowance of time for drainage results in better separation. At a pH of around 5.5, the separation of binary proteins was observed to be maximum. The concentrations of BSA and HG in bulk solution were found to be maximum at 1.8 mg/ml and 1.0 mg/ml respectively.

Keywords: BSA, Foam Fractionation, Enrichment, Hemoglobin, Separation factor

INTRODUCTION

The area of fluid-fluid systems has been well researched with various studies on transport processes and novel separation techniques being reported. The achievement of separation through foam fractionation, in general, can be understood by studying the transport in gas-liquid systems. The pioneering works were conducted by Chang *et al.*[1] wherein they confirmed the enhanced performance of the foam separation method for biological components. Lemlich [2] brings into perspective more such adsorption-based bubble separation methods. There are two essential features of foam that carry importance in its separation ability: large liquid-gas interfacial area and lowered liquid holdup (LH). From reviews conducted and reported, the LH is typically below 0.05. Grieves [3] concluded that a lower protein concentration and pH favor better separation by comparing the effect of different parameters on the enrichment factor S, (defined as the ratio of protein concentration in foam to that in the feed). No model for predicting the enrichment factor was reported until Beuker *et al.*[4] who also looked into the BSA enrichment with regard to continuous foam fractionation, which was comparable with the earlier works of Ahmad [5,6]. Ever since[1] first reported, many studies have been done on the subject of foam separation of various biomaterials.

A rather systematic approach of predicting the dynamic concentration of bioorganic materials through foam has been researched [7]. A bulk-interface equilibrium has conventionally been assumed[4], but Bhattacharjee *et al.*[8] demonstrated that, with different mass transfer rates of proteins and bubble absorption rates through the pool passages below the foam, it is quite unfeasible to achieve equilibrium. Beuker *et al.*[4] analyzed foam fractionation in the case of binary protein mixtures like casein–BSA (C-BSA), BSA–lysozyme (BSA-L) and casein–lysozyme (C-L). Different foamate rates and their influence on different protein combinations and concentrations were investigated. The foam stability [9] however, was enhanced by the addition of a greater quantity of lysozyme. Roselet *et al.*[10] first reported co-adsorption studies on lysozyme-casein, where it was noted that the individual protein component adsorption did not occur in a conventional fashion.

A study conducted by [5] predicted the adsorption kinetics of L-BSA at an air–water interface, and also that lysozyme adsorption was at a significantly slower rate in comparison with BSA. It was found that the surface coverage almost approaching equilibrium after 15 hours of adsorption time rendered a surface concentration measurement of 0.8 mg/ml for BSA, in comparison to 0.001 mg/ml for lysozyme, in a 1:1 BSA-lysozyme mixture with 1.5 mg/ml individual bulk concentration. The surface concentrations for experiments done with pure components were particularly interesting, on this note: 0.9 mg/ml for BSA and 0.6 mg/ml for lysozyme. Blesken *et al.* [11] used guided foam fractionation technique to enhance the production of rhamnolipid from hydrophilic carbon compounds by separation of the biocatalyst from bacterial matter with the help of a foam

fractionation column. In another study [11], made use of an external fractionation column for the purpose of production of biocatalysts, in a manner to upscale the surfactant separation process. Qin *et al.*[12] utilized form fractionation for the purpose of effective removal of copper from a disposed PCB of a PC computer. Sunkesula *et al.*[13] studied the effect of two key feed variables: initial concentration of the proteins and pH, on the recovery and selective enrichment of cheesy whey proteins, for which foam fractionation was utilized. Chen *et al.*[14,7] utilized milk as a source of immunoglobulin and enriched the concentration of immunoglobulin with the help of foam fractionation, whereas, De Rienzo *et al.*[15] studied the production and recovery of rhamnolipids from Pseudomonas aeruginosa ATCC 9207 and Burkholderia thailandensis E264 by means of form fractionation.

Xiao *et al.*[16] performed the extraction of saikosaponins by means of batch foam fractionation and obtained 77.2% recovery rate and 3.68 enrichment ratio under optimal process parameters. Yi *et al.* [17] produced surfactin from bacillus strains by performing repetitive batch fermentation processes by means of the fill-and-draw process and the foam fractionation column. Chen *et al.* [18] and Zhang *et al.*[19] utilized continuous foam fractionation technique to perform a study predicting the foam volume and surface tension of methyldiethanolamine. Ghosh *et al.*[20] and made use of ion foam fractionalization to achieve separation of tetra hexavalent Chromium (Cr(VI)) using the cationic surfactant cetyl trimethyl ammonium bromide (CTAB) [21]. The authors also developed a 3-stage ion foam fractionation column which demonstrated a Cr(VI) % removal efficiency > than 99.

Chen *et al.*[18] utilized foam fractionation to achieve continuous production of the biosurfactant surfactin by the use of glucose as the source of carbon. The rate of surfactin generation was found to be influenced by the dilution rate as well as the glucose feed concentration. Blesken *et al.*[22] managed the production of mono rhamnolipids and HAAs (3-3-hydroxy-alkanoyloxy alkanoic acids) by the usage of Escherichia coli as a host. It was concluded that the fatty acid dimer moeity profile in rhamnolipids and HAAs was mainly dependent on the specificity of [23] investigated the feasibility of using foam fractionation in order to separate total whey protein, as well as the single fractions. It was observed that at a pH level ranging from 2 to 3, a nearly complete enrichment of whey protein was possible [24]. With the addition of a surfactant [18],sodium dodecyl sulphate (SDS), a transfer of components of whey protein: albumin bovine, α-lactalbumin and β-lactoglobulin, into the foam fraction at enrichment ratios of about 30, and recovery rates ranging from 64.5% to 99.8%, was achieved. Xiao *et al.*[16] designed a foam fractionation system for the removal of Cu_2^+ from its aqueous solution. The optimal separation conditions were determined and the removal rate in these conditions was found to be 97.2%, the enrichment rate being 53.

MEASUREMENTS REQUIRED FOR EVALUATING SEPARATION FACTORS

There are two successive steps involved in the operation of the batch foam column:

- Generation of the foam upto the desired height by the passage of gas bubbles into the liquid pool.
- Draining of the foam

Step 1 consists of the adsorption of protein molecules at the gas-liquid interface of the bubbles as the bubbles rise through the liquid pool. The protein remains in a state of being adsorbed as bubbles combine with the foam. A negligible amount of liquid is simultaneously carried by the bubbles. Since the amount adsorbed in the foam is low [11] it can be assumed that the protein concentration in the pool bulk liquid does not undergo any change from the initial value.

The factor E (Enrichment or separation factor) is defined as the ratio of the concentration of a desired protein in the foam (C_p) to the concentration of the same protein in the bulk liquid mixture (C_b):

$$E = \frac{C_p}{C_b}$$

Thus, the quantity of each specific protein and volume of the liquid in the column needs to be observed after draining, in order to calculate the E factor. The adsorption equilibrium of the protein affects the amount of protein adsorbed and the adsorption rate. Thus, the equilibrium data (adsorption isotherm) through experiments should be made available in order for the modeling of the enrichment factor [25].

MATERIALS USED

The binary protein used in this work is Bovine Serum Albumin and Hemoglobin which were purchased from Hi media chemicals. The globular BSA has an isoelectric point of 4.7, whereas that of globular Hemoglobin is 6.8.

EXPERIMENTAL PROCEDURE

Bovine Serum Albumin and Hemoglobin of protein mixture were taken into the column, fabricated as given in Fig. 1. The initial liquid level was noted in the column. To attain the desired height, Nitrogen gas is passed. As soon as the foam attained the desired height, the gas supply was bypassed. The liquid, present in the foam, began to sink into the liquid pool. The level of the foam-liquid interface was measured against time function. After allowing the water to flow for some time,

the foam is separated from the remaining liquid and the foam-breaking liquid and the residual pool liquid were then analyzed to measure the protein concentration.

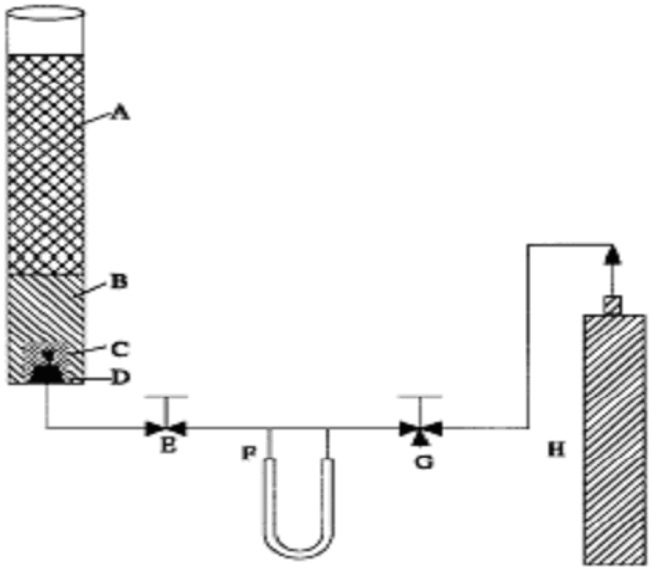
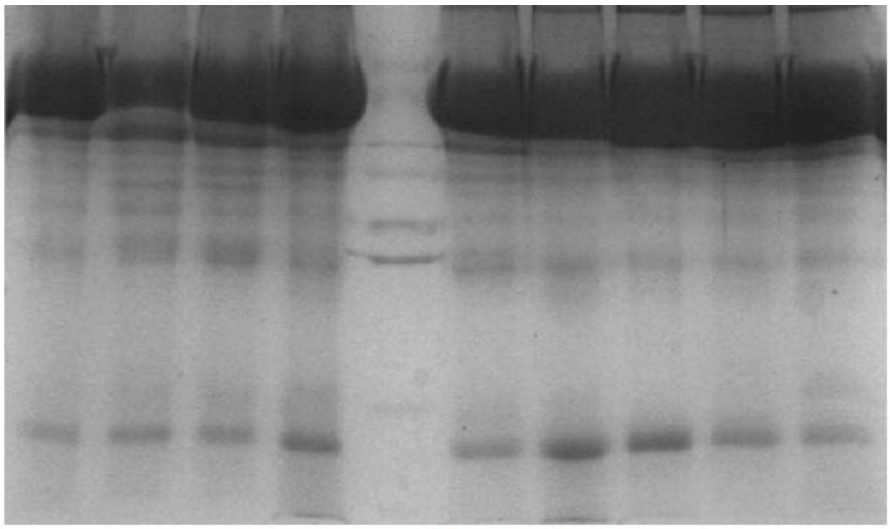


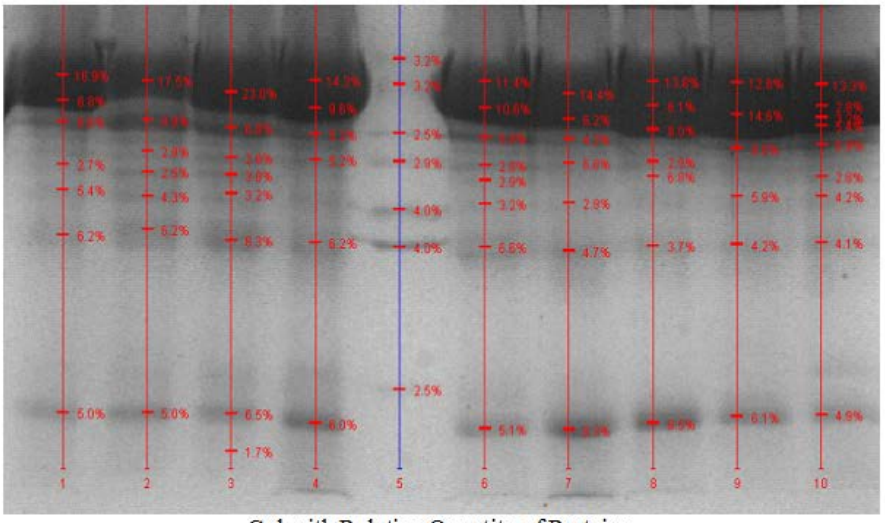
Fig. 1: Experimental Set up

CONCENTRATION IN PROTEIN MIXTURES

SDS-PAGE was utilized for the quantitative estimation of individual components in the protein mixture and is given in Fig. 2. The method involved transfer of the mixture through a gel containing cross-linked polyacrylamide. Each protein-SDS complex, with a definite mobility depending on its size and consequently, navigates in the gel matrix. Based on size differences as in Figure 2, the separation of protein mixture can be carried out. For the purpose of estimation, the deposited protein was stained with a Coomasie blue dye. Laser densitometry was utilized to decipher the precise amount of deposited protein.



Gel loaded with BSA and Hemoglobin



Gel with Relative Quantity of Proteins

Fig. 2: Gel loaded with BSA and Hemoglobin

RESULTS AND DISCUSSION

Examination of the single protein in the collection column by Foam separation showed that the adsorption rate played an important role. In addition, the concentration of excess protein in the pool affects the concentration of the right area in the bubble. Therefore, the effect of different combinations of concentration and depth of the lake has been investigated.

The following parameters are varied to show the effect of it on separation factor: concentration of the bulk solution, pH of the solution, height of the liquid pool, flow rate of air.

Effect of Concentration of Bulk Solution

The experimental study performed BSA concentrations at a maximum solution of 2 mg/ml while Hemoglobin concentration was 0.2 mg/ml and maintained at an air flow rate of 0.2 l/min. The pH of the bulk solution varies as 7.5, 6.5 and 5.5 to study the effect of pH on the separation of binary

proteins. The height of the liquid pool is adjusted from 5 cm to 20 cm and the separation factor is calculated. The results obtained from the studies are presented Fig. 3.

From the studies, it can be shown that as the liquid level of the liquid increases the amount of adsorbed proteins in the foam increases. This is because as the height increases, the duration of the bubble settlement in the liquid pool increases, which is why more advertising occurs in the bubble area.

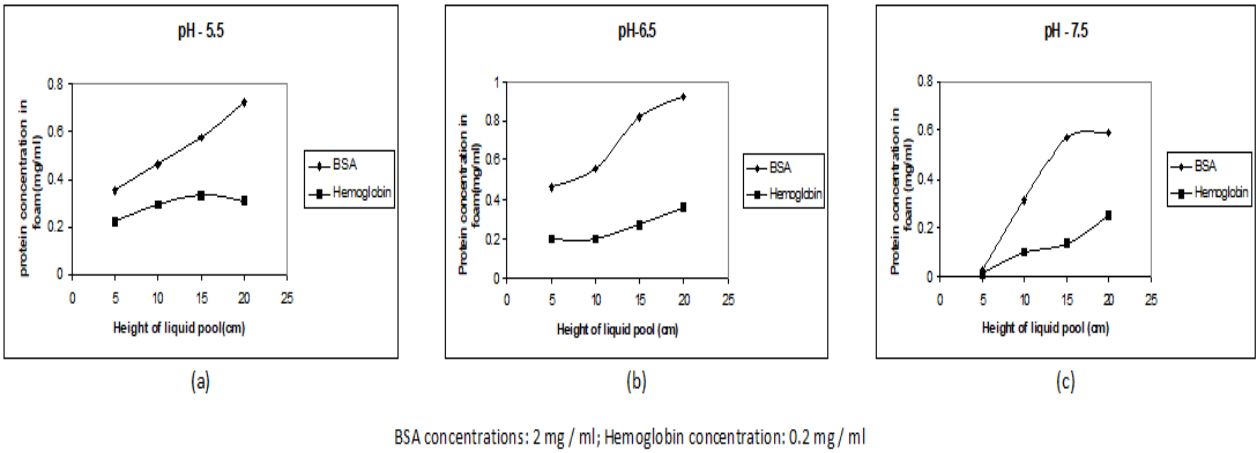


Fig. 3: Effect of concentrations on Liquid pool (BSA-2 mg/ml; HG-0.2 mg/ml)

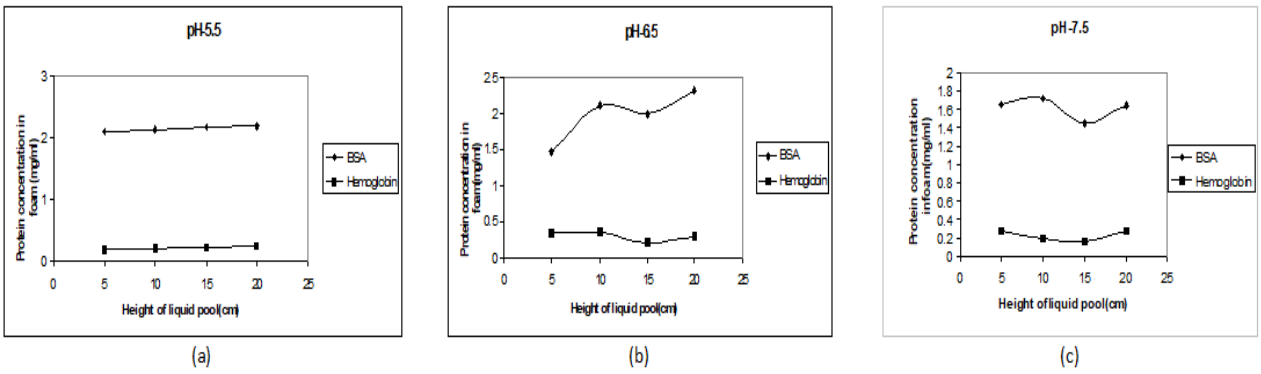
It can also be noted that, when the pH varies from 7.5 to 6.5 and 5.5, the differentiation factor increases, due to the increased hydrophobicity of the protein. The electrostatic repulsive energy and the attractive energy of the vander Waals interact between the adsorbed proteins in the optical fluid connector. The power of the vander Waals is very complex and facilitating conversation is focused on electrical energy. Excess charging of a protein molecule results in the separation of amino acid residues.

Electrostatic depletion between protein molecules advertised in the bubble area is considered to be very weak in this isoelectric region and the proteins are required to be strongly concentrated in the bubble area. Also, in an alkaline or acidic pH environment, proteins will not be attracted to each other due to strong electrostatic repulsion. From Fig. 3 it can be shown that the concentration of Hemoglobin foam has doubled to pH 6.5 for the reason mentioned above.

Effect of Concentration Variation of Hemoglobin on Separation Factor

The flow rate of the air is maintained at 0.2 l/min by using a Rotameter. The concentration of BSA in the bulk solution is kept at 2 mg/ml whereas the concentration of Hemoglobin is changed to 0.5 mg/ml. The pH of the initial feed is varied as 7.5, 6.5, and 5.5 to study the effect of it on the

separation of binary proteins. The height of the liquid pool is changed as 5 cm, 10 cm, 15 cm, 20 cm and the separation factor is represented in Fig. 4. It can be shown that the separation is very low in pH 7.5 and it is clearly understood that as Hemoglobin concentration is elevated in the base mixture, its advertising decreases but there is a simultaneous increase in BSA adsorption. This is because the occurrence of Hemoglobin in the bulk mixture helps BSA advertising in the bubble area. Also evident is that the concentration of BSA foam is elevated in the presence of Hemoglobin.

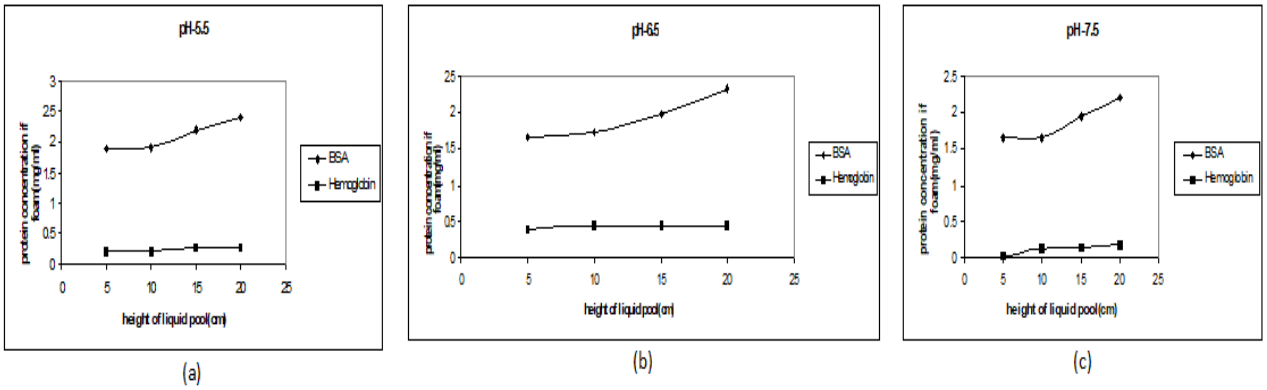


BSA concentrations: 2 mg / ml; Hemoglobin concentration: 0.5 mg / ml

Fig. 4: Effect of concentrations on Liquid pool (BSA-2 mg/ml; HG-0.5 mg/ml)

The concentration of BSA in the bulk solution is kept at 2 mg/ml whereas the concentration of Hemoglobin is changed to 0.8 mg/ml. The pH of the initial feed is varied as 7.5, 6.5, and 5.5 to determine the potential effect of it on the extent of separation of binary proteins. The height of the liquid pool is changed as 5 cm, 10 cm, 15 cm, 20 cm and the separation factor is calculated.

The flow rate of the air is maintained at 0.2 l/min by using a Rotameter. From Fig. 5 it can be shown that the separation factor is very less at pH 7.5. In this case also, the concentration of BSA increases in the foam as the amount of Hemoglobin in the feed is increased.



BSA concentrations: 2 mg / ml; Hemoglobin concentration: 0.8 mg / ml

Fig. 5: Effect of concentrations on Liquid pool (BSA-2 mg/ml; HG-0.8 mg/ml)

The concentration of BSA in the bulk solution is kept at 2 mg/ml whereas the concentration of Hemoglobin is further increased to 1.0 mg/ml. The pH of the initial feed is varied as 7.5, 6.5, and 5.5 and the effect of it on the separation of binary proteins were analyzed by varying the height of the liquid pool as 5 cm, 10 cm, 15 cm, 20 cm.

The flow rate of the air is maintained at 0.2 l/min by using a Rotameter. From Fig. 6, it can be shown that at a pool depth of 15 cm and 20 cm the adsorption of Hemoglobin in the foam is completely stopped. At pH 5.5 the concentration of BSA in the foam is raised to 3.75 mg/ml and Hemoglobin is completely absent.

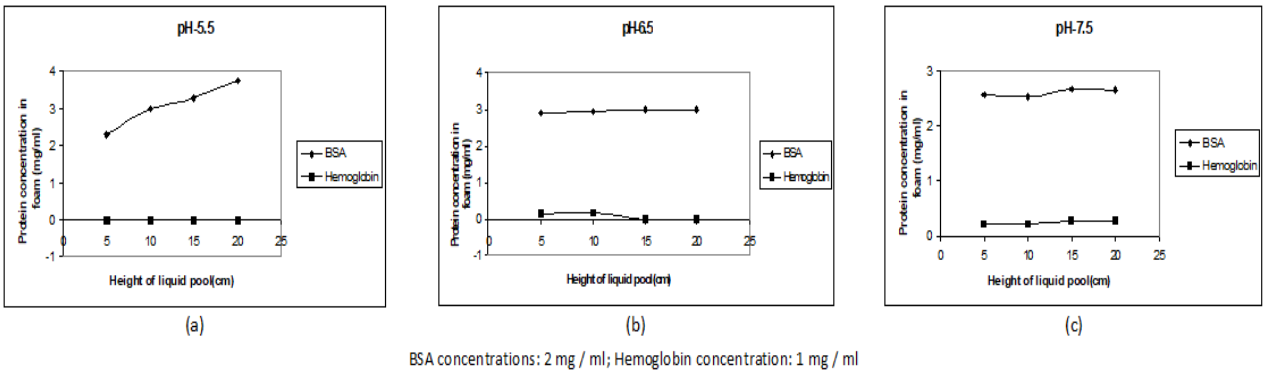
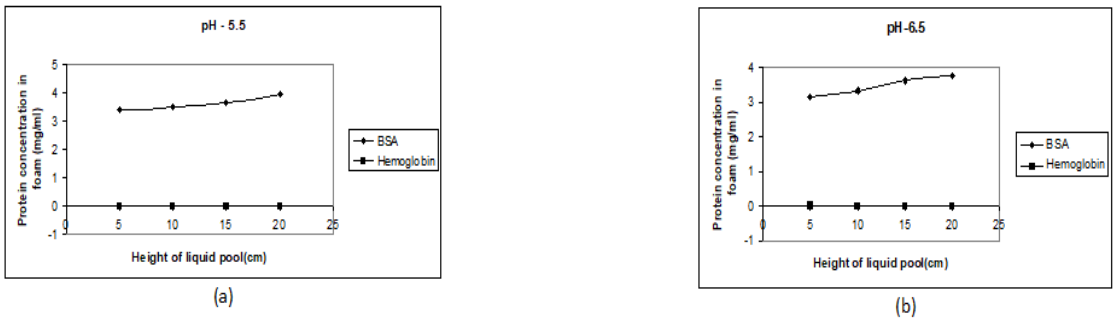


Fig. 6: Effect of concentrations on Liquid pool (BSA-2 mg/ml; HG-1 mg/ml)

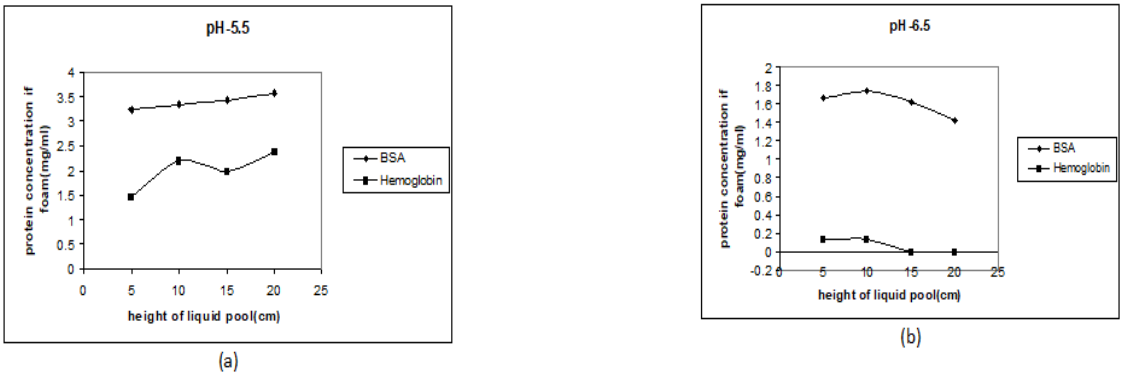
Effect of increase in Air Flow rate on Separation Factor

The flow rate of the air is increased from 0.2 l/min to 0.5 l/min by using a Rotameter. The concentration of BSA in the bulk solution is decreased to 1.8 mg/ml whereas the concentration of Hemoglobin is 1.0 mg/ml. The pH of the initial feed is varied as 6.5, 5.5 to study the effect of it on the separation of binary proteins. It is clearly seen that both the protein concentration in the foam decreases as the air flow rate is increased. This is due to the fact that as the air flow rate is increased the bubble residence time in the liquid pool decreases which results in less adsorption in the bubble surface.

From the Fig. 7, it can be shown that as the concentration of Bovine Serum Albumin in the bulk solution decreases, its adsorption on the foam layer increases due to increases in the surface tension. Since the surface tension is related with foam stability, as the surface tension decreases, foam will be unstable and hence the separation factor also decreases. At this condition the concentration of BSA in the foam is 3.9 mg/ml for a pool depth of 20 cm and the separation factor is 1.42. From Fig. 8, it is indicated that the separation factor is high at pH 5.5 at all protein concentration at a poll height of 20 cm.

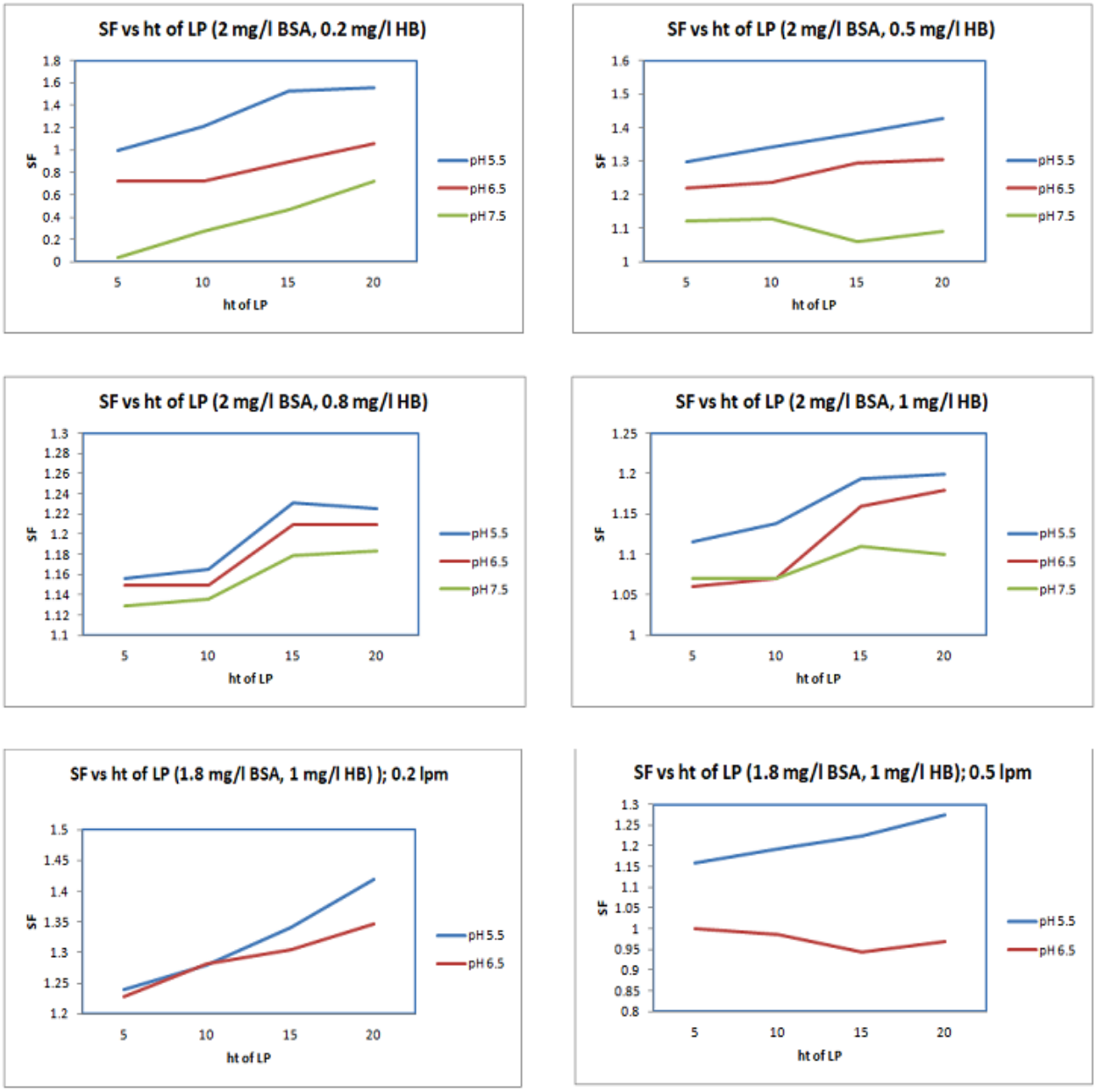


BSA concentrations: 1.8 mg / ml; Hemoglobin concentration: 1 mg / ml, Air flow rate: 0.2 lpm



BSA concentrations: 1.8 mg / ml; Hemoglobin concentration: 1 mg / ml, Air flow rate: 0.5 lpm

Fig. 7: Effect of Air flow rate on Separation Factor



Variation of Separation Factor (SF) with height (ht.) of Liquid Pool (LP)

Fig. 8: Effect of height of liquid pool on Separation Factor

CONCLUSION

Based on the studies conducted, an optimum pH of 5.5 was obtained as the value at which maximum separation occurs, which is attributed to an elevated hydrophobic character of proteins, particularly at the isoelectric point. In the bulk solution, a gradual increase in the hemoglobin concentration results in a significant increase in the amount of BSA adsorbed in the foam. This could be due to presence of hemoglobin, which could be attributed to the fact that the presence of one protein may help in the adsorption of another. With the Hemoglobin concentration approaching 1 mg/ml, there is a cessation of adsorption on the bubble surface. When the concentration of BSA is 1.8 mg/ml its concentration on the foam phase is doubled (i.e.) it reaches 3.97 mg/ml at a pool depth

of 20 cm. The optimum airflow rate is found to be 0.2 l/min. An enhanced recovery of binary protein (BSA and Hemoglobin) shall be obtained wherein only BSA is concentrated in the foam phase without any presence of Hemoglobin.

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Figure captions:

Fig. 1: Experimental Set Up

Fig. 2: Gel loaded with BSA and Hemoglobin

Fig. 3: Effect of concentrations on Liquid pool (BSA-2 mg/ml; HG-0.2 mg/ml)

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Fig. 7: Effect of Air flow rate on Separation Factor

Fig. 8: Effect of height of liquid pool on Separation Factor