Research Article

Quantification of BSA in therapeutic dairy waste enriched by foam fractionation

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Abstract

Objective: In this study, experiments were carried out to enrich medicinal proteins from dilute dairy waste by using anionic surfactant namely sodium dodecyl sulphate (SDS) as collector for separation of surface active proteins by adsorption on bubbles surface using foam fractionation method and quantified one single protein specifically bovine serum albumin(BSA) in enriched foamate by RP-HPLC. **Materials and Methods:** Dairy waste water collected from industry was processed and foam fractionated to foamate of concentrated proteins by using a 8cm. diameter and 100 cm. long glass column fitted with sintered glass G-3 sparger(15-40 micron porosity) used for distribution of bubbles generated by passing N_2 gas. Foamate were analysed spectrophotometrically as well as by RP-HPLC. The method was evaluated through experiments by varying process controlling parameters like pH, initial and ionic concentration of feed, gas flow rate through feed, waste-surfactant mass ratio in feed by taking 1litre of feed for every experiment. **Results:** The process was optimised at initial concentration (500µg/ml) , gas flow rate (350ml/min) ,ionic concentration(0.1M of NaCL), pH (5.5) and waste–surfactant mass ratio(1.5:1) with highest enrichment ratio (49.09) , percent recovery (98.18%) in foamate. BSA was quantified by RP-HPLC analysis as 4.9% (w/w) of enriched proteins in foamate at optimised condition. Mass transfer co-efficient was determined 12.68×10^{-9} mol/cal/cm^2/sec at pH 8.5, feed concentration of 600µg/ml and gas flow rate at 350ml/min. **Conclusion:** The study focused foam fractionation as lucrative unit operation to concentrate thermo labile medicinal proteins as well as eliminate pollutant proteins from dairy waste water.

Keywords: Foam fractionation; sodium dodecyl sulphate; enrichment; BSA; medicinal proteins; RP-HPLC

Introduction

International milk production is increasing every year more than 1% that approached approximately 800 tons in the year of 2017 (Bulletin IDF, 2017). Europe and South Asia were the chief milk producing area, about 350 tons of milk production (44% of overall milk production) till 2016. India will become the leading milk producing country for the coming year 2026 (UN-OECD, 2017). Under this circumstances, huge amount of dairy wastes are generated from various dairy industries because milk is transformed into a variety of dairy products such as milk, yoghurt, desserts and custards as well as self-life products namely cheese, butter, milk powders, etc. Dairy waste water from industrial process streams contains a variety of pharmaceutical bio molecules along with other compounds (Durham and Hourigan, 2007; Bulletin IDF, 2017; UN-OECD, 2017).

In this regard, application of low cost technique for co-product recovery from dairy waste has become of paramount importance in order to serve the dual purpose for controlling environmental pollution by eliminating pollutant from waste streams as well as recovery of medicinal and nutritional proteins for the benefit of health of man and animal kingdom. Now a day's some of the present applied techniques like ultrafiltration, nano-filtration, electro-dialysis, ion-exchange, gel-filtration, precipitation and coagulation are costly. Therefore, it needs to find out lucrative techniques for the benefit of coming days. Foam fractionation is one of the cheapest techniques with some advantages. It is under the purview of foam branch of "Adsorptive Bubble Separation Method" projected by Robert Lemlich in his edited book (Lemlich, 1972). The principle of separation of the technique

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is based on physical or chemical adsorption of surface active molecules on the gas-liquid interface (bubble's surface). The amount of surface active species adsorbed can be quantitatively expressed by Gibb's Equation of Adsorption Isotherm (Lemlich, 1972; Yuki and Chou, 1979).

Several researchers applied this technique in the field of pharmaceutical biotechnology for enrichment, purification and extraction of thermo labile medicinal proteins like digestive enzymes, natural antioxidants, thrombolytic streptokinase, polypeptide antibiotic-antiviral such as Nisin from explicit culture media and a variety of natural pharmaceuticals from plant extracts and bio sources such as, human placenta, rennin, catalase and industrial waste streams (Datta et al., 2015). This technique has also been successfully applied for removal of toxic heavy metals by metallic detoxification of industrial waste streams to control environmental pollution (Rubio and Tessele, 1997; Kim et al., 2001; Qu et al., 2008).

Foam fractionation provides various benefits over other methods, e.g., easy scale up, continuous operation, suitable for concentration and purification of thermo labile molecules like proteins in the biotechnological process pathway without application of heat, limited space, low power consumption and no extra cost of solvent and high output for dilute feed. Dairy waste water chiefly contains a multi-component mixture of medicinal proteins these are major proteins such as bovine serum albumin (BSA), alpha lactalbumin (α-LA), beta lactoglobulin (β-LG), immunoglobulin (IG) and minor proteins for instance bovine lactoferrin (BLF) and bovine lactoperoxidase (BLP) .The above proteins have wide medicinal role for a variety of disease such as immunity enhancer ,anti-cancer, anti caries ,anti-convalescent ,natural preservative as well as useful diet for AIDS patient (Durham et al., 1997; Jam and Van 2000; Giansanti et al., 2016; Kussendrager and Van, 2000) . Some of the proteins are also used in infant formula for nutritional value (Durham et al., 1997).The major and minor dairy proteins have iso electric pH (pI) approximately 5.5 and 9.0 respectively (Michel et al., 2002; Marshall, 1982). Proteins become neutral and undergo hydrophobic adsorption on the bubbles surface at isoelectric pH.

In this study, we evaluated the batch process of foam fractionation to enrich multi component medicinal proteins from dilute dairy waste. Additionally, HPLC analysis has been performed to find out the quantity of single protein (BSA) present in enriched multicomponent protein mixture obtained by foam fractionation.

Materials and Methods

Chemicals, Instrument and Apparatus

Dairy waste water was collected from local dairy industry (Kolkata).AR grade sodium dodecyl sulphate (SDS) and sodium chloride(NaCL) purchased from E. Merk Ltd. Standard protein namely bovine serum albumin (BSA) were purchased from Sigma Aldrich (USA).Acetonitrile, methanol, trifluoroacetic acid (HPLC grade), concentrated hydrochloric acid and sodium hydroxide were procured from E. Merck (Mumbai, India). All other solvents used were of analytical grade, procured from E. Merck.

The foam fractionation apparatus (figure 2) was purchased from local manufacturer (Kolkata). The acrylic rotameter was procured (50-500 cm^3/min) from Rivotek instruments, digital pH meter from Toshniwal instruments, centrifuge and foam breaker from Remi, Eyela Rotary Evaporator from Indosathi Scientific Lab and tensiometer by Deeksha Instrument Corporation (India), the spectrophotometer Shimadzu UV-1800 from Shimadzu Corporation(Japan).

Initial processing of dairy waste water

Dairy waste water was filtered through muslin cloth and centrifuged several times for removal of fat from the protein till constant absorbance was recorded and 250 ml of such processed dairy protein solution was evaporated at 45°C for 4 hrs by using Eyela Rotary Evaporator. The obtained dry protein mass (195 gms) was preserved in a refrigerator at -18°C until use.

Preparation of standard curve for total protein quantification

The protein mass was diluted in the concentration range of 50- 900 μg/ml in double distilled water and absorbance of each concentration was determined in a spectrophotometer at 280 nm to draw the standard curve of protein waste powder processed from dairy waste water.

Determination of critical micelle concentration and isoelectric pH of total and target protein by surface tension (γ) method at operating temperature 20- 25°C

Surface tensions of different concentrations (50-900 μg/ml) of processed protein waste and BSA (5-150μg/ml) in double distilled water were determined in a tensiometer by interfacial surface tension method to find out critical micelle concentration (CMC). The isoelectric pH of target protein (BSA) as well as dairy waste protein solution were determined at different pH by using 0.1(N) HCL and 0.1(N) NaOH below CMC through tensiometer (Noel et al., 2002).

Foam Fractionation

The experimental set up (Figure 1) consists of a glass column (100 cm long), internal diameter (8cm) and thickness (0.5cm) attached with nitrogen cylinder as the source of gas supply through a glass porous frit no. G_3 (15-40 micron porosity) fused at the base of the column. Gas bubbles generated by the sparger ascend through the

column and deposit as foam over the dilute feed. Foam moves through the column by gas pressure and finally deposits as foamate at the top outlet of the column. The dilute feed (1 litre) was prepared from processed dairy protein waste by adding SDS with mass ratio 1.5(waste):1(SDS) at different concentrations. 9 lots of experiments with each lot consisting of 3 experiments (total 27 experiments) were carried out at different pH of feed (2.5, 5.5 and 8.5), GFR (250, 300 and 350ml/min) and feed concentration (400,500and 600µg/ml) at ionic concentration by adding 0.1 gram mol of NaCL per litre of feed .The pH of dilute feed was adjusted with 0.1(M) of hydrochloric acid or sodium hydroxide solution. GFR was kept under observation by rotameter for every experiment. The gas flow rate, pH and initial concentration of feed were varied to find the impact on separation efficiency of total protein in foamate during every experiment. The weight and volume of foamate and foam breaking time were accurately calculated. Column was run for 1hr and foamate samples were collected from sample port at different time intervals (5, 10, 15, 25, 35, 45 and 55 minutes) for spectrophotometric analysis of total protein in foamate and RP-HPLC analysis of BSA.

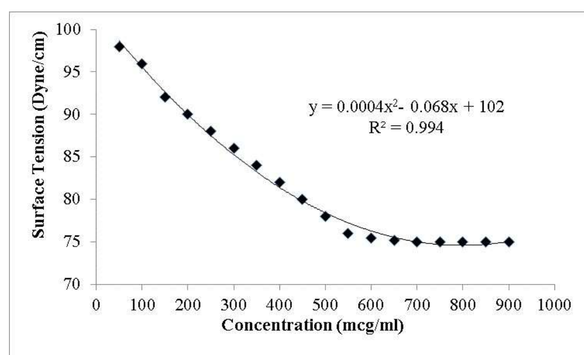

Figure 1. Plot of Surface Tension vs. Concentration of dairy protein waste Solution

Table1. Initial study of surface characteristics of dairy protein waste

Medicinal protein in dairy waste	Mol. wt. (Da*10^3)	Isoelectric pH(pI)	[dγ/dc] (dyne cm^2/µg)	Range of conc. (µg/ml) of constant slope	CMC (µg/ml)
BSA	69	5.1	-0.189	5-100	100
BLF	84	9.0	-------	------	-----
BLP	89	9.6	-------	------	-----
α-LA	14	5.3	-------	------	------
β-LG	18.30	4.8	-------	-----	------
IG	15	5.5	-------	------	------
Dairy protein waste	25.60	5.2	-0.329	50-750	750
Dairy protein waste +SDS (1.5:1,w/w)	-------	-------	-0.301	50-800	800

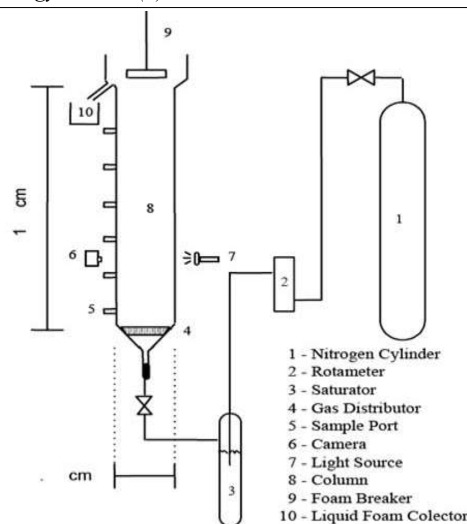

Figure 2. Experimental set up for foam fractionation

Quantification of Multicomponent Protein Mixture by UV Absorbance

The total protein concentration in foamate, feed and residue were quantified from standard curve by UV absorbance at 280 nm by using UV-1800 spectrophotometer (Saleh and Hossain, 2001).

Evaluation of performance

Efficiency of foam fractionation are governed by three parameters namely (i) enrichment ratio (E_R) is equal to the ratio of C_S/ C_I, where C_S is the concentration of protein in foamate and C_I is the initial concentration in feed, (ii) percent recovery (%Rp) calculated by [(A_{FM} / A_{FD})*100], where A_{FM} and A_{FD} are the total amount of protein (mg) in foamate and feed respectively (iii) separation ratio (S_R) is equal to the ratio of C_S/C_R, where C_R is expressed as concentration in residual solution. The highest values indicate the optimum efficiency of separation.

Studies on interfacial area of adsorption

Evaluation of available surface area of foam phase for adsorption is important to determine the mass transfer coefficient (K) which is the indicator of molar mass transfer to foam phase from dilute feed (Saleh and Hossain, 2001). The interfacial area is calculated by the following in equation 1.

$$As = \frac{6A_C H\varepsilon}{d_{32}} \text{---------------} (1)$$

Where H is the height of liquid feed in the column, A_c is the column cross sectional area (in this case 50.25cm^2), A_S is the interfacial area of foam phase for adsorption, ε is the void fraction determined from % gas hold up and $\overrightarrow{d_{32}}$ is bubble sauter mean diameter for individual location determined by the equation 2.

$$\overline{d_{32}} = \frac{\sum d^3}{\sum d^2} \text{-------------- (2)}$$

d_{32} is the bubble mean sauter diameter given by equation3,where (k) is the number of locations (in this case 4) of the column where bubbles photograph taken.

$$d_{32} = \frac{k}{\sum \frac{1}{d_{32}}} \text{------------- (3)}$$

A feed solution of 1litre at pH5.5 was taken in the column with concentration 500µg/ml of dairy waste with waste surfactant mass ratio (1.5:1 w/w). The ionic concentration of feed was adjusted by adding 0.1 gm mole of NaCL per litre. The gas was passed through the column at different flow rates (250,300,350ml/min).

As per the different flow rates as stated above, bubbles were photographed at 4 different locations namely 5, 15, 25 and 35 cm vertical distances from the sparger respectively by placing a Canon 450D Camera of 55mm focal length from a distance of 20cm. The photograph was developed in the computer and bubbles diameter was determined manually by super pixel scale after enlargement. 165-170 such bubbles were measured per plate and mean sauter bubble diameter (d_{32}) was calculated for respective flow rate and related data recorded in table 2. The percentage gas hold up was calculated for different superficial gas velocities in the liquid feed from the drop of liquid level by sudden stoppage of the gas supply. The maximum drop in level (ΔL) in each case was recorded and gas hold up fraction (ε) was determined by equation (4).

Table 2. Effect of superficial gas velocity on interfacial area

SGV (cm/s)	Gas flow Rate (ml/min)	Sauter mean Diameter d_{32} (cm)	%gas hold up (ε*100)	Interfacial area (cm²)	Percent Recovery (%Rp)	Feed density (gm/cc)	Feed Viscosity (Poise)
0.083	250	0.0621	0.90	845.09	90.98	1.235	0.0095
0.099	300	0.0705	1.19	1012.85	93.99		
0.116	350	0.0821	1.38	1117.29	98.18		

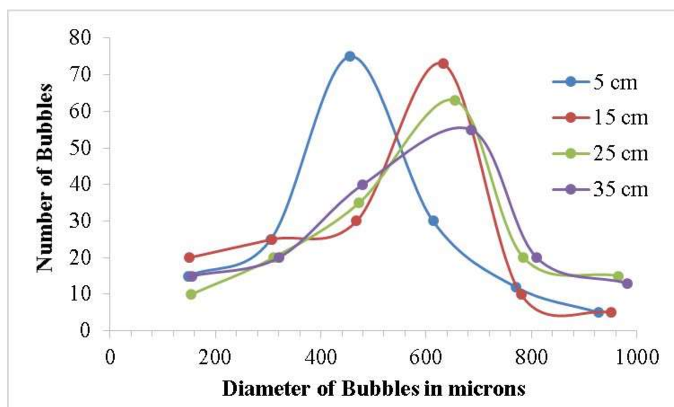

Figure 3. Bubble size distribution

$$\varepsilon = \frac{\Delta L}{L} \text{ ----------------------- (4)}$$

Where L is the initial level of liquid pool. Void fraction (ε) multiplied by 100 will give the percentage gas hold up. Mean sauter bubble diameters and gas hold up volumes were recorded in ascending order of gas flow rate. Data were represented in figure 3 and 4 and recorded in table 2.

Studies of effect of ionic concentration on gas hold up

The effect of ionic concentration in feed on gas hold up, enrichment ratio (E_R) and percent recovery (%Rp) was studied at a fixed GFR (250ml/min), initial concentration C_I (500µg/min), pH5.5 and waste surfactant ratio (1.5:1). Four different concentrations namely 0.0125,0.05 0.10and 0.15gm- mole of NaCL /litre of feed were chosen for the study and their impact of E_R and %Rp were recorded in table 3 and represented by figure 5.

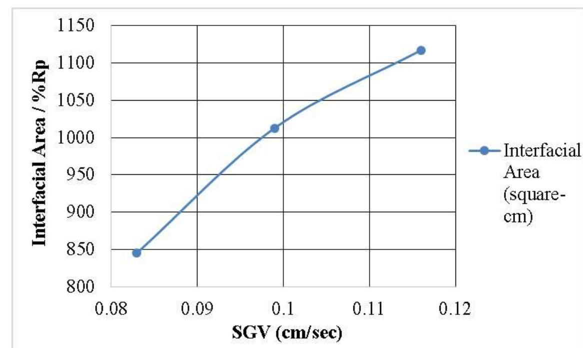

Figure 4. Effect of superficial gas velocity on Interfacial Area and %R_p

Table 3. Effect of ionic concentration on enrichment and percentage recovery

Molar Ionic Concentration	Percent gas hold up (ε*100)	Enrichment ratio (E_R)	Percent Recovery (%Rp)
0.0125	0.70	32.80	70.40
0.05	0.785	33.39	78.80
0.1	0.905	40.45	90.99
0.15	0.810	36.52	80.35

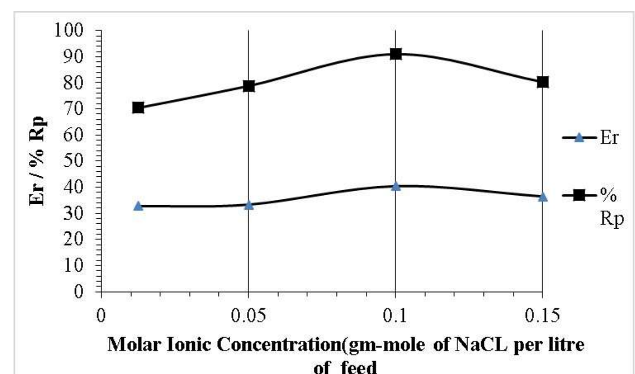

Figure 5. Effect of ionic concentration on E_R and % Rp

Theory of molar mass transfer to the foam phase

Molar adsorption of a surface active protein on bubbles surface from dilute feed can be quantitatively expressed by Gibb's equation of adsorption isotherm, τ=1/RT [dγ/dc]*C

Where, τ = quantity of surface active molecule adsorbed per unit area of bubble's surface [gm mole/ cm^2], T= operating temperature in Kelvin, C= concentration of molecule in feed (gm/cc) and distributing factor for adsorption. γ= surface tension of experimental molecule (dyne/cm), R= gas molar constant (8.3143 x 10^7ergs/°C/mole or 1.987 cal/°C/mole).

In Gibb,s equation, the negative slope [-dγ/dc] of γ vs. c curve indicates the surface tension of the molecule reduces with the increase of bulk protein concentration .The value of τ will be zero, when [dγ/dc] becomes zero and the curve is parallel to concentration-axis and this point on concentration axis is called critical micelle concentration(figure1) of the surface active molecules and γ-value will be least at this concentration. At dilution below CMC, surface tension on free surface active solute remains high and adsorption on the bubble's surface [gas-water interface] becomes high. At CMC, molecules form micelle between them and no free molecules remain in the solution to get adsorbed on the bubble's surface.

By application of material balance of the surface active protein in the two phase system (liquid and foam), we can determine experimentally the latent heat of desorption (λ) by equation (5) and mass transfer coefficient (K) by equation (6) respectively.

$$\ln\left[\frac{V_O}{V_B}\right] = \frac{1}{\left(e^{\lambda/RT} - 1\right)} \ln\left[\frac{C_O}{C_B}\right] \text{------------------} (5)$$

$$\int_{C_{So}}^{C_S} \frac{d(Cs)}{\left[\lambda - RT \ln \frac{C_S}{C_B}\right]} = K\left(\frac{A_S}{V_S}\right)\int_0^{\theta} d\theta = K\, As\theta / Vs \text{-----------} (6)$$

Where, V_0= initial bulk volume of liquid at zero time (θ=0) ,V_B= bulk volume of liquid after any time(θ),V_S= volume of liquid of foam phase at any time (θ),C_0 =concentration in bulk at time(θ=0),C_B= protein concentration in bulk after any time (θ),C_S=protein concentration in foam phase after any time (θ), K= mass transfer coefficient in mol/cal/cm^2/s, λ= latent heat of desorption in cal/mol can be determined from the slope of ln[V_0/V_B] vs. [ln C_0/C_B] plot(figure no 6), T(K) =absolute temperature, A_S= interfacial area in cm^2 , θ= residence time in of foam phase in relation to C_S inside the column obtained from the flow rate of bubbles and the height of the column. The left hand side integral can be determined by graphical integration from (λ-RT [ln C_S/ C_B])$^{-1}$vs. $C_S$plots initiating from $C_{S0}$to C_S to get the area under curve between two time intervals (θ_1 andθ_2). (As/Vs), the interfacial area per unit volume of foam phase in cm^{-1} may be computed from the size and number of bubbles in the feed at any time. The different graphically integrated values were plotted

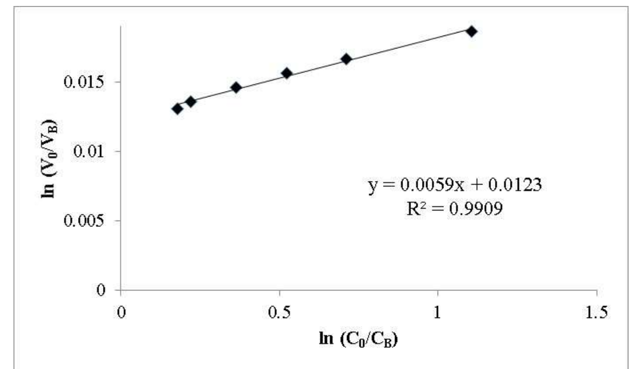

Figure 6. ln (V0/VB) vs. ln (C_0/C_B) Curve for (λ) determination

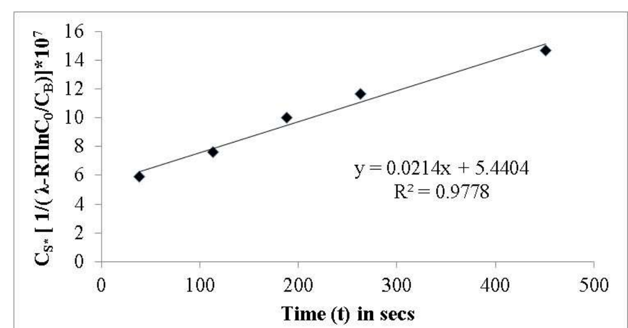

Figure 7.C_{S*} [1/ (λ-RTlnC_0/C_B)]*10^7 vs. time (t) plot for K determination

against different collection times (θ) (Figure 7) and the slope of the line was determined to measure the value of Kx(A_S/V_S). The thickness of foam phase [t] = [Kx(A_S/V_S)] was determined by Gibb's equation, ($e^{\lambda/RT}$-1) = (1/t RT) *(-dγ/dC) and t can be calculated from R, T, (-dγ/dC), λ and mol. wt. of protein. The mass transfer coefficient (K) was determined from known values of t and (A_S/V_S) by the relation, t=K (A_S/V_S) (Lemlich, 1972). In this study, the mass transfer co-efficient was computed on the basis of average molecular weight of total proteins (in this case, 25,600) calculated in according to the respective mass fraction of individual protein component multiplied by the respective molecular weight of processed dairy protein waste (Bovine serum albumin- 5%, β-lactoglobulin-50%, α-Lactalbumin-12%, Immnoglobulin-10%, Bovine lactoferrin-1%, Bovine lactoperoxidase- 0.5%.).

Quantification of bovine serum albumin in foamate by HPLC analysis

The HPLC system (Waters, MA, USA) was consisted of Symmetry 300 C_4 protein analysis column (50 × 4.6 mm; particle size 5 μm; pore size 300 Å) and equipped with a guard column. The temperature of the column was kept at 25°C. The analysis was consisted of a 600 controller pump, a multiple-wavelength ultraviolet-visible (UV-Vis) detector equipped

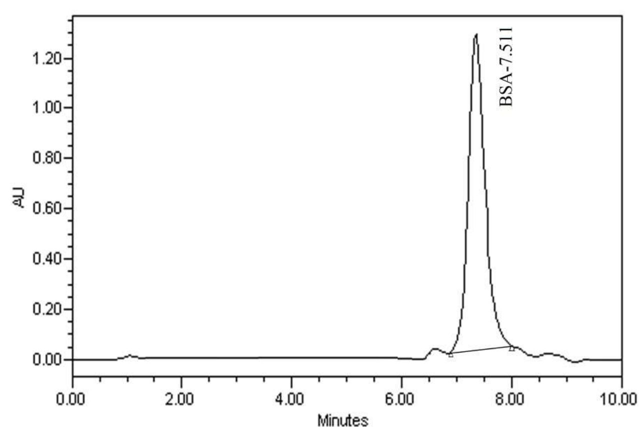

Figure 8. RP-HPLC standard chromatogram of BSA

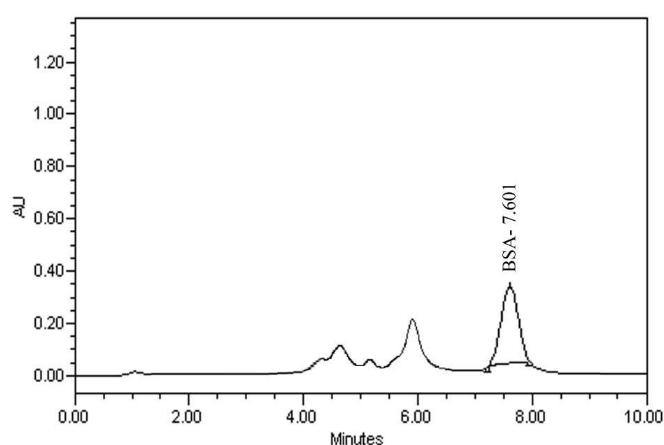

Figure 9. RP-HPLC chromatogram of BSA in enriched protein sample analysed from foam fractionation experiment

with 50 µl loop injector (Rheodyne±, Cotati, CA, USA). The outputs were processed and recorded in a compatible integrator (model 486, Waters, MA, USA).

HPLC assays were performed using a gradient system of 0.1% trifluoroacetic acid (TFA) in water (A) and 0.08% TFA in acetonitrile (B) with the ratio of 80:20 (v/v), which changed linearly to the final ratio of A and B was 35:65. The run time was set at 15 min. The Flow rate was set at 1.0 ml/min and the absorbance was detected at 220 nm. The sample solutions were prepared by taking 1mg of sample in 1 ml methanol. Respective chromatograms of standard and sample were represented in figure 8 and 9 respectively.

Results

Effect of pH at a fixed GFR of 350ml/min

Effect of pH of feed solution at a fixed GFR was recorded in table 5 and represented in figure 10, it was noted the maximum value of enrichment ratio (49.09) and percent recovery (98.18%) for total protein as well as BSA of 4.9%(w/w) in enriched protein of foamate at pH 5.5 . All data were found of the order of pH5.5>2.5>8.5 respectively.

Effect of GFR at a fixed pH 5.5 of feed

From table 4 and figure 11, it was found that enrichment ratio (ER) and percent recovery (%Rp) of total protein as well as the component BSA%(w/w) in foamate enhanced with the increase of GFR at fixed pH 5.5.From the experimental results, it was observed that the enrichment ratio(ER) and percent recovery (%Rp) increased when GFR changed gradually from the values of 250,300 and 350 ml/min respectively.

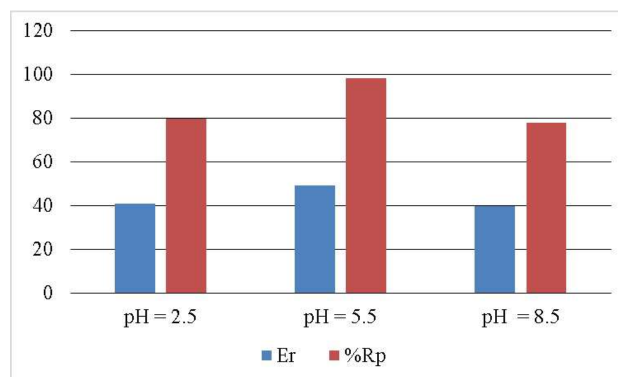

Figure 10. Effect of varying pH (2.5; 5.5 and 8.5) on E_r and $\%R_p$

Table 4. Notable experimental results of foam fractionation

Lot No.	Exp. No.	pH	Feed conc. (µg/ml) (C_I)	Gas Flow Rate (ml/min)	Concentration in foamate (CS) (µg/ml)	Enrichment ratio (E_R)	% Rp (Total protein)	(BSA) (% w/v)	Heat of desorption λ (cal/mol)
1	2	5.5	400	250	13478	33.70	77.50	3.88	2907
2	2	5.5	500	250	20898	34.84	91.95	4.60	2833
3	2	5.5	600	250	22295	37.16	81.75	4.09	2991
4	2	5.5	400	300	15087	37.72	86.75	4.35	3420
5	2	5.5	500	300	20207	40.41	92.95	4.65	2878
6	2	5.5	600	300	22595	37.65	82.85	4.14	3204
7	2	5.5	400	350	16718	41.80	91.85	4.59	3127
8	2	5.5	500	350	24545	49.09	98.18	4.90	3360
9	2	5.5	600	350	24585	40.98	83.75	4.10	3326

Effect of ionic concentration(I_C) on % gas hold up at a fixed GFR 250ml/min

From table 3 and figure 5, it was inspected the maximum adsorption at ionic concentration of 0.1(M) of NaCL for 1litre of feed at a fixed GFR. The indicators of adsorption(ER, %Rp) increase gradually from 0.0125(M) to 0.1(M) and then reduces at 0.15(M). The maximum % gas hold up was noted 0.905 at 0.1(M) of NaCL concentration in feed.

Effect of superficial gas velocity on% gas hold up

The effect of superficial gas velocity on % gas hold & % Rp were represented in figure 4. Gas hold up enhances linearly up to the superficial gas velocity (SGV) of 0.199 cm/sec. In the present study, SGV was maintained in the range of 0.0829 – 0.116 cm/s as shown in table 2. Percent recovery (% Rp) is enhanced with the increase of interfacial area.

Discussions

The surface tension of dairy protein waste solution was determined in the concentration range of 50-900 μg/ml and the experiment was repeated by adding SDS in the same concentration range with1.5:1 mass ratio (dairy waste protein : SDS). Result shows [dγ/dc] value (-0.0329 dynes /cm^2 / μg) more than that of dairy waste and SDS mixture solution (0.0301 dynes/ cm^2 /μg). So, from [dγ/dc] value (table1 & figure 1), it was concluded that CMC-value of dairy waste protein solution was unaffected by addition of SDS. Ekichi et al. (2005) have also indicated that optimum foaming process is achieved below 750 μg /ml with appropriate gas flow rate which will have positive effect on protein enrichment.

In foam fractionation experiment, pH plays vital role in

Table 5. Notable experimental results showing the effect of pH at GFR 350ml/min

Lot No.	Exp No	pH	Feed conc. (μg/ml) (C_1)	Gas flow rate (ml/min)	Concentration in foamate (CS) (μg/ml)	Enrichment ratio (E_R)	%Rp (Total protein)	(BSA) %(w/w)	Heat of desorption λ (cal/mol
8	1	2.5	500	350	20440	40.90	79.91	3.99	3330
8	2	5.5	500	350	24545	49.09	98.18	4.90	3360
8	3	8.5	500	350	19950	39.90	77.85	3.89	3270

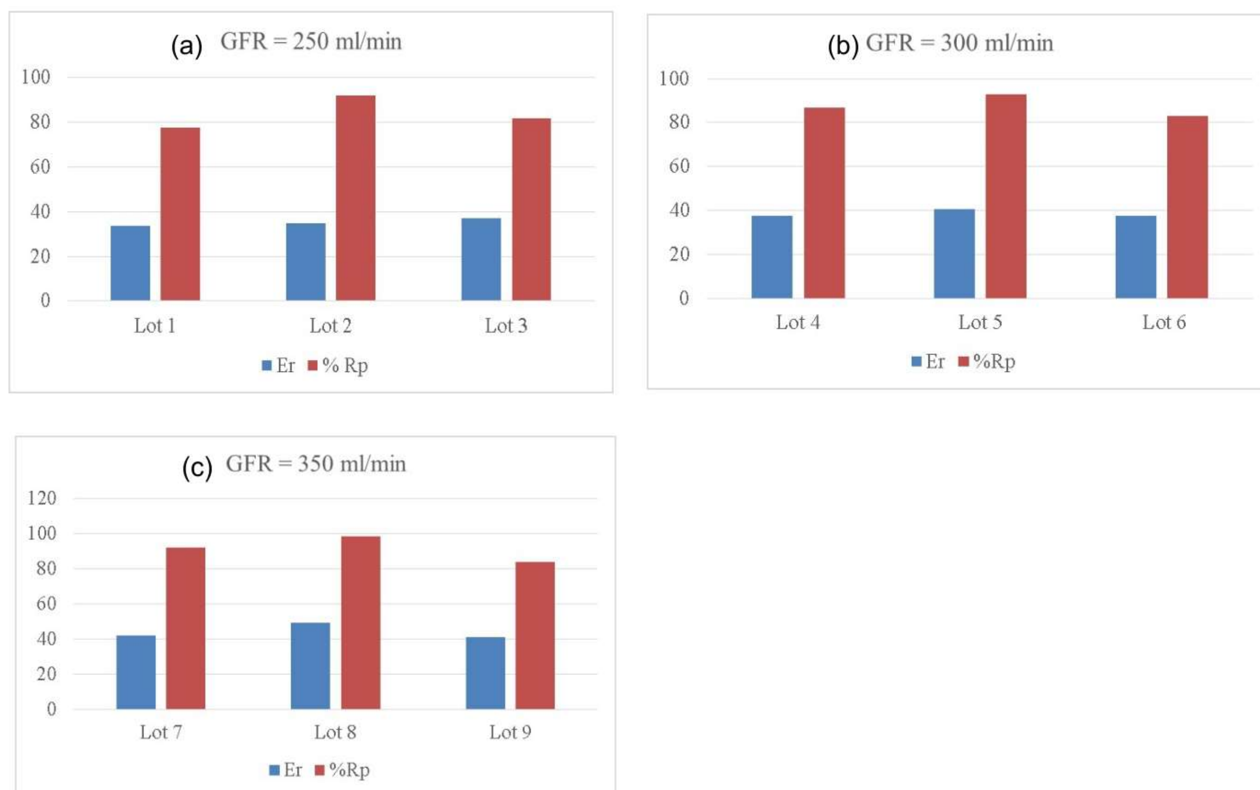

Figure 11. Effect of varying GFR [(a) 250, (b) 300 and (c) 350] ml/min on E_r and $\%R_p$ for different lots of experiment

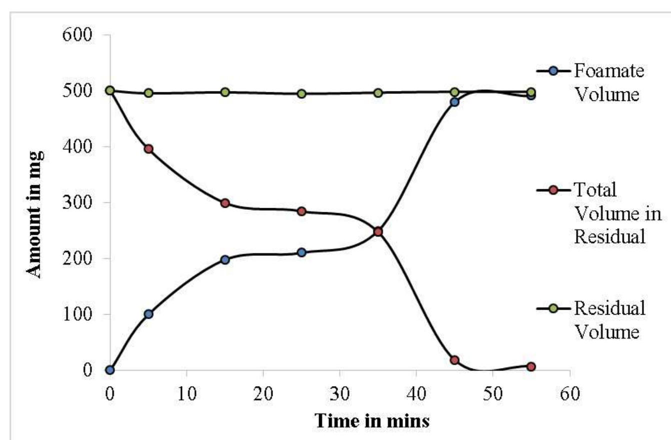

Figure 12. Material balance diagram of experiment no 2 of lot 8

controlling adsorption of protein at the gas-liquid interface (bubble's surface). Iso-electric points (IEP) of all the major dairy waste proteins are near about at pH 5.5 mentioned in Table1 (Michel et al., 2002; Marshall, 1982). Net charge on protein is zero at pH (=IEP) of protein, while pH < IEP and > IEP will create net positive and negative charges respectively on the protein molecules. For BSA as well as other major proteins at pH 2.5 (<IEP), negative head groups of SDS (anionic surfactant) are attached by columbic attraction with the positively charged regions of protein and form surfactant bridges between lamella (intra space of foam). This will enhance the foam's tackiness and rigidity which will resist the rising flow of liquid through lamella with less quantity of liquid cramped in the lamella of polyhedral foam causing reduction of enrichment and hindrance from recovery. The two minor heavy proteins BLF and BLP will behave cationic at pH 5.5 and form sturdy hydrophobic complex with anionic surfactant (SDS) and get adsorbed at pH5.5 (Fuda et al., 2004; Suzuki et al., 2002).It is rather difficult for hydrophobic adsorption at their IEP(=9) due to heaviness of these molecules. At pH 8.5(>IEP), BSA and other major proteins become anionic and columbic repulsion between proteins and SDS-protein complex molecules will repel each other. As a result, thickness and viscosity of film is less than that at pH 2.5. So, foam is quite wet at pH 8.5. Foam intrusion time and volume of foamable liquid directly proportional to enrichment and recovery is in the order of pH 5.5> pH 2.5> pH 8.5 (table 5 and figure 10).

The presence of inorganic ions (NaCL) enhances the gas hold up volume below critical concentration of NaCL [0.145 (M)] due to inhibition of coalescence between the bubbles and increases interfacial area available for adsorption by formation of micro bubbles (Besagni and Inzoli, 2015). Percent recovery (% Rp) is enhanced with the increase of interfacial area at a given concentration of feed & pH. This can be explained by the fact that the increase of gas velocity creates large number of bubbles with simultaneous enhancement of interfacial area for adsorption (table 2 and figure11). Distribution of bubbles was observed to be positively skewed as shown in figure 3 which indicates bubbles gets larger in size gradually from the distribution point. The SDS – protein complex may change the foam properties like width, flexibility, and solidity of the interfacial membrane and foaming ability of protein.

Total 9 lots of 27 experiments were done. Each lot consists of 3 experiments .Optimum performance criteria for each lot are tabulated in (Table 4). Performance of experiment no 2 of lot number 8 was observed best among all the experiments. Material balance was performed in each experiment. Amount of protein in foamate (M_S) and remaining liquid (M_R) and total mass (M_T=M_S+M_R) are plotted against the time of foaming (θ) for exp. no 8(2) (Figure 12). Linearity indicates the principle of material balance and least loss of material. Rate of removal and time for 50% removal ($t_{50\%}$) were obtained from the slope and point of intersection of curves. The heat of desorption (λ) was determined with help of slope of figure 7 and equation 6 and mass transfer co-efficient (K) was determined with the help of “t” (foam thickness)” determined from slope of the line in figure 7 (already mentioned in molar mass transfer section). Based on the experimental condition of exp no 2 of lot no 9, K was determined at the value of 12.686 * 10^{-9} gm mol cal^{-1} cm^{-2} s^{-1}.

The highest performance criteria [E_R = 49.09, % Rp = 98.18] was observed in lot No 8 of exp no 2 at pH 5.5, GFR = 350 ml/min, WSR = 1.5:1, I_C =0.1gram mole of NaCL /litre of feed.

Quantification of BSA by HPLC method

The mean R_t was observed for BSA at 7.51 ± 0.05 min by comparing between standard (figure 8) and dairy protein waste chromatogram (figure 9). The calibration range of BSA was found to be 100-1000 µg/ml, with the linear equation Y= 333402.75.X + 54304, with coefficient of determinants (R^2) of 0.996. The amount of BSA was found 4.9% (w/w) of enriched dairy protein of foamate 4.9% (w/w) at optimised condition of foam fractionation experiment.

Conclusion

It is noted that the foam fractionation is a useful unit operation to enhance concentration of medicinal proteins from dilute dairy waste as well as to eliminate pollutant proteins to certain level from dairy waste water to control environmental pollution. The method was found to have optimal effectiveness at initial concentration of 500µg/ml, gas flow rate 350ml/min, waste surfactant mass ratio 1.5:1 and ionic concentration 0.1gm-mole of NaCL per litre of

feed at pH5.5. Superficial gas velocity and ionic concentration enhance interfacial area for adsorption by increasing the size and number of micro bubbles. Mass transfer coefficient inspected in this study was to some extent high than that of earlier studies. The difference in value may be due to the impact of SDS and inorganic electrolyte (NaCL) resulting adsorption of high molecular weight proteins such as lactoferrin and lactoperoxidase. Evaluation of performance of experiment number 2 of lot 8 was found acceptable. The study focused foam fractionation as lucrative unit operation to concentrate thermo labile medicinal proteins as well as eliminate pollutant proteins from dairy waste water.

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Conflicts of interest

The authors do not have any conflicts of interest as of such with each other or any other publications either in print or published online.

References

Besagni G, Inzoli F. 2015. Influence of electrolyte concentration on hold up, flow regime transition and local flow properties in a large scale bubble column. Proceeding of the 33rd Italian Union of Thermo fluid–dynamics, Heat Transfer Conference, Journal of Physics: Conference Series 655,012039.

Bulletin IDF, International Diary Federation. 2017, Vol. 489.

Datta PK, Ghosh A, Chakraborty P, Gangopadhyay A. 2015. Foam fractionation in separation of pharmaceutical bio molecules: a promising unit operation for industrial process and waste control. Journal of Fundamental Pharmaceutical Research, 3(1):33-41.

Durham RJ, Hourigan JA, Sleigh RW, Johnson RL. 1997. Whey fractionation: wheying up the consequences. Food Australia, 49(10):460-465.

Durham RJ, Hourigan JA. 2007. Waste management and co-product recovery in diary processing. In: Waldron K (Eds), Handbook of Waste Management and Co-product Recovery in Food Processing, England, Woodland Pub. Ltd., 332-387.

Ekichi P, Backleh SM, Palar H. 2005. High efficiency enrichment of total and single whey proteins by pH controlled foam fractionation. International Journal of Food Sciences and Nutrition, 56(3): 223–229.

Fuda E, Jauregi P, Pyle DL. 2004. Recovery of lactoferrin and lactoperoxidase from sweet whey using colloidal gas aphrons (CGAs) generated from an anionic surfactant, AOT. Journal of Biotechnology Progress, 20(2):514-525.

Giansanti F, Panella G, Labolfe L, Antonini G. 2016. Lactoferrin from milk: nutraceutical and pharmacological properties'. Journal of Pharmaceuticals, 9(61):1-15.

Jam MS, Van Hooijdonk ACM. 2000. Occurrence, structure, biochemical properties and technological characteristics of lactoferrin. British Journal of Nutrition, 84 (Suppl. 1): S 11 – S17.

Kim YS, Choi YS, Lee YL. 2001. Determination of zinc and lead by solvent sublation using ion-pair. Bulletin, Korean Chemical Society.

Kussendrager KD, Van Hooijdonk ACM. 2000. Lactoperoxidase: physico-chemical properties, occurrence, mechanism of action and applications. British Journal of Nutrition, 84(Suppl. 1):S19-S25.

Lemlich R. 1972. Adsorptive Bubble Separation Techniques .In (Eds), Academic Press, New York, Elsevier, 159-173.

Marshall KR. 1982. Industrial isolation of milk proteins: whey proteins. In Fox PF (Eds), Development in Diary Chemistry, Proteins, Chap. 11, Applied Science Publishers, London.

Michel MJ, Pouliot Y, Britten M, Noel I, Lebrun R. 2002. Study on the molecular mass changes of bovine k-casein glycomacropeptide and on its separation in ultra-filtration with and without application of electric fields. Journal of Diary Science, 85, Suppl. 1(Abstract).

Noel J, Prokop A, Tanner RD. 2002. Foam fractionation of a dilute solution of bovine lactoferrin. Applied Biochemistry and Biotechnology, 98-100(2):395-402.

Qu YH, Zeng GM, Huang JH, Xu KE, Fang YY, Lee XUE, Liu LH. 2008. Recovery of surfactant SDS and Cd^{2+} from permeate in MEUF using a continuous foam fractionator ,Science Direct, Journal of Hazardous Materials,155(1-2):32-38.

Rubio J, Tessele F. 1997. Removal of heavy metal ions by an adsorptive particulate floatation. Journal of Mineral Engineering, Elsevier Science Ltd., Great Britain, 10(7):671-679.

Saleh ZS, Hossain Md M. 2001. A study of the separation of proteins from multi-component mixtures by a semi-batch foaming process. Chemical Engineering and Processing, Elsevier Publishers Ltd., 40:371-378.

Suzuki A, Yasuhara HS, Maruyama H. 2002. Selective foam separation of binary proteins solution by SDS complexation method. Journal of Colloidal Interface Sciences, 253:402-408.

UN-OECD (Organization for Economic Cooperation and

Development). 2017. Special Report.

Yuki YO, Chou J. 1979. Foam Fractionation Process, McGraw Hill Publishers, London.