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Protein Removal from Whey Waste by Foam Fractionation in a Batch Process

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The work investigates the separation of proteins from whey waste collected from a local confectionery by the foam fractionation technique in batch mode. The purpose of this work was to evaluate performance criteria of protein separation. The effects of pH, the concentration of initial feed solution, the nitrogen flow rate, the % gas hold up, the bubble diameter, the breaking time of foam, and the optimization of the protein–surfactant ratio (1.5:1) were investigated in detail. Maximum enrichment ratio (48.189), %Rp (96.378) were observed at a gas flow rate of 330 ml/min and pH 5 that is closest to isoelectric point of observed proteins (Bovine serum albumin, β-Lactoglobulin, α-lactalbumin).

Keywords breaking time; foam fractionation; pH; whey waste

INTRODUCTION

Whey was considered previously as a waste product of the cheese industry. Later potentiality of whey proteins had been recognized by the dairy industry. Today, the demand for whey protein, whey protein hydrolysate as sports drinks, and as a disease preventing agent (antihypertensive), has been increasing in developed countries. Therefore, a large-scale enrichment and purification of whey protein at low cost becomes the necessity for the dairy industry not only for the utilization of its nutrition value but also to reduce waste stream BOD (1,2). Most of the current techniques (ultrafiltration, gelfiltration, ion exchange, precipitation, and coagulation) used in the separation of dissolved/dispersed materials from aqueous stream are tedious, expensive, and complicated. So far, the large-scale manufacturing units of whey proteins utilize membrane filtration techniques to supply more than one lakh tons per year of whey protein concentrates (WPC) and isolates (WPI). Foam fractionation is an alternative method with an economical and ecological point of interest. Though foaming operation may cause the unfolding of the structure of proteins but it can be reversed by some other techniques (3).

Nowadays, functional and biological properties of individual whey proteins have become the focus of commercial interest. The whey proteins differ widely in their functional properties (solubility, gelation properties, dispersibility, water holding capacity, stability, adhesion, emulsification properties, film formation, foaming properties, organoleptic properties, viscosity, binding properties, etc.). The functionality of protein is basically dependent on the molecular structure. Factors that cause variability in functional properties of whey proteins are the source of whey, season-dependent variations of its components, and the processing steps under different conditions such as pH, ionic strength, heat treatments, and the presence of minerals. α-lactalbumin has good emulsifying properties, but its gelation ability is poor. Instead, β-lactoglobulin has excellent gelling and foaming properties.Whey protein concentrate has been reported to have poor foaming stability, emulsifying properties at low concentration. So, foaming of whey is made possible with the use of surface active agents in a foam fractionation experiment (4,5).

Charged balanced protein at isoelectric point (IEP) becomes hydrophobic in nature that favors its adsorption at the interface. The packing of the molecule at the interface determines the foamability and the stability of foam. The pH of the solution primarily effects bubble size, interfacial tension, and secondarily foam stability, rigidity of the surface (6), and adsorption. The foaming property, rigidity of whey protein concentrate (WPC) calcium may be attributed to the formation of a ca – bridge between the carboxyl groups of protein molecules at the interface and to the reduction in electrostatic repulsion between the adsorbed layer of proteins and other adsorbing protein molecules. Rigidity of the interface, and foaming intensity have been correlated with the power consumption of a different mechanical foam breaker. It is a factor that determines enrichment and the % recovery of protein. Researchers investigated foam fractionation of pure proteins (Bovine serum albumin, β-lactoglobulin, α-lactalbumin, casein, α-lactoferrin,

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lysozyme) in a synthetic feed to see the effect of pH, and the gas flow rate (7,8). Bramaud et al. (9) showed that α-la undergoes isoelectric precipitation (pH 4.2) due to the dissociation of calcium ions and hydrophobic interactions at 55–65°C. Some investigators chose a pair of model proteins with a wide gap in IEP to facilitate the separation of the protein in complexation with the surface active agent (7). Lysozyme forms a hydrophobic complex with sodium dodecyl sulfate at low pH and gets adsorbed more at the interface in comparison to ovalbumin. Adsorption of lysozyme-SDS follows the Langmuir isotherm. Kamalesh and Anand (5) established the mechanism of the adsorption of BSA and lysozyme from a synthetic feed. Bulk phase molecules cannot exchange adsorbed molecules of their own due to their difference in chemical potential. Structural changes in the protein molecule upon adsorption had been suggested by A K Brown (10). Zaid S. Saleh et al. (11) observed a preferential separation of a mixture BSA and α-lactalbumin while maximum part of α-lactoferrin were left in the residual feed. Other important parameters determined by them are the mass transfer coefficient, heat of desorption of BSA, effects of operating variables in a semi-batch foaming process. Very few works have so far been reported on whole whey foam fractionation (9). Enhanced enrichment of proteins as a whole was observed with increasing concentration of SDS at low pH (2) other than IEP. L. Du reported a similar observation (12).

The aim of the present work was to separate proteins as a whole by SDS complexation from commercial whey waste obtained from the local confectionery. This was performed by the foam fractionation technique in batch mode. It is an age old technique. But its application is still continuing. Its feasibility at various operating conditions had been elucidated. The role of pH on the performance characteristics was the prime objective here. In the present work, the breaking time of foam was studied in relation to pH.

EXPERIMENTAL

Materials, Instruments, Equipment

Whey was supplied by a local confectionery, and Sodium dodecyl sulfate (SDS) was obtained from Loba (India). Double distilled water was prepared at the laboratory. The instruments used were UV spectrophotometer (UV 1700 Shimadzu), pH meter (satorius), Centrifuge (remi), and Foam fractionation apparatus which was supplied by a local glass fabricator.

Quantification of Whey Protein

The protein fraction is determined by spectrophotometric analysis at wavelength 280 nm. The standard curve is prepared by whey protein powder and that is prepared by evaporating the aqueous part of the solvent from treated whey under a controlled condition in a BOD.

Treatment of Whey

Raw and fresh whey was collected from a local confectionery. It was filtered through a cheese cloth. The filtrate was centrifuged and filtered several times until the absorbance becomes constant at a wavelength of 280 nm. Thus fat was removed. Fat has a detrimental effect on foaming property. Film rigidity decreases when fat competes with protein molecules. It was then diluted as per requirement and the concentration was checked by a spectrophotometer. The operating temperature was maintained at 25°C.

Determination of Surface Tension (γ) of Experimental Liquid

In any study of surface phenomenon, it is essential to know the critical micelle concentration of the surface active agent, surface excess concentration, $d\gamma/dc$. Surface tension was measured by the interfacial surface tension method (14) in a tensiometer (Jencon, Du nouy). After treatment the whey was diluted in a range of concentration at particular pH. Since the present foaming experiment was performed with the help of Sodium lauryl Sulfate (SDS), so the surface tension of whey along with SDS was measured. The data was calculated and tabulated in Table 1. Isoelectric pH was determined by plotting the surface tension against pH. The operating temperature was maintained at 25°C.

TABLE 1
Characteristic of proteins

Protein	Mol. wt.	IEP	$d\gamma/dc$ (dyne cm^2 per μg)	Concentration range (μg/ml) of constant slope	CMC, μg/ml at IEP	Ki, cm	$\tau i * 10^{-10}$, mole/cm^2 at cmc
BSA	69000	4.8	−0.603	5–24	24	1.646	5.72
α-Lactalbumin	14200	5.1	−0.674	10–30	30	0.3786	7.99
β-Lactoglobulin	37200	5.3	−0.517	10–31	31	0.7610	6.34
Whey	25600	4.7	−0.0359	100–700	700	0.0319	9.979
Whey + SLS (1.5:1)	–	–	−0.0291	1166–1666	{700(whey) + 466(SDS)}	–	–

Foam Fractionation

The experimental set up (Fig. 1) consisted of a glass column, nitrogen cylinder, humidifier (glass set), air flow meter, foam receiver, and stirrer. The glass column was designed and fabricated by a local fabricator. It was of 1 meter length having an internal diameter of 8 cm. A porous glass sparger (frit no. 3, pore size 16–40 micron) was fitted on the top of a small glass tube and that was attached at the bottom of the column by a standard joint. Feed was prepared by diluting treated whey with post addition of SDS. Optimization of the protein**:** surfactant ratio has been performed at particular condition (pH 5, gas flow rate 290 ml/min, Ci = 500 μg/ml). Concentration of protein in the foamate and the amount of protein recovered from aqueous feed were determined and the effect of the protein: surfactant ratio on the performance criteria of foam fractionation had been shown in Fig. 2. The pH of the feed had been fixed at various levels by adding either hydrochloric acid or sodium hydroxide solution. The column was filled upto a certain height by 1 liter of feed. Nitrogen gas was humidified to prevent loss of solvent at the outlet. The gas flow rate was monitored by an air flow meter. The gas flow rate (superficial gas velocity) and the pH of the feed were varied at different levels to observe their effects on performance criteria. Gas bubbles were generated by a sparger and ascend through liquid feed and then transformed to foam when it left the liquid column. The foam moved upward and escaped through the outlet. It was collected and collapsed by a foam breaker, and a mechanical stirrer. The weight of the foamate and its breaking time was carefully noted. Samples were withdrawn at intervals from the sample port. Samples were analyzed by a spectrophotometer. Data were presented in Table 5 as the average of the three experimental results.

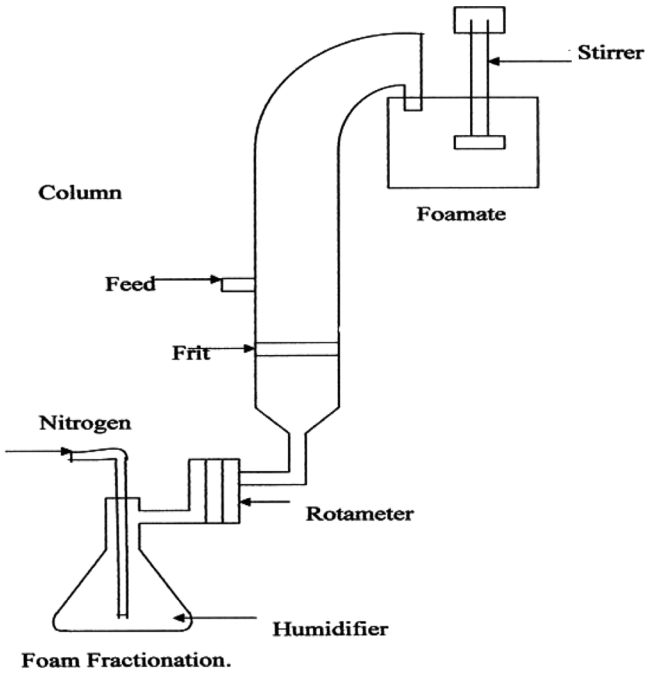

FIG. 1. Experimental setup of foam fractionation method.

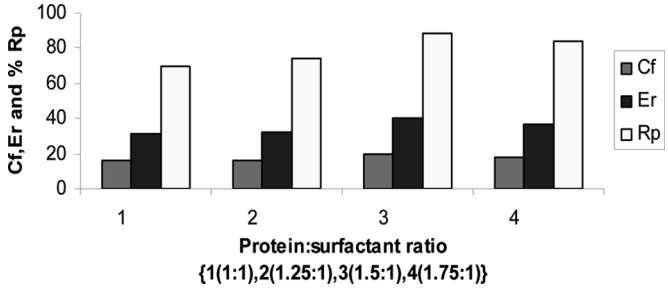

FIG. 2. Performance characteristic of protein surfactant ratio.

Determination of Gas Hold Up

The percent gas hold up was measured in a batch of liquid when gas passed through the column. The height of the liquid pool was measured. Again, the height of the liquid pool was measured after shutting off the gas flow. The percentage of the gas bubble entrapped in a liquid column was calculated and tabulated as % gas hold up (Table 2). Percent gas hold up was plotted against the superficial gas velocity (SGV) in Fig. 3. The operating temperature was maintained at 25°C.

Determination of Bubble Diameter

A Cannon 450D Camera with 15–55 mm lens and a shutter speed of 1/30 (focal length 55 mm) was used for photography of the bubbles from a distance of 15 cm. The illumination was provided by two 250 W lamps located 30 cm away from the column. There was a 135° angle between the camera and each light lamp. A light diffuser was used to provide indirect light in the column. There was a mega pixel scale on the outside surface of the column for determining the bubble size. Bubbles were photographed at different locations of the column such as 5, 10, 20, and 30 cm from the frit. The pictures were developed into a computer, where they were enlarged and the bubble diameter was measured manually with an actual mega pixel scale.

Around 160–163 bubbles were measured per plate. The average sauter diameter (d_{32}) was calculated. The operating conditions were: Ci 500 mcg/ml, PDR 1.5, pH 5, temperature 25°C.

$$d_{32} = \frac{k}{\sum \frac{1}{d_{32}}} \qquad \overline{d_{32}} = \frac{\sum d^3}{\sum d^2} \qquad A = \frac{6AH_L\varepsilon}{\overline{d_{32}}}$$

d_{32} is the sauter diameter (6), k is the number of section (4), $\overline{d_{32}}$ is the average sauter diameter, A is the interfacial area,

TABLE 2
Effect of interfacial area of bubble on % Recovery, at initial concentration, 500 mcg/ml and pH of 5, temperature of $25 \pm 2^{\circ}C$

SGV, cm/s	Gas flow rate, ml/min	% gas hold up	Sauter d_{32} cm	Interfacial area sq.cm.	Density of feed, gm/cc	Viscosity of feed, Poise	% Rp
0.0828	250	1.000	0.0664	903.61	1.225	0.00946	88.99
0.0961	290	1.140	0.069	991.3			91.99
0.1094	330	1.285	0.0702	1098.29			96.38

Akira Suzuki and his co workers (14) used the following equations for the calculation of surface phase, S_b (cm^2/s) and diameter (d_b, cm) of bubble.

$S_b = 6A^{*}\varepsilon(1-\varepsilon)4.65\{(4/225)(\rho_L-\rho g)^2 g^2/\mu_L\rho_L\}^{1/3}$

$d_b = Ug/\varepsilon(1-\varepsilon)4.65\{(4/225)(\rho_L-\rho g)^2 g^2/\mu_L\rho_L\}^{1/3}$

A' is the cross sectional area of column and, ε is the void fraction of bubble in liquid column.

TABLE 3
Calculation of total surface area of bubble

S_b, cm^2/s	d_b, cm	Residence time, s	Total surface area, cm^2
373.70	0.0668	2.40	897.91
423.23	0.0685	2.36	998.83
473.82	0.0696	2.337	1107.21

ρ_L and ρg are the densities of liquid and gas at operating condition, g is the acceleration due to gravity. In this work, residence time (θ') of bubble within liquid is obtained from superficial gas flow rate (SGV) and height of liquid. Same value of SGV is assumed for liquid filled column. Using the above equations, total area of surface face at a particular residence time is calculated by multiplying S_b with θ'. d_b is also calculated. These values are close to that (Interfacial area, sauter diameter) obtained from the above table.

H_L is the height of liquid within the column, ε is the void fraction of bubble obtained from % gas hold up (see Tables 2, 3 and Fig. 4).

Study of Breaking Time of Foam

The breaking time of foam was performed to assess the relative strength of the foam film at various conditions. The foam volume of 500 ml was collected and then it was broken mechanically by a stirrer at various speeds. Breaking time and the weight of the collapsed foam (foamate) were noted and tabulated in Table 4 and shown in Fig. 5. The operation was performed at 25°C.

Theory of Mass Transfer

Adsorption of a protein at the interface can be expressed by Gibb's equation.

TABLE 4
Breaking time of foamate θ (min), gas flow rate (290 ml/min), Ci (400–600 mcg/ml) Volume of foamate 500 ml

Operating condition	θ in min at rpm 150	θ in min at rpm 450	θ in min at rpm 900	Foamate wt, mg
Ci400, pH 2	10	4	1.2	300
Ci400, pH 5	14	6	2	380
Ci400, pH 8	12	5	1.5	330
Ci500, pH 2	12	6	2	330
Ci500, pH 5	16	8	4	400
Ci500, pH 8	13	7	3	360
Ci600, pH 2	15	10	5	340
Ci600, pH 5	20	12	8	410
Ci600, pH 8	17	11	6	370

TABLE 5
Foam fractionation in batch process

Exp. no	Ci, Feed conc.^{n} μg/ml	pH	Gas flow rate cm^3/min	Cf μg/ml	Amount in foamate mg	Time at 50% removal min	Rate of removal mg/min	Er (= Ci/Cf)	%Rp	heat of desorption λ cal/mole
1(1)	400	2	250	11814	259.92	35	4.13	29.54	64.98	2738
1(2)	400	5	250	13302	305.94	33	4.78	33.26	76.49	2868
1(3)	400	8	250	10779	247.92	38	4.06	26.95	61.98	2578
2(1)	500	2	250	15907	349.95	35	5.66	31.81	69.99	2815
2(2)	500	5	250	20224	444.94	32.4	6.84	40.45	88.99	2803
2(3)	500	8	250	14769	324.93	37.2	5.27	29.54	64.99	2706
3(1)	600	2	250	18932	425.97	35.1	7.34	31.55	71.00	2855
3(2)	600	5	250	21544	473.98	33.66	7.85	35.91	79.00	2890
3(3)	600	8	250	18259	419.96	36.14	7.22	30.43	69.99	2890
4(1)	400	2	290	17439	294.8	33.64	4.86	36.85	73.70	3327
4(2)	400	5	290	15634	343.96	31.43	5.37	39.09	85.99	3390
4(3)	400	8	290	13307	292.75	34.4	4.73	33.27	73.19	3125
5(1)	500	2	290	16814	369.92	34.25	5.95	33.63	73.98	2869
5(2)	500	5	290	19997	459.93	30.1	7.4	39.99	91.99	2848
5(3)	500	8	290	17142	359.98	34.69	5.85	34.28	72.00	2815
6(1)	600	2	290	20452	449.94	34.32	7.59	34.09	74.99	2966
6(2)	600	5	290	21817	479.97	33.83	7.98	36.36	80.00	3094
6(3)	600	8	290	19677	432.89	36.52	7.1	32.80	72.15	2919
7(1)	400	2	330	14474	303.96	34.43	4.83	36.19	75.99	3000
7(2)	400	5	330	16742	259.95	30.79	5.66	41.86	89.99	3064
7(3)	400	8	330	14534	312.49	31.91	5.1	36.34	78.12	3104
8(1)	500	2	330	20438	398.55	33.61	6.31	40.88	79.71	3326
8(2)	500	5	330	24094	481.89	26.33	7.99	48.19	96.38	3350
8(3)	500	8	330	19939	388.82	33.61	6.15	39.88	77.76	3269
9(1)	600	2	330	23091	461.82	34.52	7.4	38.49	76.97	3230
9(2)	600	5	330	23989	479.8	34.23	7.57	39.98	79.97	3256
9(3)	600	8	330	22572	451.44	34.78	7.29	37.62	75.24	3181

Feed-1 liter; Whey: surfactant = 1.5; Foaming time, 55 min; Temperature-25 ± 2°C.

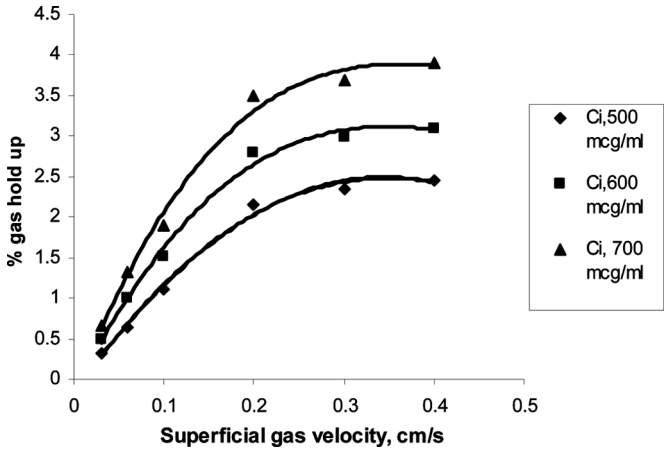

FIG. 3. Representation of % gas hold up at different superficial gas velocities.

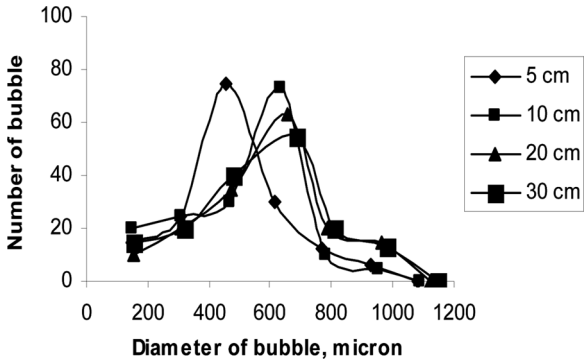

FIG. 4. Representation of bubble size with bubble numbers.

$\tau = -\frac{1}{RT}\frac{d\gamma}{d\ln c}$, here τ (gmmole/cm^2) is the surface excess concentration at equilibrium state, γ (dyne /cm) is the surface tension, c is the concentration, R is the universal gas constant ($8.3143 * 10^7$ erg per degree per mole or 1.987 cal per degree per mole). T is the absolute temperature.

At low temperature τ varies with c linearly and the corresponding equation is $\tau = k_i c$, here k_i is the equilibrium constant. The difference between the chemical potential of the protein at the interface and bulk phases acts as a driving force for the movement of protein molecules from bulk to interface. At zero time there is no concentration gradient between the surface and the bulk phases but the driving force is at a maximum and at equilibrium though the concentration gradient is there but the driving force is zero. Applying Gibb's equation to a two-component system (protein, water) an equation relating the thickness (t, cm) of the surface face and surface tension is developed that is later coupled with the mass balance equation in batch foaming. Mathematical analysis of batch foaming is based on mass balance. The heat of desorption (λ) can be determined from the following equation. Mass transfer coefficient, K (moles/cal/cm^2/s) is determined by a graphical integration of this equation.

Considering foaming of the differential volume of the liquid an equation is obtained after integration.

$$\ln\frac{Vo}{V} = \frac{1}{(e^{\lambda/RT} - 1)}\ln\left[\frac{(C)B_0}{(C)B}\right]$$

V and CB are the volume and the concentration of the bulk phase at time t respectively. 0 denotes zero time.

The mass transfer coefficient (K) was determined by a method as described in the "Adsorptive bubble separation techniques by Robert Lemlich (p. 161–173) (14).

$\int_{Cf0}^{Cf} dCf/(\lambda - RT\ln Cf/CB) = K(A/Vf)\int_0^{\theta} d\theta$ was derived from the mass balance equation.

CB, Cf, Cf0 are the bulk liquid concentration, concentration of surface adsorbed protein (foamate) at any time and zero time respectively and Vf is the volume of the surface adsorbed protein (foamate). R and T are the universal gas constant and absolute temperature, respectively. A/Vf is the surface area per unit volume in the surface phase (reciprocal of thickness (t) of the surface phase). θ is the residence time of the surface phase within the column. It can be determined by the flow rate of the bubble and the height of the column. In the present study, a foamate residence time or collection time was used. The above equation was integrated graphically by plotting $1/(\lambda\text{-RTlnCf/CB})$ against Cf (see Fig. 7) The resulted graphically integrated values were plotted against the residence time (θ) (see Fig. 8). K (A/Vf) was obtained from the slope of the linear curve. The heat of desorption may be related to the surface tension by the Gibbs equation.

$(1/tRT)[-d\gamma/(dc_B)] + 1 = e^{\lambda/RT}$. Here γ is the surface tension of the experimental liquid. Thus A/Vf (=1/t) was calculated by this equation. Finally, the mass transfer coefficient, K was calculated. The average molecular weight of proteins as per respective fraction of individual proteins (Bovine serum albumin −5%, β-Lactoglobulin-50%, α-lactalbumin-25%) was used in this calculation.

RESULTS AND DISCUSSIONS

In the present experiment pH plays a pivotal role in controlling the adsorption of protein at the interface. Since the isoelectric points (IEP) of the selected whey proteins (BSA, β-lg,α-la) are very close to pH 5, so all three will be adsorbed appreciably at this pH. Table 1 shows the preliminary surface properties of different proteins. The surface tension of whey is determined in the concentration range of 100–800 μg/ml and the same experiment is repeated by adding SDS to each sample of the whey (100–800 μg/ml) at a ratio of 1.5:1 (protein:SDS). The total concentration in the samples of protein plus SDS varies from 166 to 1666 μg/ml. Whey (100–800 μg/ml) showed higher value of $d\gamma/dc$ (0.0359 dynes cm^2/μg) in comparison to whey with SDS (0.0198 dynes cm^2/μg) at the ratio of 1.5:1. Critical micelle concentration (CMC) of whey was found at 700 μg/ml and CMC of whey with SDS was found at a total concentration of 1166 (g/ml μ = 700 μg/ml of whey +466 μg/ml of SDS). Therefore, the CMC of whey was not affected by SDS at the optimum ratio. Apparently it seems that CMC is raised, but this is not true. CMC of whey gives a guideline in fixing the upper limit range of concentration of whey and SDS. In the present study the concentration range of whey was 400–600 μg/ml that is below its CMC. Table 1 listed the calculated values of the surface excess concentration (τ) of different proteins, that varies with the molecular weight of protein. A sufficient quantity of SDS was added as a collector of proteins. Use of excess quantity of SDS causes uncontrollable foam and wastage. In Fig. 2 the effect of protein: surfactant upon the performance criteria had been exhibited. The ratio of 1.5:1 was found to be the best. This optimized value was used for further experiments. Adsorption of protein is dependant upon the pH of the solution, the interfacial area of the bubble, and the residence time of the bubble. The Interfacial area is another important factor that depends on the sparger type, gas velocity, and gas hold up capacity of the liquid (void fraction, ε), property of liquid and gas. In the present study a particular type of ceramic sparger (frit size 3, 16–40 micron pore size) was used. The effect of gas velocity and concentration of feed on % gas hold up had been shown in Fig. 3. Gas hold up (%) increased linearly upto a superficial gas velocity (SGV) value of 0.125 cm/s. In this experiment SGV was maintained within 0.0828–0.1094 cm/s. In Table 2, Rp (% recovery of protein) increased with the increase of interfacial area. Increase of gas hold up with the increase of gas velocity generated a larger number of bubbles, so there

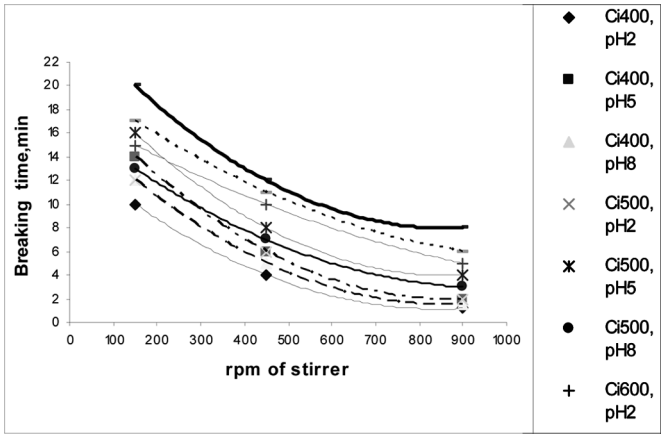

FIG. 5. Representation of breaking time at different rpm.

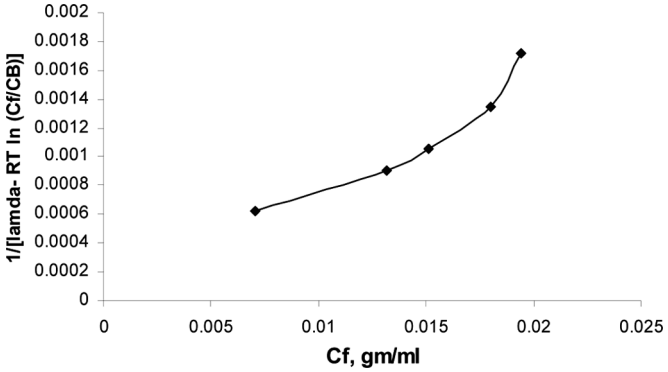

FIG. 7. Representation of graphical integration.

was an increase in the interfacial area. The average sauter diameter of the bubble was calculated as described earlier. This value and the interfacial area are in good agreement with that calculated by the equations. The calculated data were shown in Table 3. Distribution of bubble size varied with the distance of the moving bubble from the point of generation (sparger). Bubbles were smaller near the frit and became larger at a higher height due to coalescence. (see Fig. 4). Distribution of the bubble is skewed to the right. Mean is influenced by a few large-size bubbles. Here the median, and the mode are in the positive side of mean. Such distribution was found to be positive skewed. Table 4 and Fig. 5 displayed the effects of rpm of stirrer and pH of solution upon breaking time of foam at different conditions. It is obvious that the breaking time decreased with the increase of rpm and decrease of concentration of the feed. The weight of the foamate and the breaking time were highest at pH 5 for each concentration of the feed. Interaction between SDS and protein is generally utilized in the formulation of the protein/surfactant mixture to induce the stability of foam and emulsion. The SDS-protein complex may alter the properties like thickness, viscoelasticity, density of the interfacial membrane and foamability of protein. Sometimes proteins get unfolded when adsorbed at the interface. The formation of the SDS-protein complex may be attributed to its electrostatic interaction that binds negatively charged head groups of SDS with any positively charged groups on protein surfaces. While the non-polar tail of the surfactant molecule may also interact with the non-polar region on the exposed surfaces of the protein molecules. Structure of protein is very complicated and shows peculiarity in aqueous solution at different pH. The net charge on protein is negative, zero, and positive when the pH is above, equal to, and below IEP respectively. Below IEP (pH 2) the negative head groups of SDS are attached with the positively charged regions of the protein surfaces, and this decreases electrostatic repulsion between them and there is the possibility of the formation of surfactant bridges between bubbles, within the lamella of foam. This adherence or aggregation leads to an increase in viscosity. It is expected that the film strength will be

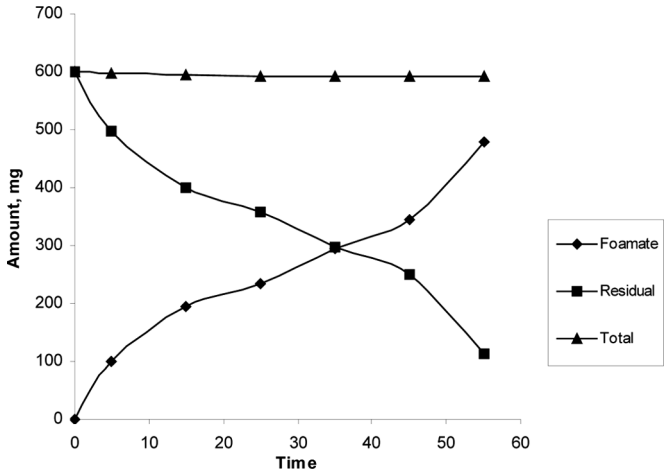

FIG. 6. Representation of mass balance at 9(2) condition.

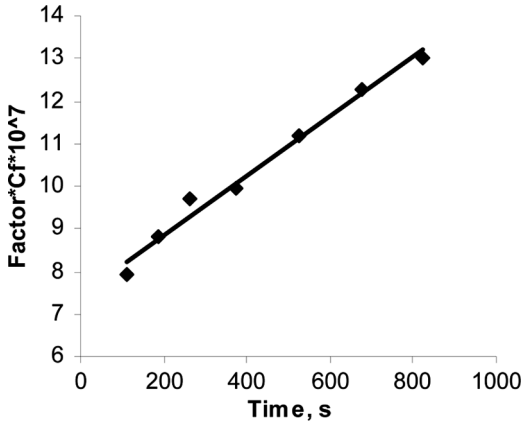

FIG. 8. Representation of mass transfer coefficient.

enhanced. This resists the upward flow of liquid through foam and less quantity of liquid is entrained in the channels of polyhedral foam. This causes reduction in enrichment and percent recovery (see Table 5). At IEP (pH 5), charges on protein is totally balanced and the protein molecule adsorbed more at the gas bubble interface due to hydrophobic nature. In this case, the strength of the interface film is not enhanced due to the absence of the protein–surfactant complex at the interface. Therefore there is a possibility of more entrainment of liquid within the channels of polyhedral foam. From Table 4, it is evident that the collapsed foam contains more liquid and it takes more time to break foam into foamate when pH is 5. When pH is 8 (above IEP) the breaking time of the foam and the liquid content of the foamate are greater than that of pH 2. Above IEP, the net negative charge increases when SDS binds with the protein and the electrostatic repulsion between the protein-SDS complex molecules repel others. So, the thickness and the viscosity of film will be less than that of pH 2. So, foam is moister at pH 8. Breaking time and the liquid content of the foamate is in the order of pH 5 > pH 8 > pH 2.

Foam fractionation of whey was performed at various conditions: like concentration of feed (400–600 μg/ml), gas flow rate (250–330 ml/min) and pH (2–8). A total of 27 experiments carried out at different conditions were shown in Table 5. Samples were collected at various time intervals (5, 15, 25, 35, 45, and 55 min).Time intervals were not included in Table 5. Each experiment was performed in triplicate. The average value of data had been tabulated. Ci was the initial concentration of protein in feed and Cf is the concentration of protein in foamate. Er (enrichment ratio) = Cf/Ci, %Rp (100 * Amount of protein in foamate/amount of protein in feed) is the percentage of recovery or removal of protein from aqueous feed. The performance criteria of this method were tabulated in Table 5. Performance of exp.8(2) was quite satisfactory.

Mass balance was carried out in each experiment. The amount of protein mass in foamate (M_f), residual liquid (Mr) and total mass ($= M_f + Mr$) were plotted against the time of operation (Fig. 6). The profile of the total quantity presented almost a linear horizontal line which indicates material loss is least and the total quantity balances with the initial amount of protein. The rate of removal and time for 50% removal were obtained from the slope and the point of intersection of curves. λ, the heat of desorption was calculated with the help of the equation as mentioned earlier.

The performance criteria such as the enrichment ratio Er and %Rp increase with concentration of feed and volumetric flowrate of gas within selected range of pH. Highest Er and % Rp were found at pH 5. Proteins become comparatively more hydrophobic at pH 5 and were absorbed most at the interface (Er-48.19, %Rp-96.38) compared to pH, 2 and 8.

The mass transfer coefficient was calculated with the data of experiment 9(3). Concentration of protein in the foamate (Cf) and the residual liquid (C_B) collected at various intervals of time were used to calculate the factor, $1/\{\lambda\text{-RTln}\ (Cf/C_B)\}$. This factor was plotted against Cf as shown in Fig. 7. The area of the plot covered data corresponding to time 15 min. Now graphically integrated values of $Cf/\{\lambda\text{-RTln}\ (Cf/C_B)\}$ was plotted against time in Fig. 8. The thickness (t) of the surface phase was calculated with the help of the equation as mentioned in the method. The mass transfer coefficient, K was calculated with the help of t and slope of the line in Fig. 8. Based on one experimental condition {9(3)}, the value of K is found as $6.07 * 10^{-9}$ gm moles/s cal. sq cm. This value is condition specific. $d\delta/dc$ of whey was used as 0.0359 dyne $cm^2/\mu g$ and the operating temperature was 25°C.

CONCLUSION

It is observed that the foam fractionation is a useful method either to concentrate proteins from dilute solution of whey or to decrease the protein level of a waste solution. The method was found to be more effective at initial concentration of whey of 500 μg/ml and gas flow rate of 290–300 ml/min at pH 5.0. Increase of superficial gas velocity caused increase in bubble diameter and generation of more number of bubbles. This resulted in the increase in the interfacial area and adsorption of protein. Study of mass balance assured no significant loss due to operational fault or any protein degradation. Mass transfer coefficient as observed here was comparable with earlier studies that varied from 10^{-8} to 10^{-12} gm moles/s cal. sq cm depending upon SGV, column dimension and the nature of the colligent. Breaking time of foam was related to the pH of the feed solution, and the presence of the ionic surfactant. Performance of exp.no. 8 (2) was quite satisfactory. Optimization of data adopting a suitable model may provide more precise operating conditions for the maximum recovery of protein.

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NOMENCLATURE

Ci	Initial Feed Concentration (μg/ml)
Cr	Residual concentration (μg/ml)
Cf	Foamate concentration (μg/ml)
Er	Enrichment ratio (Cf/Ci)
Sr	Separation ration (Cf/Cr)
%Rp	Percentage recovery
PDR	Protein Drug ratio
IEP	Isoelectric Point
WPC	Whey protein concentration (μg/ml)
SDS	Sodium dodecyle sulphate
BSA	Bovine serum albumin

d_{32}	Sauter diameter (cm)
A	Interfacial area (sq cm)
ε	Void fraction
d_{32}^{-}	Average Sauter diameter (cm)
γ	Surface tension (dyne/cm)
K	Mass transfer coefficient (gm moles/s cal. sq cm)
t	Thickness (cm)
θ	Time (min)

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