ORIGINAL RESEARCH PAPER

Pharmaceutical

SEPARATION OF MAJOR WHEY PROTEIN BY BATCH ABSM METHOD

KEY WORDS: Foam Fractionation, Enrichment Ratio, Bovine Serum Albumin, Batch Mode.

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ABSTRACT

The foam fractionation is very fast, simple, effective process for separation of chemical compound and recovery of waste materials from aqueous solution and can be treated as pre-concentration method in their analytical determination. In the food industry the waste materials needed for the reuse purpose because of the conservation policy of the various aqueous product is taken by the authority. The treatment of whey waste as Bovine serum albumin by foam fractionation technique in batch mode is dealing with the recovery of whey protein from the aqueous product in the food industry very effectively. The research is concerns the fundamental recovery of whey waste by describe in the process. Result found that at fixed Ci 500mcg/ml, pH 3.8, GFR 200, Fomate volume 300ml, the highest enrichment found 10 in 15 min at PSR 1.25. At higher pH and lower GFR the Enrichment found 9 (highest). At 150 GFR, Ci500, pH5.8,PSR 1 FV300, the enrichment ratio is 8, which is lowest. The research is concerns the fundamental recovery of BSA from whey waste through adsorption chemistry.

Introduction

One of the low cost and environmental friendly methods of waste water treatment is foam fractionation technique. Foam fractionation is a member of group of process known as adsorptive bubble separation technique and it is wildly use for removal of minute hazardous materials (like-dyes, organic materials, metal ions) where surfactant plays the role as a collector. It uses bubbles as a separation medium and concentrates the surfactants from its aqueous solutions by difference of adsorption properties of the surfactant on gas-liquid interfaces, here gas creates the dispersion of rising bubbles. Then, surfactant is adsorbed onto gas-liquid interfaces of the rising bubbles. When adsorption is as possible as sufficient, the bubbles leave the bulk liquid phase and form the foam phase. The foam tends to have a higher liquid hold up when it just leaves the bulk liquid phase. Liquid hold up decreases quickly to an asymptotic value as the foam rises then the foam goes out of the column and is collapsed to form the liquid, of a referred as the foamate. The surfactant is enriched in the foamate and enrichment ratio is an important factor for evaluating efficiency of foam fractionation. In order to increase enrichment ratio the drainage model was proposed first by Leonard and Lemlich[1]. Foaming may be defined as the creation and stabilization of gas bubbles in a liquid. Proteins defuse to the air-water interface and reduce surface tension. At the interface they partially unfold and associate to produce an intermolecular cohesive film with some degree of elasticity. Foam expansion and stability improved when Bovine Serum Albumin interacted with basic proteins like lysozyme and clupeinedue to cross-links formed between Bovine Serum Albumin and lysozyme at the interface. Bovine Serum Albumin on its own performs best near its isoelectric point when electrostatic repulsion is at its minimum. When its interacts with lysozyme, the greatest expansion and stability was found between pH 8 and 9, which is between the isoelectric point of Bovine Serum Albumin and lysozyme when the proteins are oppositely charged[2] .Clupeine with a pH at 12 was found to be more effective than lysozyme. Lipids inhibit foaming by displacing protein molecules the air-liquid interface and by disrupting the integrity of the protein film. This lipid inhibition was counteracted when Bovine Serum Albumin interacted with clupeine at the air-lipid interface.

Physical-Chemical properties of BSA:

The albumin molecule is not uniformly charged within the primary structure. At neutral pH, Peters (1985) calculated a net charge of -10, -8 and 0 for domains I, II, and III for bovine serum albumin.

Homogeneous property of BSA:

Bovine serum albumin (BSA) is one of the most commonly used carriers for conjugation in antibody production. It belongs to the class of serum protein called albumins, which make out about half of the proteins in plasma and are the most stable and soluble proteins in plasma. It is very popular with laboratories developing immunoassays, mostly due to its availability, solubility and the numerous functional groups present for coupling to heptans.

Solubility:

Solubility in aqueous solution, BSA is easily dissolved in an aqueous solution. It can be highly purified to become homogeneous. BSA is commonly used as protein standard in protein assay and as a molecular weight standard for SDS-PAGE and gel permeation chromatography[3] .It is recommended to reconstitute the lyophilized BSA in sterile 18M cm H_2O for 20 minutes at room temperature and at a concentration no greater than 200 mg/ml.

Materials

Bovine serum albumin (BSA):

Bovine serum albumin (BSA) is a single polypeptide chain consisting of about 600 amino acid residues and non-carbohydrates. Also known as "Fraction V", is a serum albumin protein that has numerous biochemical application including ELISAs (Enzyme-Linked Immunosorbent Assay), blots, and immunohistochemistry. It is also used as a nutrient in cell and microbial culture. In restriction digests, BSA is used to stabilize some enzyme during digestion of DNA and to prevent adhesion of the enzyme to reaction tubes and other vessels. This protein does not affect other enzymes that do not need it for stabilization. BSA is used because of its stability, its lack of effect in many biochemical reactions, and its low cost since large quantities of it can be readily purified from bovine blood, a byproduct of the cattle industry. Bovine serum albumin has a good essential amino acid profile. It binds free fatty acids, other lipids and flavor compounds. BSA's primary function has been associated with its lipid binding properties. It may play role in mediating lipid oxidation. Denatured BSA might reduce the probability of a person acquiring certain disease, such as insulin dependent diabetes or auto-immune disease.

ß -Lactoglobulins:

This group, including eight genetic variants, comprises approximately half the total whey proteins. ß -Lactoglobulin has two internal disulfide bonds and one free thiol group. β-lactoglobulin is approximately 50% of the total whey protein content.

This protein has numerous binding sites for minerals, fat soluble vitamins and lipids, and acts as a transport protein for desirable lipophilic compounds such as tocopherol and vitamin A.This protein, a carrier of small hydrophobic molecules including retinoic acid, has the potential to modulate lymphatic responses[4] .Modification of β-lactoglobulin results in products that have strongantiviral activity against human immunodeficiency virus types 1 and 2, its molecularweight is about 18000 Da.

alpha-Lactalbumins:

These proteins contain eight cysteine groups, all involved in internal disulfide bonds,and four tryptophan residues.Thermal denaturation and pH<4.0 results in the release of bound calcium[5] .α- lactalbumin comprises about 25% ofthe total whey protein

content. This protein possesses an excellent amino acid profile,which is rich in lysine, leucine, threonine, tryptophan and cystine. The main knownbiological function of - lactalbumin is to modulate the synthesis of lactose in themammary gland. Addition of this protein is strongly advocated in the formulation of "humanized" infant formulas and to create other products for people with limited orrestricted protein intakes. its molecular weight is about 14178 Da.

Foam Fractionation:

Adsorptive bubble separation method, depends upon the differences in physiochemical properties of particles[7]. The particles of interest must selectively attach to the gas-liquid interface of foam bubble rising through the liquid pools. If the particles are not surface active, can be made active by using collectors. Under equilibrium conditions of dilute solutions (assuming activity to unity), adsorption of surface active species from bulk solution at a gas-liquid interface can be quantitatively described by the "Gibb's equation" as-

$$\tau/C=(-1)/RT\cdot dY/dC$$

τ is the surface excess of the absorbed solute (i.e. concentration at the surface g.mole/cm^2). C is the bulk equilibrium concentration, and can be considered as distribution factor,is the surface tension, 'R' is the gas constant, and T is the absolute temperature[8].

Surface inactive-species can be removed from solution if an appropriate surface-active material is added to unite with this material so that it can be adsorbed at the bubble surface. This can occur through the formation of chelate, electrostatic attraction or some other mechanism.

Driving force for movement of protein from bulk phase to surface phase:

When a surface is presented to a protein solution, after sometimes has elapsed, protein molecules move to interface, increasing the concentration there. At equilibrium due to differences between force fields at the interface and bulk solution, a concentration gradient exists. The chemical potential associated with the greater concentration of protein at interface equals the chemical potential of that in bulk solution at equilibrium, which is given by following equation- $(\mu_1)_B = (\mu_1)_S$

Where (μ^1)Bis the chemical potential of protein in bulk solution, and (μ^1)S is the chemical potential of protein component in surface phase. The time required to achieve equilibrium for proteins may vary from minutes to hours. The difference in chemical potential between the surface phase and bulk phase supplies the energy of driving force for the movement of protein molecules from the bulk to surface or interface phase.

Collectors[9]

In an adsorption bubble separation method, collectors are used when the particles of interest (colligend) are not having surface active properties by their own, they made effectively surface active or collected by attaching to the collectors.

In ion floatation, ion surfactant moves to the surface of gas bubbles rising through the liquid and the surface becomes charged. The material of interest (colligend) which is oppositely charged adsorp to the bubble interface as a counter ion creating an electrical double layer. Owing to the foams large ratio of surface areas to liquid volume, the liquid that results upon collapse of the foam is manifold concentrated in the ion compared to the initial solution. Thecolligend must be selective for charged surfactant's interface. The molecule of surfactant consists of two parts: one is hydrophobic part and other end is hydrophilic part come outward and the hydrophobic part remained inside. The hydrophilic part joined or attached to the material of interest and carried off at the interface of foam bubble.

A. TYPES OF COLLECTORS USED:

1.Sodium laurate.
2. Sodium lauryl sulphate.
3. Cetyltrimethyl ammonium bromide.
4. Octadecyltrimethyl ammonium chloride.
5. Dodecylamine.

11. Factors affecting the efficiency of Foam Fractionation:

The performance and efficiency of a foam separation method depends on many factors. The relative importance of each factor depends on the specific condition. These include basic variables such as-

A. Effect of pH:

In changes in pH have marked effects on the nature and charge of both collector and colligend, ion flotation is particularly susceptible to several of this parameter. Many investigators refer to some aspect of this dependence; the following are the effects to be expected on varying the pH.

a) A change may occur in the charge on the colligend, due to hydrolysis or the formation of other complexes.
b) Changes may occur in the ionization of the collector; acids and amines, for example may lose their charge at low or high pH values respectively. They either then cease to be collectors, or their mode of collection changes.
c) Flotation may be suppressed by the increased ionic strength which arises on adjusting the pH to extreme values.
d) The stability of the foam supporting the sublate may change, leading to re-dispersion.

B. Temperature:

Temperature has been suggested as on operating variable for cases foam stability of surface-active components is different at different temperature. In the case of froth flotation of minerals, surfactant adsorption and hence flotation could be excepted to decrease with an increase in temperature if the binding of the collector to the mineral surface is due to physical adsorption. Adsorption is an exothermal process, an increase in temperature leads to a decrease in the amount of surfactant on the bubbles, and thus efficiency is reduced. However in many cases, temperature was found to have little effect in ion flotation and foam fractionation process.

C. Gas flow rate:

The gas flow rate strongly effects the removal of dissolved substances without significantly affecting steady state removals. The removal of dissolved substance involves their distribution or partition between gaseous and aqueous phase. The amount and size of gas bubbles depending on the gas flow rate which increase interfacial area, causes an increase in removal at any given time.

D. Foam height:

The foam height has significantly effects on the separation of albumin, the effect was very pronounced near the foam liquid interfaces. A change in the foam height from 3 to 17 cm produced a drastic change in the foam stream.at foam height of 30 cm, the volume of the solution carried away in the form of foam was 24 ml/min, while at 17 cm foam height; a volume of 10 ml/min was obtained. Further it was observed that an increase in foam height produced a small decrease in the efficiency of the process.

E. Foam density:

Foam density is important to get success in the operation. If the foam density is too much high, the bubbles may not rise up by the pressure, thereby causing no separation. The high densities of bubbles are seen at too much high concentration of molecules present in solution and high concentration of collectors.

1.Equipment design:

Equipment design is one of the important factors for the efficiency of this method. As described earlier, there should be a sufficient foam height to get dry foam, the diameter and height of column should be in specific proportion for liquid distribution, the frit size should also be minimized to get the sufficient area of bubbles.

Experimental Procedure:

A. Preparation of standard curve for Bovine Serum Albumin:

Required quantity of BSA powder was weighed and dissolved in double distilled water and then suitably diluted to get the desired the concentration range from 50-800 mcg/ml. the optical density value (OD) was then observed using UV-spectrophotometer at max of 280 nm. The resultant value was plotted against concentration to obtain a standard curve. (fig no.1)

B. Determination of Critical micelle concentration (CMC) of BSA:

The BSA powder was weighed and dissolved in double distilled water and then suitably diluted to obtain a desired concentration range of 100-800 mcg/ml. The surface tension of those samples was measured by using drop count method, and a plot of Concentration Vs Surface tension was constructed. (fig no.2)

C. Determination of percentage gas Hold up:

For determining percentage gas hold up different concentration of feed are prepared but maintaining the same Protein surfactant ratio. The Percent gas hold up was measured in a batch of liquid when gas passed through the column. The height of pool was measured. Again height of liquid pool was measured after shutting off the gas flow. Percentage of gas bubble entrapped in liquid column was calculated and tabulated as %55 gas hold up. Percent gas hold up was plotted against gas flow rate (GFR)(fig no.3). Operating temperature was maintained at 27o C.

D. Foam Fractionation (Batch process)[9]:

Initially feed at particular concentration was prepared by suitably the diluting the stock BSA with water. Required quantity of Sodium Lauryl Sulphate (SLS) was added to the to get the desired Protein surfactant ratio (PSR), Then the pH of the feed was measured and adjusted by adding either concentrated acetic acid as per requirement. The foam fractionation column was then filled with 300 ml. of feed solution and Nitrogen gas was passed through the feed at desired gas flow rate (GFR). As per '% gas holdup study' GFR was maintained in the range of 100-200 cc/min. Bubbles are formed by the frit initially which then rise through the liquid column. Bubbles form foam when it leaves liquid. Foam moves up the column due to gas velocity, foam was collected continuously through the top outlet into a receiver. The receiver was attached with 500ml beaker. The foam was continuously collected for required period of time. Foam was then allowed to collapse by a stirrer until the foam breaks down to form foamate (collapsed foam). 2ml residual solution collect and then 2ml Surfactant mixed solution push into the feed. The total volume of foamate was measured, suitably diluted and absorbance was noted, The gas was turned off after the experiment was over and the remaining substance in the column which is referred as residual liquid had been collected. The volume of the residual liquid was measured and its concentration was also calculated. Mass flow rate (MFR) was then determined using regression equation obtained by plotting amount of protein Vs time. Performance criteria of foam fractionation such as Enrichment ratio (Er), separation ratio (Sr), Recovery percentage (Rp) were also calculated.

Discussion

This is a type of a foam technique. The present work deals with the separation and removal of BSA proteins from the feed of the liquid solution by the help of N_2gas. This is a type of batch mode of operation where performed andthe effects of several parameters such as Protein-Surfactant ratio (PSR), pH of the feed, Gas flow rate (GFR), Initial concentration of feed (Ci) on the separation process studied thoroughly. An effort was also made to optimize those various operating conditions so that the maximum separation efficiency can be achieve. Below the effect of flowing parameter is discussed.

Effect surface active agent recovery process:

From the graph (figure no. 4-6) the recovery of surface active agent changes as the GFR increased then the recovery process is also increased. Where we see the table no 1-4, that first 15 min the recovery is excellent and the last 15 min the recovery percentage is reduced. So, we decided the recovery of BSA protein in the first 15 mins is very much effective in batch mode

Effect of pH to the recovery of BSA protein:

pH is very much effective in batch mode process for recovery of BSA protein. The control of pH is very sensitive as per graphically obtained %RP Vs PSR we can see that at pH 3.8 the Gas flow rate at first 15mins is giving good result but the results is not good for recovery process at last 15 mins. At the pH 5.8 the %RP is changing in various manner, we can see that graph figure 2 the recovery is high in the first 15 mins then the last 15 mins. Sameas from the graph figure 3 the recovery of first 15 mins and the last 15 mins is same.

Effect of Gas flow rate to recovery of BSA protein:

The effect of Gas flow rate is also very much effective in recovery of BSA. According to batch mode Gas flow rate is 100-200 cc/min because the column is small and its height is short only 780 ml capacity of the column. If we select high gas flow rate the feed volume is decreased rapidly; so the result will be manipulated. Depending upon column the gas flow rate is selected 100-200 cc/min is very prominent because it gives good quantity of recovery percentage of BSA protein in batch mode.

Effect of feed Concentration of BSA:

From the table 1-12, the selected feed concentration (Ci) is 500 mcg/ml. if we take 400 mcg/ml then the recovery process is very low and satisfactory result is not obtained. If we take 600 mcg/ml, the recovery process is high then the selective percentage. So, we taking the 500 mcg/ml for feed concentration to get the proper result.

Effect feed volume in the recovery process ofBSA protein:

Feed volume selection is vital point for the recovery of BSA protein. We took only 300 ml of the residual volume for getting better result from the system. If we take 200 ml residual volume then the Optical density value can not suck properly from the column because we have a specific column height and specific solution through out the whole batch process.

Conclusion

The Bovine serum albumin often from fetal bovine source is used as a nutrient cell and microbial culture. In molecular biology BSA used to stabilize some restriction enzymes during digestion of DNA and prevent adhesion of enzyme to reaction tubes, and other vessels. The large percentage of BSA found in milk. It comprises about to 2-5% of whey protein. This whey protein contains sulphur amino acids and glutamylcycsteine, both of which are precursors of glutathione. Glutathione has the ability to recycle the other anti-oxidant so that they can be reduced by the body. Bovine serum albumin binds with long chain fatty acid and also prevent the glutathione concentration indicator illness. So, the recovery of BSA is very important for the various food industries. The present work deals with the recovery of BSA from commercial waste by foam fractionation technique in batch mode posses high efficiency and reuse of whey protein is very effective in the food industry.

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Table-1: Performance criteria of foam fractionation of BSA in batch mode under following condition Ci- 500 mcg/ml, pH- 3.8, GFR- 200 cc/min, PSR- 1, Feed volume- 300 ml.

TIME (min)	FOAMATE VOLUME (ml)	RESIDUAL VOLUME (ml)	Cf (mcg/ml)	Cr (mcg/ml)	TOTAL AMOUNT in FOAMATE (mg)	TOTAL AMOUNT in RESIDUAL (mg)	ER	SR	%RP
15	19	275	1257	71.96667	79.61	65.96944	8	17	53
30	31	265	983.3333	49.73333	101.6111	43.93111	6	20	68
45	43	249	668	59.76667	95.74667	49.606333	4	11	64
60	56	234	511	64.2	95.38667	50.076	3	7	63

Table-2: Performance criteria of foam fractionation of BSA in batch mode under following condition Ci- 500 mcg/ml, pH- 3.8, GFR- 200 cc/min, PSR- 1.25, Feed volume- 300 ml.

TIME (min)	FOAMATE VOLUME (ml)	RESIDUAL VOLUME (ml)	Cf (mcg/ml)	Cr (mcg/ml)	TOTAL AMOUNT in FOAMATE (mg)	TOTAL AMOUNT in RESIDUAL (mg)	ER	SR	%RP
15	16	278	1520.333	68.7	81.08444	63.662	10	22	54
30	28	267	1053.667	49.76667	98.34222	44.29233	7	21	65
45	38	257	834.6667	44.6	105.7244	38.20733	5	18	70
60	46	246	750.3333	38.86667	115.0511	31.87067	5	19	77

Table-3: Performance criteria of foam fractionation of BSA in batch mode under following condition Ci- 500 mcg/ml, pH- 3.8, GFR- 200 cc/min, PSR- 1.5, Feed volume- 300 ml.

TIME (min)	FOAMATE VOLUME (ml)	RESIDUAL VOLUME (ml)	Cf (mcg/ml)	Cr (mcg/ml)	TOTAL AMOUNT in FOAMATE (mg)	TOTAL AMOUNT in RESIDUAL (mg)	ER	SR	%RP
15	19	289	1148	71.16667	72.70667	68.55722	8	16	48
30	31	280	1045.333	42.06667	108.0178	39.26222	7	25	72
45	43	278	720.3333	31.26667	103.2478	28.97387	5	23	68
60	57	259	632.6667	23.86667	120.2067	20.60489	4	26	80

Table-4: Performance criteria of foam fractionation of BSA in batch mode under following condition Ci- 500 mcg/ml, pH- 3.8, GFR- 200 cc/min, PSR- 1.75, Feed volume- 300 ml.

TIME (min)	FOAMATE VOLUME (ml)	RESIDUAL VOLUME (ml)	Cf (mcg/ml)	Cr (mcg/ml)	TOTAL AMOUNT in FOAMATE (mg)	TOTAL AMOUNT in RESIDUAL (mg)	ER	SR	%RP
15	21	271	953.6667	86.83333	66.75667	78.43944	6	11	44
30	32	262	901.3333	56.8	96.14222	49.60533	6	16	64
45	43	250	830.3333	30.83333	119.0144	25.69444	5	27	79
60	54	239	689.6667	21.26667	124.14	16.94244	4	32	83

Table-5: Performance criteria of foam fractionation of BSA in batch mode under following condition Ci- 500 mcg/ml, pH- 5.8, GFR- 100 cc/min, PSR- 1, Feed volume- 300 ml.

TIME (min)	FOAMATE VOLUME (ml)	RESIDUAL VOLUME (ml)	Cf (mcg/ml)	Cr (mcg/ml)	TOTAL AMOUNT in FOAMATE (mg)	TOTAL AMOUNT in RESIDUAL (mg)	ER	SR	%RP
15	6	291	1660.333	71.16667	26.02667	121.6057	9	10	17
30	16	279	1553.667	51.1667	61.65333	83.421	8	13	41
45	24	260	967.6667	46.76667	88.37333	55.98667	7	17	59
60	31	259	687	64.73333	108.6722	33.49733	7	27	72

Table-6: Performance criteria of foam fractionation of BSA in batch mode under following condition Ci- 500 mcg/ml, pH- 5.8, GFR- 100 cc/min, PSR- 1.25, Feed volume- 300 ml.

TIME (min)	FOAMATE VOLUME (ml)	RESIDUAL VOLUME (ml)	Cf (mcg/ml)	Cr (mcg/ml)	TOTAL AMOUNT in FOAMATE (mg)	TOTAL AMOUNT in RESIDUAL (mg)	ER	SR	%RP
15	10	286	1046	112.7333	34.86667	107.4724	6.9	9	23
30	18	276	998	87.9	59.88	80.868	6.9	11	40
45	26	265	943	67.9	81.72667	59.97833	6.2	14	54
60	34	253	842	56.43333	95.42667	47.59211	5.6	15	64

Table-7: Performance criteria of foam fractionation of BSA in batch mode under following condition Ci- 500 mcg/ml, pH- 5.8, GFR- 100 cc/min, PSR- 1.5, Feed volume- 300 ml.

TIME (min)	FOAMATE VOLUME (ml)	RESIDUAL VOLUME (ml)	Cf (mcg/ml)	Cr (mcg/ml)	TOTAL AMOUNT in FOAMATE (mg)	TOTAL AMOUNT in RESIDUAL (mg)	ER	SR	%RP
15	5	291	1242	128.8	20.7	124.936	8	10	14
30	17	284	1123	88.73333	63.63667	84.00089	7	13	42
45	25	269	1079.333	62.26667	89.94444	55.83244	7	17	60
60	31	253	897.6667	57.53333	92.75889	48.51978	5	16	62

Table-8: Performance criteria of foam fractionation of BSA in batch mode under following condition Ci- 500 mcg/ml, pH- 5.8, GFR- 100 cc/min, PSR- 1.75, Feed volume- 300 ml.

TIME (min)	FOAMATE VOLUME (ml)	RESIDUAL VOLUME (ml)	Cf (mcg/ml)	Cr (mcg/ml)	TOTAL AMOUNT in FOAMATE (mg)	TOTAL AMOUNT in RESIDUAL (mg)	ER	SR	%RP
15	4	291	1292	128.7	17.25333	124.839	9	10	11
30	11	282	1243	104.5667	45.57667	98.29267	8	12	30
45	18	273	1188	81.23333	71.28	73.92233	8	15	47
60	23	269	1122.33	64.73333	86.04556	58.04422	7	17	57

Table-9: Performance criteria of foam fractionation of BSA in batch mode under following condition Ci- 500 mcg/ml, pH- 5.8, GFR- 150 cc/min, PSR- 1, Feed volume- 300 ml.

TIME (min)	FOAMATE VOLUME (ml)	RESIDUAL VOLUME (ml)	Cf (mcg/ml)	Cr (mcg/ml)	TOTAL AMOUNT in FOAMATE (mg)	TOTAL AMOUNT in RESIDUAL (mg)	ER	SR	% RP

15	18	276	1143.667	84.2	68.62	77.464	8	13	45
30	31	263	956	49.8	98.78667	43.657	6	19	66
45	41	250	875.667	31.06667	119.6744	25.88889	5	28	79
60	52	239	747	21.23333	129.48	16.91589	4	35	86

Table-10: Performance criteria of foam fractionation of BSA in batch mode under following condition Ci- 500 mcg/ml, pH- 5.8, GFR- 150 cc/min, PSR- 1.25, Feed volume- 300 ml.

TIME (min)	FOAMATE VOLUME (ml)	RESIDUAL VOLUME (ml)	Cf (mcg/ml)	Cr (mcg/ml)	TOTAL AMOUNT in FOAMATE (mg)	TOTAL AMOUNT in RESIDUAL (mg)	ER	SR	%RP
15	10	288	816.3333	122.0667	27.21111	117.184	5	7	18
30	23	272	735.3333	96.6	56.37556	87.584	4	8	37
45	34	259	757	63.8	85.79333	55.08067	5	12	57
60	40	244	712	56.43333	94.9333	45.89911	4	13	63

Table-11: Performance criteria of foam fractionation of BSA in batch mode under following condition Ci- 500 mcg/ml, pH- 5.8, GFR- 150 cc/min, PSR- 1.5, Feed volume- 300 ml.

TIME (min)	FOAMATE VOLUME (ml)	RESIDUAL VOLUME (ml)	Cf (mcg/ml)	Cr (mcg/ml)	TOTAL AMOUNT in FOAMATE (mg)	TOTAL AMOUNT in RESIDUAL (mg)	ER	SR	% RP
15	14	281	1660.333	71.16667	42.94889	103.6266	6	8	29
30	26	266	1553.667	51.16667	72.97333	69.30778	5	11	49
45	36	256	967.6667	46.76667	86.28	53.21956	5	12	67
60	43	248	687	64.73333	95.93778	41.11289	4	13	63

Table-12: Performance criteria of foam fractionation of BSA in batch mode under following condition Ci- 500 mcg/ml, pH- 5.8, GFR- 150 cc/min, PSR- 1.75, Feed volume- 300 ml.

TIME (min)	FOAMATE VOLUME (ml)	RESIDUAL VOLUME (ml)	Cf (mcg/ml)	Cr (mcg/ml)	TOTAL AMOUNT in FOAMATE (mg)	TOTAL AMOUNT in RESIDUAL (mg)	ER	SR	%RP
15	21	270	920.3333	91.63333	64.42333	82.47	6	10	43
30	32	261	904.3333	56.4	96.46222	49.068	6	16	64
45	49	243	811.3333	16.4	132.5178	13.284	5	49	88
60	57	231	688	14.2	130.72	10.934	4	48	87

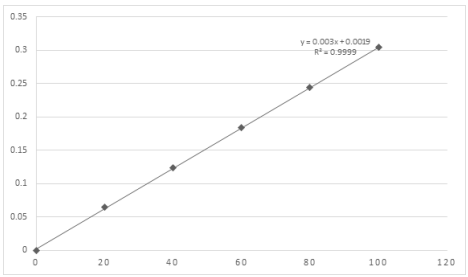

Figure-1: Standard curve of Bovine serum album

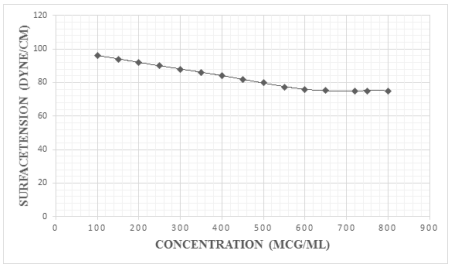

Figure-2:Determination of critical micelle concentration (CMC) of BSA

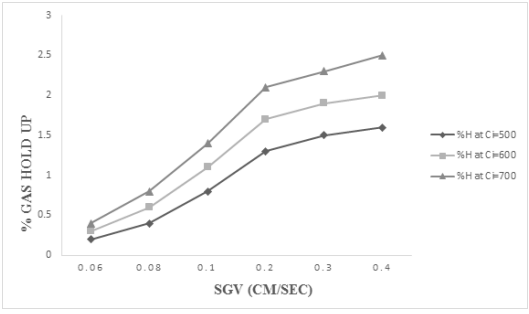

Figure-3: Percentage of gas hold up at different Ci at fixed PSR

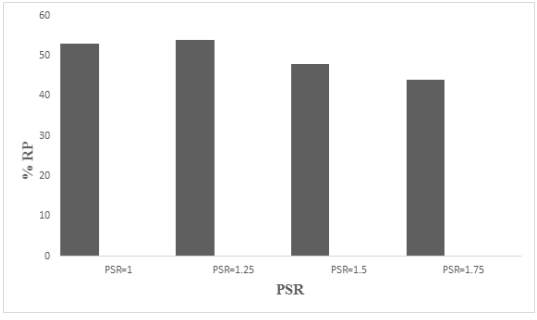

Figure-4: First 15 min Comparison of % RP at different PSR GFR200.

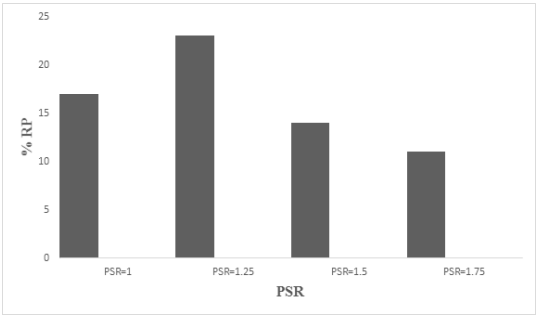

Figure-5: First 15 min comparison of % RP at different PSR GFR 100.

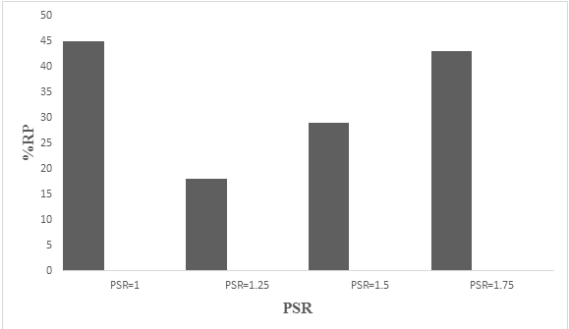

Figure-6: First 15 min comparison of % RP at different PSR GFR 150.

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