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Statistical analysis in enrichment of total whey protein by continuous foam fractionation method

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ABSTRACT

The objective of the present study was to optimize the operating conditions in the separation of the total whey proteins from whey by continuous foam fractionation method using response surface methodology (RSM). The effects of the different process variables such as pH (X_1) of proteins in feed, gas flow rate, (X_2) of initial feed solution, protein: surfactant ratio (X_3) and volumetric flow rate (X_4) where investigated on the performance criteria of fractionation of raw processed whey. Four factors, three levels Box-Behnken design was used for the optimization procedure. Quadratic model regression equations and response surface plots correlate independent variables (X_1, X_2, X_3 and X_4) and dependent variables (response) such as concentration of Foamate (C_f), Enrichment ratio (E_r), and percentage Recovery (% R_p) of total whey proteins. All the four factors had significant effects on the response variables. The model predicted that the optimized values of the factors (X_1, X_2, X_3 and X_4) such as 5, 290, 1.5 and 14, respectively. The predicted responses were (concentration of foamate, enrichment ratio, and percentage recovery) such as 6647.32, 13.27, and 78.02, respectively. Experiments were performed with the predicted values of factors.

1. Introduction

Whey protein is a rich source of essential amino acid [1]. In the cheese industry, whey is produced as a byproduct containing substantial amount of proteins that are either added to dairy products or animal fodder or are discharged as waste, resulting in high Bacteriological Desiccators (BOD) [2,3]. Its importance was extensively realized by the industrialists and whey proteins had been recovered from whey by different methods. It is the renewed interest of scientists to improve the quality of product coupled with efficiency of cost [4]. Whey proteins impart some functional advantages such as enhancement of solubility, viscosity, gel forming capacity, foaming ability etc. to food products [5]. Functionality of whey proteins is influence by a number of compositional factors, physicochemical factors such as composition of proteins, temperature, pH, ionic strength, concentration of Ca^{2+} and other ions, molecules. Solubility of whey proteins vary in isoelectric pH (IEP) range, heat induced state [6]. Protein-surfactant complex show different hydrophobicity at a pH other than IEP and at IEP. Whey protein concentrate had been reported to have poor foaming stability, emulsifying ability at low concentration. So, foaming of whey is assisted by the surface active agents in foam fractionation experiment of separation to maximize enrichment [7]. The separation of proteins from a culture medium or whey is usually carried out by adsorption, ion exchange, chromatography and various membrane separation methods [8-10]. Foam fractionation in adsorptive bubble separation method offers several advantages over these methods e.g. ease of scale up, flexibility in continuous operation, very high separation efficiency and cost effectiveness [11]. So far, some investigators reported their works with pure concentrate and characterized the separation experiment ratio, % recovery of product, selectivity and separation ratio. Selective separation of protein from a multicomponent system was also made possible partially if there is wide gap in their isoelectric pH [12].

In the present work, processed native whey has been chosen to study separation by continuous foam fractionation and its optimization by the Response surface methodology. In the past decades, many have used RSM in food process design for the optimization of variables owing to the ease of operation, reliability and reproducibility of the model parameters as well as the availability of uses friendly computer software packages [13-14]. The Response Surface Methodology (RSM) encompasses the use of experimental design, generation of polynomial equation, mapping of the responses over the experimental domain to determine optimum conditions to achieve desired responses [15]. Investigators get advantages by saving time in running of numerous experiments in order to achieve optimization when compared to conventional empirical method. RSM is more effective and precise if experiments are suitably designed. In this paper, we report application of RSM in the foam fractionation of native whey (waste) to study the role of pH(X_1), Gas flow rate of Nitrogen gas (GFR, X_2) and Protein Surfactant Ratio (PSR, X_3) and Volumetric Flow rate (VFR, X_4) different response variables such as concentration of Foamate, concentration of residual feed solution, enrichment

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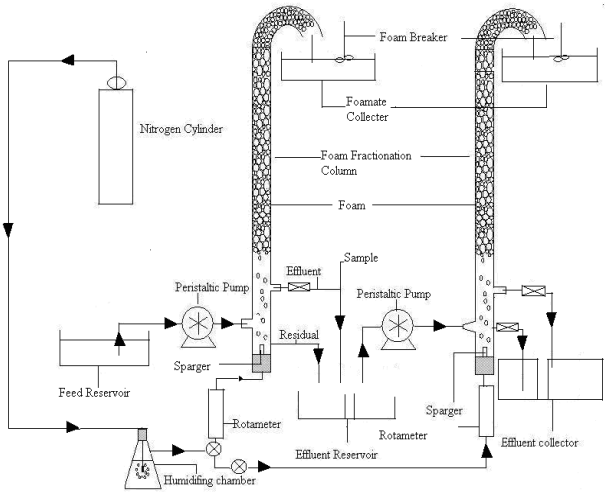

Figure 1. Foam fractionation apparatus operating in continuous mode.

ratio, percentage recovery, using Box-Behnken design. The model generates regression equations and response surface plots that correlate independent and dependent variables with these optimizes values, maximum % recovery can be achieved easily.

2. Experimental

2.1. Experimental design

The experimental design and analysis of date were performed with the help of design-expert (Version 7.1.7. Stat-Ease, Minneapolis, USA) [16]. Factorial design based on multiple regression analysis involved the main, the quadratic and interactive effects that were caused by four independent operating variables. These variables generated few response variables. The four studied parameters were pH of feed solution (whey), Gas flow rate (GFR), Protein-Surfactant ratio (PSR) and Volumetric flow rate (VFR). Response variables were concentration of protein in foamate (C_f), enrichment ratio of protein (Er = C_f/C_r), and percentage recovery of protein (%R_p). Following is the general model for response surface,

$$Y = bo + \sum_{i=1}^{i=3} b_i x_j + \sum_{i=1}^{i=3} b_{ij} x^2{}_{ij} + \sum_{i<j=2}^{i=3} b_{ij}\, x_i x_j + e \ldots\ldots\ldots \quad (1)$$

The Box-Behnken design was used for the optimization of all variables. ANOVA was performed with the coefficients related to block term, linear, quadratic and interactive terms. The model generated second order polynomials for different responses [17]. Table 1 represents levels of design parameters that were treated for experimental design.

Table 1. The levels of variables chosen for the trials at fixed (Ci = 500 µg/mL).

Level/Factor	-1	0	+1
X_1, pH	2	5	8
X_2, GFR (cm³/min)	250	290	330
X_3, PSR	1.25	1.5	1.75
X_4, VFR (cm³/min)	12	14	16

X_1, X_2, X_3 and X_4 are variables of model equation had been exhibited as in two dimensional contour plots and three dimensional response surface plots.

2.2. Materials, instruments, equipment

Whey was supplied by local confectionery; sodium dodecyl sulfate (SDS) was obtained from Loba (India). Double distilled water was prepared at laboratory. The instruments used were UV spectrophotometer (UV 1700 Shimadzu), pH meter (Satorius), Centrifuge (Remi), Foam fractionation apparatus was supplied by local glass fabricator.

2.3. 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 aqueous part of solvent from treated whey under controlled condition in a BOD.

2.4. Treatment of whey

Raw and fresh whey is collected from local confectionery. It is filtered through cheesecloth. The filtrate is centrifuged and filtered several times until absorbance becomes constant at wavelength of 280 nm. Thus fat is removed. Fat has detrimental effect on foaming property. Film rigidity decreases when fat competes with protein molecules. It is then diluted as per requirement and concentration is checked by spectrophotometer [18].

2.5. Foam fractionation

The experimental set up (Figure 1) consists of a glass column, nitrogen cylinder, humidifier (glass set), air flow meter, foam receiver and stirrer. The glass column is designed and fabricated by local fabricator. It is of 1 meter length having an internal diameter of 8 cm. A porous glass sparger (Frit no. 3, pore size 16-40 micron) is fitted on the top of a small glass tube and that is attached at the bottom of column by standard joint. Feed was prepared by suitable dilution of stock whey to get the desired feed concentration. Required quantity of Sodium Lauryl Sulphate (SLS) was added to the feed to get the desired PSR, it was then allowed to mix uniformly with the help of an ultrasonic cleaner. Then the pH of the feed was measured and adjusted as per requirement. The foam fractionation column was then filled with 1 L of feed solution and Nitrogen gas was passed through the feed at desired gas flow rate (GFR). Feed was introduced from outside through an inlet in the column with the help of a peristaltic pump to maintain a constant volumetric flow rate, and the effluent is constantly collected through a outlet from other side, the flow rate of the outgoing

Table 2. Experimental design of variables.

Std	Run	pH	GFR	PSR	VFR	Cf	Er	%Rp
24	1	0.00	1.00	0.00	1.00	6715.85	13.43	81.69
10	2	1.00	0.00	0.00	-1.00	5905.13	11.81	69.88
3	3	-1.00	1.00	0.00	0.00	5602.62	11.20	70.35
15	4	0.00	-1.00	1.00	0.00	8014.76	16.02	85.15
14	5	0.00	1.00	-1.00	0.00	5340.00	10.68	75.85
25	6	0.00	0.00	0.00	0.00	8872.94	17.74	94.27
5	7	0.00	0.00	-1.00	-1.00	6908.34	13.81	81.76
22	8	0.00	1.00	0.00	-1.00	7599.25	15.19	89.94
4	9	1.00	1.00	0.00	0.00	5549.60	11.09	78.82
8	10	0.00	0.00	1.00	1.00	6861.10	13.72	83.46
16	11	0.00	1.00	1.00	0.00	6323.52	1.64	89.82
9	12	-1.00	0.00	0.00	-1.00	5834.55	11.66	69.05
21	13	0.00	-1.00	0.00	-1.00	7194.97	14.38	85.15
6	14	0.00	0.00	1.00	-1.00	7354.33	14.70	87.04
2	15	1.00	-1.00	0.00	0.00	6856.04	13.71	72.84
20	16	1.00	0.00	1.00	0.00	5519.17	11.03	75.88
18	17	1.00	0.00	-1.00	0.00	5281.16	10.56	72.61
13	18	0.00	-1.00	-1.00	0.00	6693.05	13.38	71.11
7	19	0.00	0.00	-1.00	1.00	7081.95	14.16	86.15
23	20	0.00	-1.00	0.00	1.00	7112.03	14.22	86.51
17	21	-1.00	0.00	-1.00	0.00	5929.41	11.85	74.45
1	22	-1.00	-1.00	0.00	0.00	6181.27	12.36	77.61
26	23	0.00	0.00	0.00	0.00	8872.94	17.74	94.27
19	24	-1.00	0.00	1.00	0.00	5143.14	10.23	70.71
12	25	1.00	0.00	0.00	1.00	5381.53	10.76	65.46
11	26	-1.00	0.00	0.00	1.00	6210.42	12.42	75.54
27	27	0.00	0.00	0.00	0.00	8872.94	17.74	94.27

effluent is same as the incoming feed. Bubbles are formed initially which then rises to the top of the column leading to formation of foam. The foam is continuously collected for required period of time. Foam was then allowed to stir using a stirrer until the foam breaks down to form foamate.

The effluent was collected in a reservoir, the residual was also collected, then the collected material (effluent) was pumped into the second column, where it acts as feed for the second column. When the work with the first column is finished the gas flow into the first column was stopped and the valve is opened so that the gas now flows into the second column and samples were withdrawn at regular intervals assessed. After steady state was achieved, the effluent showed constant concentration. Whole procedure is repeated again as mentioned above. The volume of foamate is measured, suitably diluted and absorbance is noted. The total effluent and residual was collected and absorbance was noted, the total input amount, output amount, loss amount, recovery %, enrichment ratio were also calculated. Samples are analyzed by spectrophotometer. Data are presented in Table 2 as the average of experimental results.

3. Results and discussion

Table 1 represents levels of design parameters that were treated for experimental design. Operating variables used in the experiments, pH, GFR, PSR, VFR were presented in coded form, responses variables (C_f, E_r, and $\%R_p$) were calculated and tabulated in Table 3. These values were used to run the software within the chosen levels of parameters response variables have ranges that were presented along with mean values and standard deviation in Table 3.

Table 3. Response variables from experimental date along with standard deviation.

Response Variables	Range of all runs	Mean	S.D.
C_f	5143.14 - 8928.24	6647.32	1.1202
E_r	10.23 - 17.85	13.2737	2.23985
$\%R_p$	65.41 - 89.94	78.0252	7.88249

3.1. Concentration (C_f) of protein in foamate solution

It is expressed by the following model equation.

$$Y_1 = 8872.94 - 34.06A - 410.06B + 165.18C - 119.47D - 181.95AB + 256.07AC - 224.87AD - 84.55BC - 200.11BD - 166.71CD - 2120.26A^2 - 896.65B^2 - 1238.28C^2 - 774.59 D^2 \quad (2)$$

The above quadratic model was written from the values obtained from Table 4. It showed R^2 value as 0.9041. This implies 2.23% of the total variation could not be explained by the model whereas the model was found significant (p=0.0004). There is only 0.04% chance of error in '*Model F-value*' that could occur due to noise. The model had been presented after eliminating non-significant parameters ($p > 0.05$). Model exhibited the effect of main parameters A and B. There was negative regression coefficient in the quadratic effect of B (GFR). At centre value of B, highest response was obtained. Response (Y_1) decreased at pH other than isoelectric point (IEP). At IEP, protein becomes slightly hydrophobic and adsorbs more at the interface [19]. The model was adequate because of its high R^2 value. In this model regression coefficient of A was found greatest in comparison to other factors. Effect of gas flow rate (GFR) was observed very much prominent. The model was free of the effects of interactions between factors as since it had insignificant p-value. It is inferred that C_f was influenced significantly by the increased of pH and GFR. Response surface plots revel factor-response interactions as do model regression equation. Figure 2 showed two-dimensional iso-response curves. Response Y_1 increased with the increased of C_i and maximized when pH approached 5. Figure 3 revealed similar three-dimensional surface effect of A (pH) and B (GFR) on the response variable Y_1 (C_f).

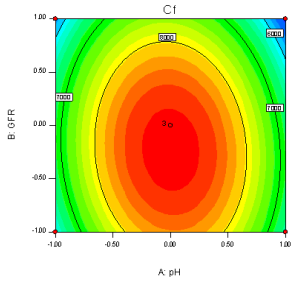

Figure 2. Two dimensional isoresponse curve of foamate (Cf).

Table 4. Regression coefficients and their p-values for the linear regression model for predicting optimized responses (C_f, E_r and $\%R_p$).

C_f (Y_1)			E_r (Y_2)			%Rp (Y_3)		
Factor	**b-coefficient**	**p-value**	**Factor**	**b-coefficient**	**p-value**	**Factor**	**b-coefficient**	**p-value**
Intercept	8872.94	0.0004	Intercept	17.74	0.0004	Intercept	94.27	0.0046
A-(pH)	-34.06	0.8201	A-(pH)	-0.63	0.8328	A-(pH)	-0.18	0.8974
B-(GFR)	-410.11	0.0161	B-(GFR)	-0.82	0.0162	B-GFR)	0.68	0.6395
C-(PSR)	165.18	0.2817	C-(PSR)	0.32	0.2899	C-(PSR)	2.51	0.0991
D-(VFR)	-119.47	0.4308	D-(VFR)	-0.24	0.4358	D-(VFR)	-0.33	0.8160
AB	-181.95	0.4872	AB	-0.37	0.4866	AB	3.31	0.1986
AC	256.07	0.3330	AC	0.52	0.3244	AC	1.75	0.4851
AD	-224.87	0.3931	AD	-0.45	0.3910	AD	-2.73	0.2842
BC	-84.55	0.7448	BC	-0.17	0.7439	BC	-0.018	0.9944
BD	-200.11	0.4458	BD	-0.40	0.4467	BD	-2.40	0.3429
CD	-166.71	0.5237	CD	-0.33	0.5255	CD	-1.99	0.4287
A^2	-2120.26	<0.0001	A^2	-4.24	<0.0001	A^2	-16.19	< 0.0001
B^2	-896.65	0.0015	B^2	-1.79	0.0016	B^2	-4.73	0.0443
C^2	-1238.28	0.0001	C^2	-2.48	0.0001	C^2	-6.09	0.0136
D^2	-774.59	0.0042	D^2	-1.55	0.0043	D^2	-5.13	0.0314
Others Statistics R^2 = 0.9041			Others Statistics R^2 = 0.9041			Others Statistics R^2 = 0.8502		
Sum of squares df			Sum of squares df			Sum of squares df		
Model	2.916E+007	14	Model	116.82	14	Model	1612.85	14
Residual	3.093E+006	12	Residual	12.41	12	Residual	284.07	12
Lack of fit	3.093E+006	10	Lack of fit	12.41	10	Lack of fit	284.07	10
Pure error	0.000	2	Pure error	0.000	2	Pure error	0.00	2
F-value of model	8.08	-	F-value of model	8.07	-	F-value of model	4.87	-
Correlation Total	3.225E+007	26	Correlation Total	129.22	26	Correlation Total	1896.92	26

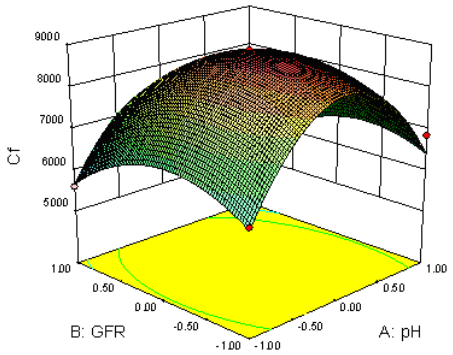

Figure 3. Three dimensional surface effect of A (pH) and B (GFR) on the response variables Y1 (Cf).

Quadratic model equations obtained by response surface methodology.

$$Y_1 = 8872.94 - 34.06A - 410.06B + 165.18C - 119.47D - 181.95AB + 256.07AC - 224.87AD - 84.55BC - 200.11BD - 166.71CD - 2120.26A^2 - 896.65B^2 - 1238.28C^2 - 774.59D^2 \quad (3)$$

$$Y_2 = 17.74 - 0.63A - 0.83B + 0.32C - 0.24D - 0.37AB + 0.52AC - 0.45AD - 0.17BC - 0.4BD - 0.33CD - 4.24A^2 - 1.79B^2 - 2.48C^2 - 1.55D^2 \quad (4)$$

$$Y_3 = 94.27 - 0.18A + 0.68B + 2.51C - 0.33D + 3.31AB + 1.75AC - 2.73AD - 0.018BC - 2.40\,BD - 1.99\,CD - 16.19A^2 - 4.73\,B^2 - 6.09\,C^2 - 5.13D^2. \quad (5)$$

3.2. Enrichment of protein in foamate

$$Y_2 = 17.74 - 0.63A - 0.83B + 0.32C - 0.24D - 0.37AB + 0.52AC - 0.45AD - 0.17BC - 0.4BD - 0.33CD - 4.24A^2 - 1.79B^2 - 2.48C^2 - 1.55D^2 \quad (6)$$

After sequential elimination of the non-significant parameters (p >0.05) from Table 4, the above equation suitably described E_r. E_r values determined in the present study ranged between 10.23 and 17.85. The model had correlation coefficient (R^2) of 0.9040 that indicated only 3.61% could not be explained by the model. Model was significant with F-value of 8.47 (p =0.0004 <0.05). With the increased of gas flow rate both A and B showed increasing effect on E_r, though effect of pH (A) on separation of protein was found maximum at pH = 5 in comparison to pH = 2 and 8. E_r increased in the order of pH 5 > 2 > 8. Proteins adsorb more at the interface at pH = 5. This fact is supported by the strong negative regression coefficient of both B and B^2. Effect of GFR on Y_2 is quadratic and it is showed negative regression coefficient indicating that highest E_r can be obtained at the centre value of GFR. In this model only one interaction (AB) had been found. The effect of pH and GFR at fixed Ci (500 mcg/mL) was further revealed both from contour plot (Figure 4) and surface plot (Figure 5), enrichment ratio was maximum near centre values of both pH and GFR. Though Y_2 is composed of primary response (C_f) but the effects of factors on Y_2 were totally different from Y_1.

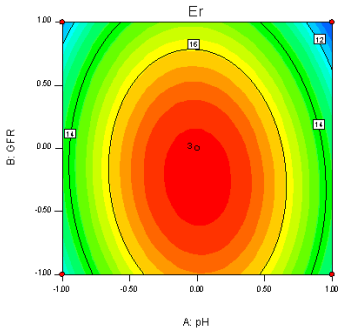

Figure 4. Two dimensional isoresponse curve of Enrichment (Er).

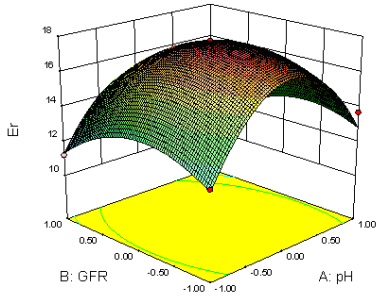

Figure 5. Three dimensional surface effect of A (pH) and B (GFR) on the response variablers Y2 (Er).

Table 5. Percent relative error between experimental and predicted values as obtained from design solution*.

Operating conditions				Cf			Er			%Rp		
pH	GFR	PSR	VFR	Pred.	Exp.	%RE	Pred.	Exp.	%RE	Pred.	Exp.	%RE
0.00	1.00	0.00	-1.00	7111.18	7110.02	0.016	14.21	13.00	2.24	87.81	86.80	1.159
0.00	-1.00	0.00	1.00	7692.45	7694.45	-0.050	15.38	15.03	2.31	85.79	84.80	1.165
0.00	0.00	0.00	0.00	8872.94	8871.92	0.011	17.74	17.20	3.04	94.27	93.59	0.721
0.40	-0.05	0.33	-0.86	8139.41	8138.00	0.017	16.27	15.29	6.04	89.75	88.50	1.401
0.30	0.07	-0.77	0.31	7616.99	7618.00	-0.013	15.22	14.26	6.36	86.45	87.50	-1.21
-0.19	-0.30	-0.31	-0.37	8549.96	8550.60	-0.007	17.09	16.0	6.40	90.74	91.75	-1.10
0.29	-0.43	-.051	0.17	835.69	838.50	-0.034	16.46	15.49	5.92	88.19	85.50	3.05
-0.37	-0.82	-0.76	0.55	7349.97	7347.50	0.033	14.69	15.50	-0.37	85.14	89.50	-5.11

* Exp = Experimental Value, Pred = Predicted value, %RE= Percentage Relative Error [i.e. (Pred-Exp)/Pred].

3.3. Concentration of protein on percentage recovery

$$Y_3 = 94.27 - 0.18A + 0.68B + 2.51C - 0.33D + 3.31AB + 1.75AC - 2.73AD - 0.018BC - 2.40\,BD - 1.99\,CD - 16.19A^2 - 4.73\,B^2 - 6.09\,C^2 - 5.13D^2 \quad (7)$$

In the above model Y_3 represents percent recovery of protein from whey waste feed by the foam fractionation method. Y_3 (%Rp) was best described by the regression equation which was obtained after sequential omission of the non-significant terms ($p > 0.05$, Table 4). The model could explain 79.99% of the behavior of %Rp. F-value (4.87) proved the model was significant. Experimental values of %Rp ranged between 65.41 and 89.94. Like other models (Y_1, Y_2), the model showed positive value of regression coefficient of C. Effect of quadratic terms (A^2, B^2) explained the enhancement of %Rp up to the optimum, beyond which it decreased. There was only one interaction term (AC), which had negative regression coefficient that implies prominent effect of A. The 2-dimensional contour plot (Figure 6) and 3-dimensional surface plot (Figure 7) explained that % Rp increased up to optimum value beyond which it declined with the increase of two vital parameters C_i, pH at fixed GFR. Maximum $\%R_p$ (89.94%) was noticed at pH 5 and C_i, 0.5 mg/mL.

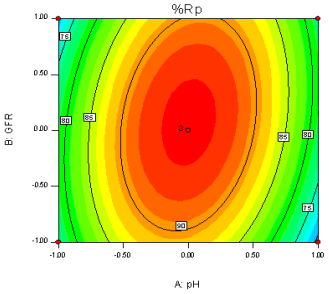

Figure 6. Two dimensional isoresponse curve of Percentage recovery (% Rp).

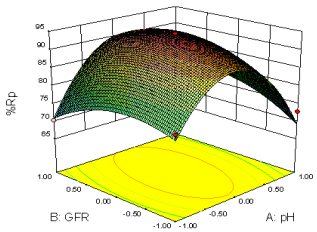

Figure 7. Three dimensional surface effect of A (GFR) and B (pH) on the response variablers Y3 (%Rp).

3.4. Optimization of the operating variables in foam fractionation experiment

Software generated number of solutions from which several were picked up. Response variables from solution were presented as predicted variables were used and foam fractionating experiment was run again. The experimental response variables were compared with the predicted values and relative percent error was presented in Table 5 [23]. There was very less deviation from the predicted values. The present work gave satisfactory result at laboratory scale foam fractionation of whey in continuous mode. Thus, the optimized values of C_f, E_r and $\%R_p$ were found 5143.14-8872.94 mcg/mL, 10.23-17.74 and 65.46-94.27, respectively.

4. Conclusion

Optimization of foam fractionation of proteins from whey in continuous mode had been successfully performed using Box- Behnken method of RSM. Operating variables were modeled and expressed in terms of leveled factors. It reduces number of experiments to a minimum fractionation at restricted level of C_i.

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