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Research Article

Optimization of Aqueous Two-Phase Systems for the Recovery of Soluble Proteins from Tannery Wastewater Using Response Surface Methodology

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Aqueous two-phase system (ATPS) composed of polyethylene glycol 6000 (PEG 6000) and sodium citrate (SC) has been proposed to recover the valuable soluble proteins from tannery wastewater. A sequential optimization strategy which included fractional factorial design (fFD) and central composite design (CCD) was employed to enhance the recovery. From this strategy, a second-order polynomial model was obtained for the protein recovery and it was validated. The optimum recovery was found as 93.46% when pH, NaCl concentration, and temperature were kept at 7.5, 0.1 M, and 33°C, respectively, for a phase system composed of 20% (w/w) PEG 6000-15% (w/w) SC. Thus the proposed ATPS can serve as an alternative to the conventional precipitation method to recover the soluble proteins from tannery wastewater.

1. Introduction

The leather industry is one of the major foreign exchange earners in India nearly over last thirty years [1]. It is reported that the Indian market has been fragmented with about 2200 tanneries [2]. During the traditional leather processing, the skin and hides are subjected to various operations such as soaking, dehairing, liming, deliming, bating, degreasing, and pickling [3]. When the skin is subjected to the alkali treatment, the soluble proteins present on the surface of the skin are discharged as waste. Yearly, nine million tons of skins/hides are being processed worldwide [4]. Literature reveals that, for every 100 kg of raw hides, 15 kg of solubilized protein is discharged as waste in the early stages of the process of transforming hides into leather [5]. The value of these solubilized proteins is enormous and they find applications in food and pharmaceutical industries [6]. The presence of these proteins in the tannery effluents increases the biological oxygen demand and chemical oxygen demand and leads to pollution. By removing these proteins from the waste, a reduction of the tax on wastewater can be achieved and the recovered proteins can be used in food and pharmaceutical industries.

There are a few reports available in the literature for the recovery of soluble proteins from industry effluents using membrane separation processes, but the major drawback of these processes is membrane fouling [7, 8]. The conventional method of protein recovery from tannery wastewater is "precipitation method" which has been well addressed by Kabdasli et al. [9] and Marsal et al. [10]. However the precipitation method has the limitation of protein denaturation and low recovery. For example, only a recovery of 50–70% and 68–78% of soluble proteins from the effluents of tannery beam-house operations was possible in the precipitation methods developed by Kabdasli et al. [9] and Marsal et al. [10], respectively.

In the context of clean environment and pollution prevention, nanotechnology could play a key role [11]. However, aqueous two-phase system (ATPS) attracts more attention because of simple process, low cost, and easy scale-up procedures. For example, very recently, ATPS composed of PEG and sodium citrate was successfully used for textile effluent dye removal [12]. It has been proven by many researchers that ATPS is an efficient and economical process when

compared to other separation processes like precipitation, chromatography, and so forth [13–15].

Therefore, a benign technique for the proteins, ATPS, has been proposed in the present study in order to recover the soluble proteins from the tannery wastewater. ATPS is a downstream processing method which uses the principles of liquid-liquid extraction. It can be formulated by mixing two hydrophilic polymers (Poly Ethylene Glycol (PEG)-Dextran) with water or one hydrophilic polymer (PEG) and inorganic salts (phosphates, sulfates, and citrates) with water [16]. It has been reported that polymer-salt-based ATPS has many advantages over polymer-polymer ATPS and few of them includes easy scale-up, low cost, low interfacial tension, possibility of process integration, and less viscous phases [17].

For the first time, Saravanan et al. [18] research group developed an ATPS made up of PEG/sulfate to recover the soluble proteins from tannery wastewater. Since then, ATPS has received much attention to recover the biomolecules from various industrial effluents such as fish industry [19], dairy industry [20], and prawn industry [21]. Recently, our research group has addressed the partitioning of tannery wastewater proteins in ATPS composed of PEG 10000 plus different citrate salts [22]. The proposed method of ATPS in this paper is environment-benign since the phase components used are nontoxic and biodegradable.

For the maximization of recovery of proteins from tannery wastewater, a sequential method of optimization using response surface methodology (RSM) has been employed which includes the following steps:

(i) screening of significant process variables which affect the protein partitioning in ATPS by a fractional factorial design (fFD),

(ii) crude optimization of the most significant variables by a full factorial design (FFD) with center points,

(iii) final optimization of the most significant variables by central composite design (CCD) using response surface methodology (RSM),

(iv) development and verification of mathematical model and expressing the relationship between the protein partitioning and significant process variables.

2. Materials and Methods

2.1. Materials. PEG 6000 was purchased from Merck and used without further purification. Tri-sodium citrate, citric acid, and sodium chloride were also purchased from Merck and Millipore-Milli-Q water was used in all the experiments.

2.2. Preparation of Tannery Wastewater. The tannery wastewater sample was prepared as discussed elsewhere [23]. In this method, known weight of raw skin/hides was treated with alkali solution. This sectional stream wastewater was used as a protein source in partition experiments to recover the soluble proteins.

2.3. Preparation of Two-Phase Systems. Calculated amounts of tri-sodium citrate and citric acid were taken, and pH of the system was adjusted. ATPS was prepared by mixing appropriate amounts of PEG and citrate solutions, with tannery waste sample as described in the previous section in 15 mL graduated tubes. By the addition of water, the weight of the system was maintained at 10 g. The systems were well mixed in a vortex mixer and left in a water bath at various temperatures for overnight.

2.4. Quantification of Tannery Wastewater Soluble Proteins. The soluble protein from the tannery wastewater was quantified by Bradford method [24]. For the determination of protein concentration, samples were withdrawn from each phase and diluted if necessary with distilled water, and its absorbance was measured using Shimadzu spectrophotometer at 595 nm.

2.5. Partition Coefficient and Recovery. The partitioning of soluble proteins in ATPS is characterized by two factors, namely, partition coefficient K_p and the percentage bottom phase protein recovery $R_{p,B}$:

$$K_p = \left(\text{Concentration of soluble protein in top phase}\right) / \left(\text{Concentration of soluble protein in bottom phase}\right)$$

$$R_{p,B}\,(\%) = \frac{\text{amount of soluble protein in bottom phase}}{\text{total amount of soluble protein in the system}} = \frac{100}{1 + \left(V_{\text{PEG}}/V_{\text{SC}}\right) K_p}, \quad (1)$$

where V_{PEG} and V_{SC} are the volume of PEG rich and sodium citrate phases, respectively.

2.6. Screening of Significant Process Variables. Partitioning of proteins in ATPS is a complex phenomenon. It depends on many factors like type and concentration of phase-forming components, pH, temperature, presence of neutral salts, and so forth. Based on prior experiments (data not shown), the following five factors namely, concentration of PEG 6000, concentration of SC, pH of the system, concentration of NaCl, and temperature were chosen as the factors that affect the protein partitioning.

Consequently, a 1/2 fraction, 2-level factorial design for five factors (2^{5-1} = 16 experiments) was employed to investigate the significant factors. Table 1 gives both coded and uncoded values of these factors in fFD with the percentage recovery. The table shows a wide variation in percentage recovery ranging from 34% to 83% which reflects the importance to attain higher percentage recovery.

2.7. Crude Optimization. It has been observed from the analysis that the three factors, namely, pH, NaCl, and temperature are the significant factors which enhance the protein recovery. Since PEG and SC do not play a significant role in partitioning, PEG was fixed at 20% (positive effect) and SC was fixed at 15% (negative effect) for all the upcoming

Table 1: Coded and uncoded values of factors of 2^{5-1} fractional factorial design (fFD).

Experiment no.	Coded[a] and uncoded[b] values of variables					Recovery, $R_{p,B}$ (%)
	PEG (% w/w)	SC (% w/w)	pH	NaCl (M)	Temperature (°C)	
1	12 (−1)	23(+1)	8 (+1)	0.3 (+1)	20 (−1)	67.24
2	20 (+1)	15 (−1)	8 (+1)	0.1 (−1)	40 (+1)	68.24
3	12 (−1)	15 (−1)	6 (−1)	0.1 (−1)	40 (+1)	33.95
4	20 (+1)	23 (+1)	8 (+1)	0.3 (+1)	40 (+1)	83.42
5	12 (−1)	23 (+1)	6 (−1)	0.3 (+1)	40 (+1)	52.71
6	12 (−1)	23 (+1)	8 (+1)	0.1 (−1)	40 (+1)	50.49
7	20 (+1)	23 (+1)	8 (+1)	0.1 (−1)	20 (−1)	52.21
8	12 (−1)	23 (+1)	6 (−1)	0.1 (−1)	20 (−1)	37.29
9	20 (+1)	15 (−1)	6 (−1)	0.1 (−1)	20 (−1)	29.89
10	20 (+1)	15 (−1)	8 (+1)	0.3 (+1)	20 (−1)	71.77
11	20 (+1)	23 (+1)	6 (−1)	0.3 (+1)	20 (−1)	54.59
12	20 (+1)	15 (−1)	6 (−1)	0.3 (+1)	40 (+1)	64.49
13	12 (−1)	15 (−1)	8 (+1)	0.3 (+1)	40 (+1)	75.33
14	12 (−1)	15 (−1)	8 (+1)	0.1 (−1)	20 (−1)	61.46
15	20 (+1)	23 (+1)	6 (−1)	0.1 (−1)	40 (+1)	48.93
16	12 (−1)	15 (−1)	6 (−1)	0.3 (+1)	20 (−1)	58.49

[a]Data in brackets; [b]Data without brackets.

Table 2: Central composite design (CCD) of variables with % recovery as response.

Experiment no.		Coded[a] and uncoded[b] values of variables			Recovery, $R_{p,B}$ (%)	
		pH	NaCl (M)	Temperature (°C)	Experimental	Predicted
1	2^3 Factorial points	6.4 (−1)	0.14 (−1)	24 (−1)	76.78	78.30
2		7.6 (+1)	0.14 (−1)	24 (−1)	87.69	86.51
3		6.4 (−1)	0.26 (+1)	24 (−1)	79.68	78.88
4		7.6 (+1)	0.26 (+1)	24 (−1)	84.35	84.82
5		6.4 (−1)	0.14 (−1)	35 (+1)	88.90	88.45
6		7.6 (+1)	0.14 (−1)	36 (+1)	89.56	90.39
7		6.4 (+1)	0.26 (+1)	36 (+1)	76.85	78.06
8		7.6 (−1)	0.26 (+1)	36 (+1)	79.21	77.72
9	I set of center points	7 (0)	0.2 (0)	30 (0)	90.66	89.81
10		7 (0)	0.2 (0)	30 (0)	90.50	89.81
11		7 (0)	0.2 (0)	30 (0)	89.65	89.81
12	Star points	6 (−1.68)	0.2 (0)	30 (0)	83.22	82.36
13		8 (+1.68)	0.2 (0)	30 (0)	88.15	88.98
14		7 (0)	0.1 (−1.68)	30 (0)	92.46	92.05
15		7 (0)	0.3 (+1.68)	30 (0)	81.51	81.88
16		7 (0)	0.2 (0)	20 (−1.68)	88.62	83.64
17		7 (0)	0.2 (0)	40 (+1.68)	78.55	78.51
18	II set of center points	7 (0)	0.2 (0)	30 (0)	89.56	89.81
19		7 (0)	0.2 (0)	30 (0)	88.62	89.81
20		7 (0)	0.2 (0)	30 (0)	89.85	89.81

experiments. The three significant variables were further optimized using a 2^3 FFD (Table 2, Experiment nos. 1–8) with three center points (Table 2, Experiment nos. 9–11) to determine the optimum operating conditions. These experiments were done to make sure that the proposed optimization process was in the appropriate region [25].

2.8. Final Optimization. In order to include the curvature, few more experiments were done by adding 6 axial (star) points (Table 2, Experiment nos. 12–17) and 3 more center points (Table 2, Experiment nos. 18–20) to the previous FFD set-up. This entire set of 20 experiments is a central composite design (CCD) for three factors, an RSM technique [26].

Table 3: ANOVA table for fFD.

Source	Degrees of freedom	Sum of squares (SS)	Mean square	F value	P value
PEG	1	83.63	83.63	5.93	0.055
pH	1	1402.88	1402.88	99.50	0.000*
NaCl	1	1324.60	1324.60	93.95	0.000*
Temperature	1	124.43	124.43	8.83	0.014*
PEG∗Temperature	1	294.29	294.29	20.87	0.001*
Error	10	140.99	14.10		
Total	15	3370.82			

S = 3.75490; R-Sq = 95.82%; R-Sq(adj) = 93.73%.
*Significant at 95% confidence level.

In this methodology, the effects of the variables on the protein recovery were fit to the second-order polynomial model according to the following equation:

$$Y = a_o + \sum a_i F_i + \sum a_{ii} F_i^2 + \sum a_j F_i F_j, \quad (2)$$

where Y is the response variable (percentage recovery), F_i and F_j are the independent variables in coded units, a_o is the average response, and a_i, a_{ii}, and a_j are the measures of the F_i, F_j, F_i^2, and F_iF_j of linear, quadratic, and interaction effects, respectively.

For the statistical calculations, the variables were coded according to the following equation:

$$F_i = \frac{f_i - f_o}{\Delta f_i}, \quad (3)$$

where F_i is the independent variable in the coded unit, f_i is the real value of independent variable, f_o is center point the real value of the independent variable, and Δf_i is the step change value. By analyzing the contour plots, the optimum values of the significant variables were obtained. The statistical analysis of the model was represented in the form of analysis of variance (ANOVA).

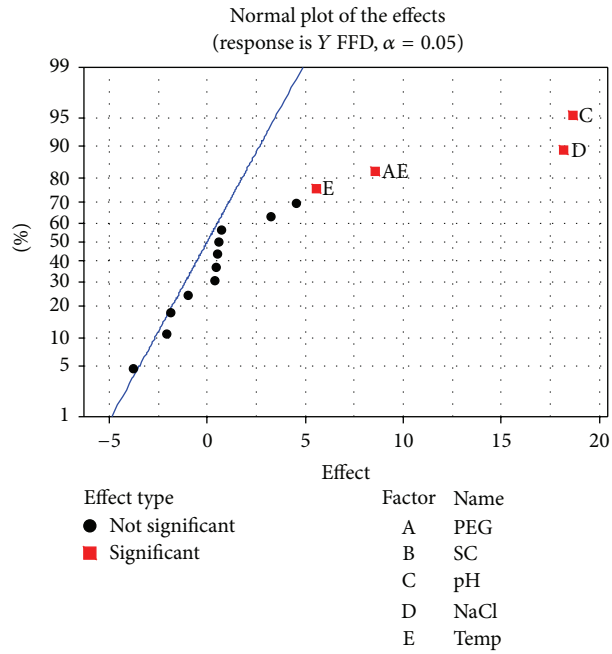

Figure 1: Normal probability plot of the effects.

3. Results and Discussion

3.1. Screening of Significant Process Variables by fFD and Crude Optimization. The fFD showing the recovery of protein from each experiment combination shown in Table 1 was used for statistical analysis. The results were analysed by MINITAB-15.0 (CA, USA). Figure 1 represents the normal probability plot of the effect estimates. This plot is used to analyze the significant factors based on the α (=0.05) value. The significant factors do not conform to the normal plot and lie away from the normal line. From the figure, it is clear that the factors pH, NaCl, temperature, and the interaction between PEG concentration and temperature are significant.

The plot of the mean percentage recovery and experiment levels (Figure 2) illustrates the main effects of the operating conditions on the recovery. PEG, pH, NaCl, and temperature increased the protein recovery at high level. A decrease in mean percentage recovery was observed for SC.

Table 3 summarizes the analysis of variance for fFD. The model sum of squares is $SS_{Model} = SS_{PEG} + SS_{pH} + SS_{NaCl} + SS_{Temperature} + SS_{PEG\ Temperature} = 3229.83$, and this accounts for 95.82% (high R^2) of the total variability in recovery. Moreover, the variables are statistically significant when the P value (defined as the smallest level of significance which leads to the rejection of null hypothesis) is less than 0.05 (95% confidence level). Based on this, pH, NaCl, temperature, and the interaction between PEG and temperature are considered as the significant factors.

The positive effect of PEG at high concentration may be because of the volume occupied by the PEG molecules with the increase in concentration decreases the free space available for the molecules in the top phase. Therefore, because of "volume exclusion effect" all the biomolecules tend to partition towards the bottom phase and thus percentage recovery in the bottom phase increases [27].

In contrast to this, SC had a negative effect on percentage recovery which can be explained based on the "salting-out effect". At high salt concentrations of salt, the ions decrease the solubility of biomolecules which makes them to move to the PEG rich top phase and therefore the percentage recovery in the bottom phase decreases [28].

Table 4: Estimated regression coefficients for percentage recovery.

Term	Coefficient	Standard error of coefficient	T	P
Constant	89.8802	1.1292	79.597	0.000
pH	1.9683	0.7492	2.627	0.025*
NaCl	−3.0216	0.7492	−4.033	0.002*
Temperature	−0.7985	0.7492	−1.066	0.312
pH∗pH	−1.9375	0.7492	−2.657	0.024*
NaCl∗NaCl	−1.4779	0.7492	−2.026	0.070
Temperature∗Temperature	−2.6800	0.7492	−3.675	0.004*
pH∗NaCl	−0.5688	0.7492	−0.581	0.574
pH∗Temperature	−1.5687	0.7492	−1.603	0.140
NaCl∗Temperature	−2.7438	0.7492	−2.803	0.019*

R^2 = 84.86%; R^2 (adj) = 71.22%.
*Significant at 95% confidence level.

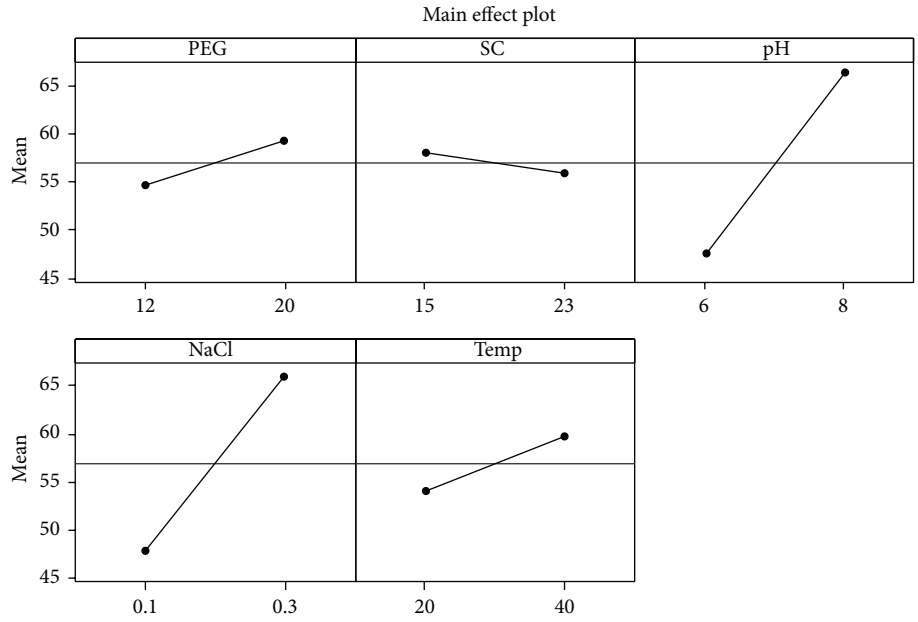

Figure 2: Main effect plot for recovery (%).

The pH presented a statistically significant positive effect for the partitioning of proteins to the bottom phase. It can be explicated with respect to the isoelectric point of the proteins. The wastewater proteins present in tannery wastewater are soluble and globular proteins [29] and therefore they have net negative charge at pH > 5. Hence, the negatively charged protein molecules partition to the bottom phase at high pH. Similar results were obtained by many researchers [30–32].

NaCl presence in the ATPS showed a significant positive effect which may be due to the alterations of hydrophobic interactions or changes in the electrostatic potential difference. For the NaCl concentrations studied in this study (0.1 M to 0.3 M), the interaction of biomolecules with the salt rich bottom phase increases because of the changes in the electrostatic potential difference [33].

The temperature also indicated a significant positive effect on the percentage recovery. The increase in temperature not only alters the structure of biomolecules but also changes the phase composition of the ATPS. Therefore the increase in temperature increases the protein recovery in the bottom phase [34].

As a conclusion from fFD, the factors pH, NaCl, and temperature are confirmed as significant factors and therefore selected for further optimization to maximize the percentage recovery. From the Table 2, it is evident that the average recovery in the center of the experimental region is 90.27%, while the average recovery at the corners is 82.88%. Since this difference is significant, the recovery will be a curved function of all three factors. Moreover, because of the presence of curvature, the response could not be explained by a linear

Table 5: ANOVA for % recovery.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	9	429.487	429.487	47.721	6.23	0.004*
Linear	3	186.308	186.308	62.103	8.10	0.005*
Square	3	160.677	160.677	53.559	6.99	0.008*
Interaction	3	82.501	82.501	27.500	3.59	0.054
Residual Error	10	76.654	76.654	7.665		
Lack-of-Fit	5	73.949	73.949	14.790	27.34	0.001*
Pure Error	5	2.704	2.704	0.541		
Total	19	506.140				

*Significant at 95% confidence level.

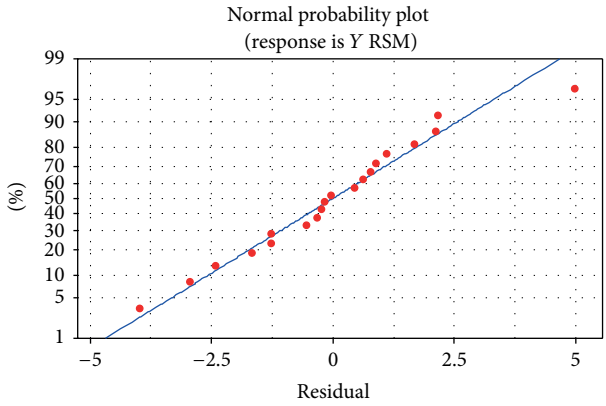

Figure 3: Residual plot with outlier.

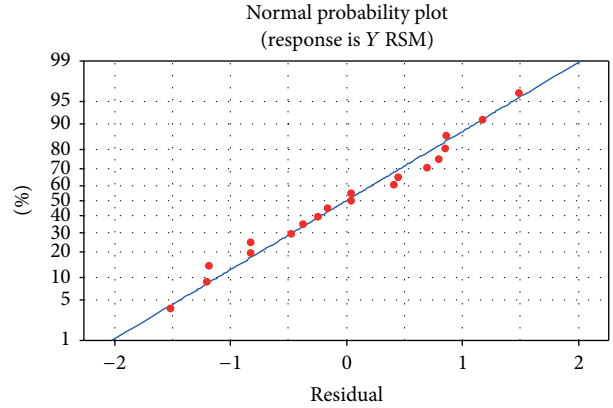

Figure 4: Residual plot without outlier.

model and there is a need for a quadratic *model* which is discussed in the following section.

3.2. Final Optimization. After crude optimization of pH, NaCl, and temperature by 2^3 FFD with three center points and ascertaining of optimal region, additional experiments were performed with 6 axial points and 3 more center points to frame a complete CCD (Table 2). The CCD of 20 experimental runs was used to analyze and optimize the significant factors. Table 4 lists the Minitab output of estimated regression coefficients, standard errors, t-values, and P values.

As discussed earlier, at 95% confidence level, the terms having P values <0.05 are considered to be statistically significant. By substituting these statistically significant parameters' regression coefficients in (2), the following model was obtained in coded units:

$$R_{p,B} = 89.88 + 1.96\,\text{pH} - 3.02\,\text{NaCl} - 1.94\,\text{pH} * \text{pH} - 2.68\,\text{Temperature} * \text{Temperature} - 2.74\,\text{NaCl} * \text{Temperature}. \quad (4)$$

In addition to the linear effects, RSM helps to evaluate the interaction and quadratic effects. It is clear from the table that interaction effects are also significant in this process. The regression coefficients R^2 and R^2_{adj} values were determined as 84.86% and 71.22%, respectively.

Table 5 represents the ANOVA for the quadratic model developed. Higher F values indicate that the term is statistically significant. Another convenient measure to test the significance of the terms is P value. It is evident that linear, interaction, and quadratic effects were statistically significant ($P < 0.05$) for the developed model. Nevertheless, a low value for lack of fit indicated that it is also statistically significant, and therefore it is necessary to identify possible outliers. It is done by examination of residual plot as shown in Figure 3 which suggested that a data point corresponding to experimental run 16 could be a possible outlier.

Consequently, this data point was omitted and the regression was repeated for the remaining data. The regression coefficients and the ANOVA values after the omission of outlier are given in Tables 6 and 7, respectively.

Substituting the new regression coefficients into (2) gives the following new modified model:

$$Y_B = 89.81 + 1.96\,\text{pH} - 3.02\,\text{NaCl} - 1.46\,\text{pH} * \text{pH} - 1.00\,\text{NaCl} * \text{NaCl} - 4.45\,\text{Temperature} * \text{Temperature} - 1.57\,\text{pH} * \text{Temperature} - 2.74\,\text{NaCl} * \text{Temperature}. \quad (5)$$

The new regression coefficients R^2 and R^2_{adj} values were determined as 97.27% and 94.54%, respectively, which were higher than the previous values. Thus, 97.27% of variation in

Table 6: Estimated regression coefficients for recovery (%) without outlier.

Term	Coefficient	Standard Error of coefficient	T	P
Constant	89.8078	0.5011	179.231	0.000*
pH	1.9683	0.3324	5.922	0.000*
NaCl	−3.0216	0.3324	−9.091	0.000*
Temperature	0.7622	0.4108	1.855	0.097
pH∗pH	−1.4647	0.3317	−4.416	0.002*
NaCl∗NaCl	−1.0051	0.3317	−3.030	0.014*
Temperature∗Temperature	−4.4476	0.4236	−10.500	0.000*
pH∗NaCl	−0.5688	0.4343	−1.310	0.223
pH∗Temperature	−1.5687	0.4343	−3.612	0.006*
NaCl∗Temperature	−2.7438	0.4343	−6.318	0.000*

R^2 = 97.27%; R^2 (adj) = 94.54%.
*Significant at 95% confidence level.

Table 7: ANOVA for recovery (%) without outlier.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	9	483.698	483.698	53.744	35.62	0.000*
Linear	3	180.715	182.794	60.931	40.39	0.000*
Square	3	220.481	220.481	73.494	48.71	0.000*
Interaction	3	82.501	82.501	27.500	18.23	0.000*
Residual Error	9	13.578	13.578	1.509		
Lack-of-Fit	4	10.874	10.874	2.718	5.03	0.053
Pure Error	5	2.704	2.704	0.541		
Total	18	497.275				

*Significant at 95% confidence level.

Table 8: Experimental verification of the model.

Optimized input process parameters			Modified value of input process parameters			Predicted recovery	Experimental recovery
pH	NaCl (M)	Temperature (°C)	pH	NaCl (M)	Temperature (°C)		
7.45	0.1	32.72	7.5	0.1	33	94.40%	93.46 ± 0.7%

yield was explained while only 2.73% was left to the residuals. Apart from this, the P value for lack of fit increased from 0.001 to 0.053 (Table 7) after the omission of the outlier. From Table 7, it is obvious that the new model is valid and linear, and quadratic and interaction terms should be included in the model. A good normal distribution of the model (Figure 4) with a linear line for the percentage recovery confirmed that the model was well fitted with the experimental results and all the major assumptions of the model [35] have been validated.

The three-dimensional response surface plots (Figures 5(a) and 5(c)) show the effects of operating parameters on the percentage recovery while the contour plots (Figures 5(b) and 5(d)) reflect the nature and degree of these effects. As seen from the figures, the response surface plots are concave, indicating that it is possible to obtain a maximum value within the range of the levels investigated. The curved lines in the contour plots confirmed that interaction between the factors ($P < 0.05$) was present and these interaction terms were included in the new model (5). The new regression model was solved for the maximum recovery using the response optimizer tool in MINTAB 15.0, and the optimum values of pH, 7.45; NaCl, 0.1 M; and temperature, 32.72°C were obtained with a maximum predicted response of 94.40% recovery.

3.3. Verification of the Model by Experiment. In order to validate these results, experiments were done in triplicates (Table 8) by using the modified optimized values (pH: 7.5; NaCl: 0.1 M; Temperature: 33°C). A minimum partition coefficient of 0.056 was obtained with a recovery of 93.46 ± 7%. The partition coefficient obtained is in good agreement with the literature for the soluble protein BSA [36]. The good correlation between the observed and predicted recoveries confirmed that the validity of the new model was adequate. This recovery is comparatively high when compared to the precipitation method as discussed earlier [9, 10]. Therefore, this ATPS can be an alternative to the conventional method of protein recovery from tannery wastewater. Moreover, in the current system studied, most of the protein is partitioned to the salt rich bottom phase and it is possible to recycle the PEG from the top phase by ultrafiltration [37, 38]. Therefore recycling of the phase components decreases the overall cost of the process.

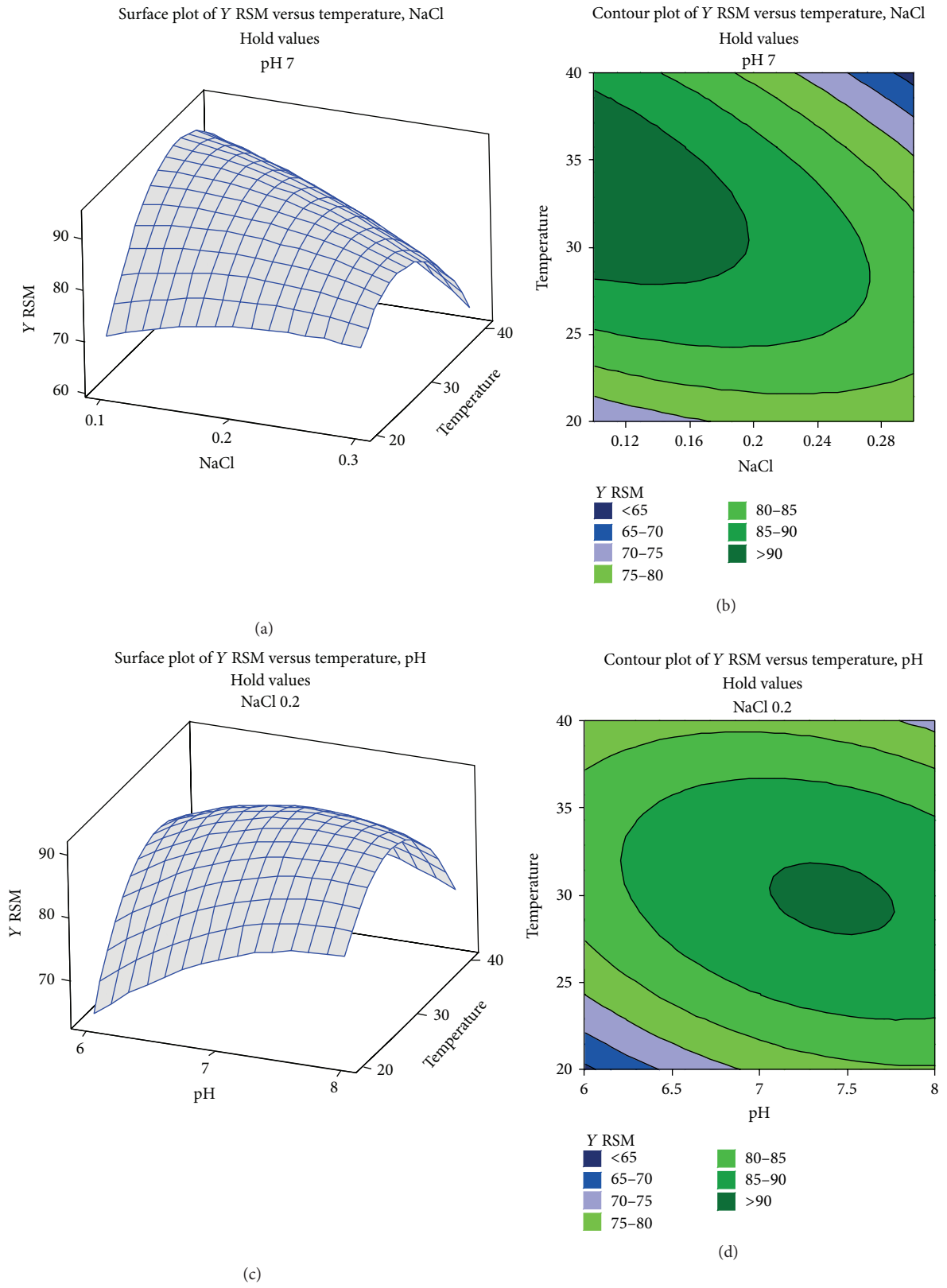

Figure 5: Response surface and contour plots showing the effect of process variables in uncoded values on % recovery. ((a) and (b)) NaCl and temperature; ((c) and (d)) pH and temperature.

4. Conclusions

A sequential optimization method which consisted of fFD and CCD was used to obtain the optimum values of significant factors for the recovery of soluble proteins from tannery wastewater in PEG 6000-SC ATPS. The fFD revealed that only pH, concentration of NaCl, and temperature were the significant factors. From the CCD studies, the optimized values of these significant factors were determined: pH 7.5, NaCl 0.1 M, and temperature 33°C for a phase system composed of 20% (w/w) PEG 6000-15% (w/w) SC. The predicted and observed recoveries were 94.40% and 93.46%, respectively, which confirmed that the proposed quadratic model was valid. Thus, it is concluded that ATPS can be used as an alternative method to recover the valuable soluble proteins from tannery wastewater.

Conflict of Interests

The authors declare that they have no conflict of interests.

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