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Design of experiments for the optimization and statistical analysis of Berberine finishing of polyamide substrates

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Abstract

Design of experiments concept was successfully applied for the determination of optimum Berberine finishing pH, temperature, concentration and time to produce Berberine treated polyamide substrates. Polyamide was treated with Berberine to impart antimicrobial functions as Berberine is a natural antimicrobial agent. The effects of process conditions on the finishing of Berberine were studied in detail using 2^4 central composite design. Experimental results were thoroughly analyzed by Analysis of Variance (ANOVA) statistical concepts. Appropriate predictable empirical models were developed incorporating interaction effects of all variables and then optimized. The significance of the mathematical model developed was ascertained using Microsoft Excel regression (solver) analysis module. The theoretical optimum conditions for maximum exhaustion were found to be pH 10.78, temperature 85 °C, concentration 0.7274% omf and time 84.96 min for Berberine. However, at the optimum conditions, maximum % exhaustion of 97.32% for Berberine was achieved experimentally.

Keywords: Polyamide polymer; Berberine chloride; Optimization; % Exhaustion; Statistical experimental design

1. Introduction

Poly(hexamethylene adipamide) popularly known as polyamide (Nylon 66) is a complex engineering polymer used for wide range of industrial applications. Polyamide polymers have been widely used in many areas, such as apparel, upholstery, floor coverings, hygiene, medical, geotextiles, car industry, automotive textiles, various home textiles and wall-coverings [1]. So, improving and imparting the special property onto the polymers by chemically modifying the functionality of the polymers i.e., functional finishing is an important concept in achieving the desirable property [2]. Durable wrinkle-free cotton [3,4], durable fire resistant cotton [5,6], durable antimicrobial cottons [2,7], antimicrobial wool [8] are examples of some of the chemical modifications of the polymers. In this context of functional finishing, Berberine, a natural cationic colorant, isoquinoline alkaloid and an excellent natural antimicrobial agent, found in roots and rhizomes of the Amur Cork tree extract, Goldenseal Coptis (*Coptis chinensis*), Oregon Grape (*Berberis aquifolium*), Barberry (*Berberis vulgaris*) Tree Turmeric (*Berberis aristata*) and Yerba Mansa (*Anemopsis californica*) [9] was applied as a functional finisher to polyamide polymer. Berberine can be used as a colorant as well as antimicrobial agent, known as natural yellow 18, being one of about 35 yellow dyes from natural sources whose measured UV spectrum is given in Fig. 1. Berberine was successfully applied to cellulose polymers, by providing anionic sites on the cellulose through the reaction with synthesized anionic bridging agent and their antimicrobial ability was tested by the author [10].

But, in the literature for finishing of polymers, only traditional methods of experimentation were followed to study the effects of all variables which are lengthy, random processes and also require large number of experimental

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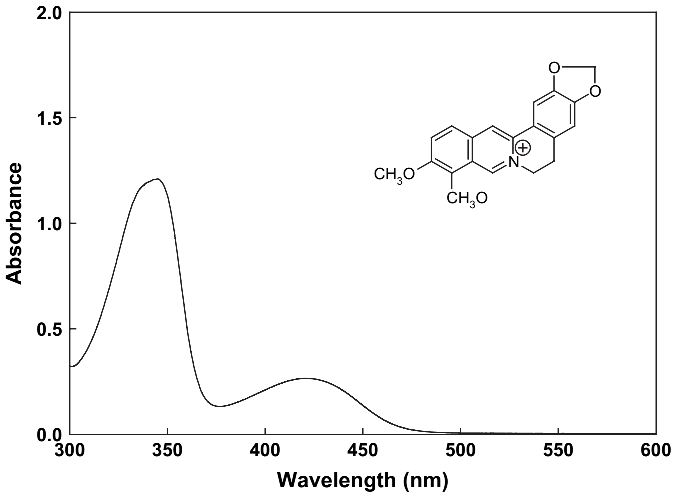

Fig. 1. UV absorption spectrum of Berberine chloride.

combinations to obtain the desired results. In addition, obtaining the optimum conditions i.e., the point at which maximum % exhaustion could be achieved is almost beyond the scope. The traditional step-by-step approach, although widely used, involves a large number of independent runs and does not enable us to establish the multiple interacting parameters. This method is also time consuming, material consuming and requires large number of experimental trials to find out the effects, which are unreliable. So, specifically designed experiments to optimize the system with lesser number of experiments are the need of the hour. These limitations of the traditional method can be eliminated by optimizing all the affecting parameters collectively by statistical experimental design [11].

So, in this research article, experiments were designed by incorporating all important process variables namely pH, temperature, concentration and finishing time using Statistical Design Software i.e., Minitab 14 (PA, USA). Experimental design allows a large number of factors to be screened simultaneously to determine which of them has a significant effect on % exhaustion. A polynomial regression response model shows the relationship of each factor towards the response as well as the interactions among the factors. Those factors can be optimized to give the maximum response (% exhaustion) with a relatively lower number of experiments. In this context, a new approach using statistically designed experiments for developing durable antimicrobial polyamide polymers through interactions with Berberine, a natural antimicrobial agent was discussed in detail. The corresponding interactions among the variables were studied and optimized using central composite design.

2. Experimental

2.1. Reagents and materials

All chemicals used were of analytical grade and doubly distilled water was always used. Polyamide substrate was purchased from Korea Apparel Testing and Research Institute (KATRI). Berberine chloride was purchased from Sigma Co.

2.2. Apparatus

A Hewlett Packard UV–vis spectrophotometer, Model HP8452 was used for measuring the absorbance and recording the normal and derivative spectra. A Corning model 220 pH meter was used for pH measurements.

2.3. Factorial experimental design and optimization of the variables

Temperature, pH, concentration and finishing time were chosen as independent variables and the % exhaustion as dependent output response variable. Independent variables, experimental ranges and levels of Berberine are given in Table 1. The formulated design matrix, shown in Table 2, is a response surface central composite design consisting of 31 sets of coded conditions. It comprises a full replication of 2^4 (= 16) factorial design plus seven center points and eight star points. All the variables at the intermediate level (0) constitute the center points and the combinations of each of the variables at either its lowest (−1) level or highest (+1) level with the other three variables at the intermediate levels constitute the star points. Thus, 31 experimental runs allowed the estimation of the linear, quadratic and two-way interactive effects of the process variables on the % exhaustion. Experimental plan showing the coded value of the variables together with % exhaustion of Berberine is given in Table 2. For statistical calculations, the variables X_i were coded as x_i according to the following relationship:

$$x_i = \frac{X_i - X_0}{\delta X} \quad (1)$$

The results of the experimental design were studied and interpreted by Minitab 14 (PA, USA) statistical software to estimate the response of the dependent variable (% exhaustion). The optimized condition was obtained from contour plot graphically and also by solving the polynomial regression equation using Monte-Carlo optimization technique.

2.4. Berberine finishing

Polyamide polymer (warp 70f24, weft 140f48, 2 g) was dyed with Berberine chloride in sealed, stainless steel dye

Table 1
Experimental ranges and levels of process variables for % exhaustion of Berberine onto polyamide

Independent variables	Ranges and levels				
	−α	−1	0	1	α
pH (X_1)	9	10	11	12	13
Temperature (°C, X_2)	41	60	80	100	117
Concentration (% omf, X_3)	2.25	0.5	3.25	6	8.75
Time (min, X_4)	15	10	35	60	85

Table 2
Central composite design matrix for % exhaustion of Berberine onto polyamide

S. no.	pH	Temperature	Concentration	Time	% Exhaustion of Berberine	
					Exp.	Pred.
1	−1	−1	−1	−1	44.35	44.54
2	1	−1	−1	−1	49.52	49.73
3	−1	1	−1	−1	62.54	60.90
4	1	1	−1	−1	65.84	64.49
5	−1	−1	1	−1	45.25	44.38
6	1	−1	1	−1	48.65	49.47
7	−1	1	1	−1	62.45	60.21
8	1	1	1	−1	66.25	63.70
9	−1	−1	−1	1	60.25	60.28
10	1	−1	−1	1	65.78	66.88
11	−1	1	−1	1	78.36	76.44
12	1	1	−1	1	83.21	81.44
13	−1	−1	1	1	61.45	60.69
14	1	−1	1	1	66.85	67.18
15	−1	1	1	1	77.2	76.32
16	1	1	1	1	84.96	81.20
17	−α	0	0	0	50.7	50.12
18	α	0	0	0	60.49	60.20
19	0	−α	0	0	64.37	61.43
20	0	α	0	0	85.85	91.82
21	0	0	−α	0	73.29	78.92
22	0	0	α	0	58.12	61.61
23	0	0	0	−α	63.29	69.45
24	0	0	0	α	72.34	75.01
25	0	0	0	0	80.24	79.47
26	0	0	0	0	80.24	79.47
27	0	0	0	0	80.24	79.47
28	0	0	0	0	80.24	79.47
29	0	0	0	0	80.24	79.47
30	0	0	0	0	80.24	79.47
31	0	0	0	0	80.24	79.47

pots of 120 cm^3 capacity in a laboratory-scale infrared dyeing machine (ACE-6000T). Dyeing was carried out as per the central composite design matrix given in Table 1. Buffer pH solution was prepared and used all through the experiments. At the end of dyeing, the dyed sample was removed, rinsed thoroughly in tap water and was oxidized in open air. Finally, the % exhaustion was measured by the following formula:

$$\%\ \text{Exhaustion} = \left[\frac{D_0 - D_t}{D_0}\right] \times 100 \tag{2}$$

where D_0 and D_t are the quantities of Berberine in the initial bath and final bath, respectively. Those values were calibrated through absorbance measurement of original bath and exhausted bath by UV–vis spectrophotometer.

2.5. Empirical modeling

Empirical model i.e., second-order polynomial regression equations were developed using Excel solver function to predict the % exhaustion, relating the process variables i.e., pH, temperature, concentration and time. Root Mean Square Error (RMSE) is the important tool to validate the model equation for its prediction capacity [12]. The RMSE is the distance, on average, of a data point from the fitted line, measured along a vertical line. If the value of the RMSE is zero, then the model is perfectly predicting the behaviour of the system i.e., ideal model. The prediction capacity of the model thus decreases with respect to the corresponding value of the RMSE from zero. So, series of equations varying the combinations of the variables like interaction effects and squared effects were run using solver function so as to get the least value of the RMSE. The goodness of fit is a measure of how well the model fits the data. Model is only developed with a sample, and the value of the model depends on the clarity and unambiguity of the relationships between the independent variables.

The behaviour of the system was explained by the following empirical model [12]:

$$Y = \beta_0 + \sum \beta_i x_i + \sum \beta_{ij} x_i^2 + \sum \beta_{ij} x_i x_j \tag{3}$$

where Y is the dependent variable, βs are the regression coefficients, and x are independent data. Root Mean Square Error (RMSE) was calculated using the following formula [12]:

$$\text{RMSE} = \sqrt{\frac{\sum_{0}^{N} (\text{Exp.} - \text{Pred.})^2}{N}} \tag{4}$$

where Exp. is the experimental value, Pred. is the predicted value from model equations and N is the total number of experiments.

2.6. Data analysis

Minitab 14 (PA, USA) was used for the statistical analysis of the experimental data obtained. The quality of fit of the polynomial model equation was expressed by the coefficient of determination R^2 and its statistical significance was analyzed by Fisher's F-test and Student's t-test (Analysis of Variance, ANOVA). The level of significance was given as values of P less than 0.0001. A differential calculation (Monte-Carlo optimization) was then employed for predicting the optimum point.

3. Results and discussion

Berberine finishing on polyamide polymer was carried out as per the central composite experimental plan given in Table 2. Important variables affecting the finishing process such as pH, temperature, concentration and finishing time were studied in detail.

3.1. Effects of process variables on Berberine finishing

The main effects plot which describes the effects of each of the variables on the Berberine finishing is given in Fig. 2. From the plot, it was observed that pH 11 was found to be the suitable condition for maximum % exhaustion. Polyamides have two different end groups, amino and carboxylate, due to

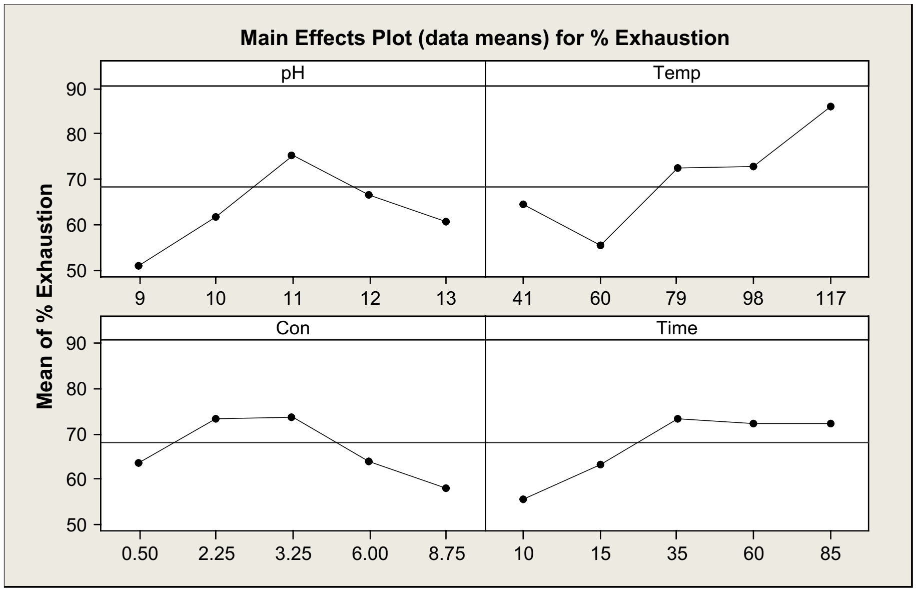

Fig. 2. Main effects plot of variables on % exhaustion of Berberine onto polyamide.

the polymerization reactions employed in preparing the polymers. Both groups are chemically active, and the amino ends have been widely employed in the dyeing of nylon fibers under acidic conditions. Carboxylic acid groups will become more interactive with cationic groups under alkaline conditions, at which these groups can form carboxylate anions (Fig. 3). However, many cationic dyes are not stable at high pH, which might be one reason that such a reaction has not been employed in cationic dyeing of nylon fibers. But, such ionic interaction can be a good access for durable antimicrobial agents, for example, Berberine dyes, which are stable under pH variations. Therefore, antimicrobial finishing of polyamide fibers with Berberine dyes is chemically feasible based on the above analysis. As expected, both acidic and neutral conditions yielded very poor % exhaustion of Berberine on fiber. In contrast, pH 11 led to a higher exhaustion since the Berberine is more attractive to the negatively charged carboxylate groups under the basic condition. Due to ionic interactions, Berberine was quickly adsorbed and diffused into fibers. The higher exhaustion of Berberine is expected in better antimicrobial functions on the Berberine treated fibers.

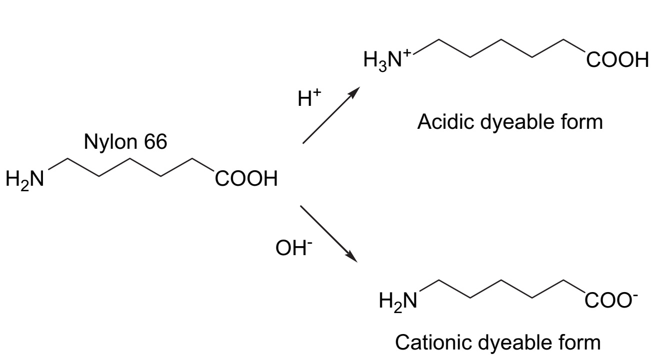

Fig. 3. Dissociation scheme of polyamide molecule at acidic and alkaline condition.

The experiments were started from 60 °C i.e., glass transition temperature of polyamide as the author indicated in previous publication [12] that significant exhaustion of the cationic salts did not occur until the finishing temperature was above the glass transition temperature (57 °C). The data show that good % exhaustion was achieved by a range of temperatures above its glass transition temperature. Above the glass transition temperature, the amorphous regions of polyamide will provide more free volume to diffuse more Berberine and as a result higher exhaustion of Berberine occurs. In addition, a swelling effect resulted from the higher temperature and alkaline conditions should facilitate diffusion of Berberine into polyamide substrate. Similar kind of results were observed for ionic interaction of cationic antimicrobial salts with polyamide fibers by the author [12].

Diffusion of Berberine into polyamide polymer depends on the concentration of Berberine in the finishing bath. The concentration of Berberine directly affects the uptake rates of salts on polyamide polymer due to ionic interactions between the carboxylate groups and Berberine. From the main effects plot (Fig. 2), it was observed that lower concentration resulted in higher % exhaustion and increase in concentration resulted in decrease in % exhaustion. Maximum % exhaustion was achieved at the concentration of 2.25% omf for Berberine. This is may be attributed to the fact that polyamides have limited carboxylic end groups and an excess amount of Berberine in the finishing bath could not produce a higher exhaustion than the maximum amount of the end groups that could react with Berberine and instead play a negative role in decreasing the exhaustion of Berberine onto polyamide.

Usually, the Berberine can only enter into amorphous regions in the polymer by diffusion, while polyamide fibers contain about 30% of amorphous areas and 70% of crystalline structure. The majority of the polymer structure is crystalline, which is tightly packed and difficult to penetrate for Berberine.

So, finishing time is quite important for the better exhaustion of Berberine onto polyamide. From the main effects plot, it was observed that higher treatment time leads to higher exhaustion of Berberine. This indicates that higher the contact time between Berberine and polyamide polymer, higher the diffusion of Berberine into the polyamide polymer and hence, higher the exhaustion.

3.2. Statistical analysis

Using the experimental results, the regression model equations (second-order polynomial) relating the % exhaustion and process variables were developed and are given in Eq. (5).

Polynomial regression equation for % exhaustion of Berberine onto polyamide polymer:

$$\begin{aligned} Y = & -769.53 + 137.43X_1 + 0.9550X_2 + 3.9989X_3 + 0.7707X_4 \\ & - 6.0775(X_1X_1) - 0.0019(X_2X_2) - 0.5842(X_3X_3) \\ & - 0.0084(X_4X_4) + 0.021(X_1X_2) - 0.0096(X_1X_3) \\ & - 0.01400(X_1X_4) - 0.00256(X_2X_3) - 0.00010(X_2X_4) \\ & + 0.002045(X_3X_4) \quad (R^2 = 0.9685) \end{aligned} \qquad (5)$$

Apart from the linear effect of the variables for exhaustion, the design of experiments gives an insight into quadratic and interaction effects of the variables. These analyses were done by means of Fisher's *F*-test and Student's *t*-test. Student's *t*-test was used to determine the significance of the regression coefficients of the variables. The *P*-values were used as a tool to check the significance of the variables, which in turn may indicate the patterns of the interactions among the variables. In general, larger the magnitude of *t* and smaller the value of *P*, the more significant is the corresponding coefficient [12]. The regression coefficient, *t*- and *P*-values for all linear, quadratic and interaction effects of the variables are given in Table 3.

The predicted values (using model equations) were compared with experimental results for Berberine and the data are shown in Table 2 and are also graphically represented in Fig. 4. It was observed that the coefficients for the linear effect of pH were highly significant ($P = 0$) and other three variables, namely temperature ($P = 0.25$), concentration ($P = 0.34$) and time ($P = 0.094$) were considered to be least significant. The squared effect of pH, concentration and time ($P = 0$) were considered to be highly significant and only temperature was the least significant parameter with *P*-value of 0.35. None of the variables were found to be significant for the interaction effect, having *P*-value greater than 0.8. The significance of this quadratic and squared effects among the variables would have been lost if the experiments were performed by conventional or traditional approach.

Although few studies on the effects of variables on % exhaustion of polyamide polymer have been reported in the literature, no attempt has been made to optimize them using statistical optimization methods. But, in this work, the model Eq. (5) was optimized using multistage Monte-Carlo optimization technique [13]. The optimum values of the process variables were first obtained in coded units and then converted to uncoded i.e., real units using Eq. (1). The optimum values of the process variables for the maximum % exhaustion are shown in Table 4. These results closely agree with those obtained by the response surface analysis i.e., graphically by contour and surface plots, confirming that the design of experiments concept could be effectively used to optimize the process variables in complex processes using the statistical design of experiments.

The contour and surface plots are model dependent and are useful for establishing desirable response values and operating conditions. In a contour plot, the response surface is viewed as a two-dimensional plane where all points that have the same response are connected to produce contour lines of constant responses. A surface plot displays a three-dimensional view that may provide a clear picture of the response surface. The stationary point or central point in the contour plot is the point

Table 3
Estimated regression coefficients and corresponding *t*- and *P*-values for Berberine

Term	Coefficients	SE coefficients	*t*	*P*
Constant	−769.53	83.5851	−7.973	0.000
X_1	137.43	13.5611	8.943	0.000
X_2	0.9550	0.5369	1.195	0.250
X_3	3.9989	3.5391	0.983	0.340
X_4	0.7707	0.3917	1.782	0.094
X_1X_1	−6.0775	0.5932	−9.171	0.000
X_2X_2	−0.0019	0.0016	−0.947	0.358
X_3X_3	−0.5842	0.1060	−5.546	0.000
X_4X_4	−0.0084	0.0013	−6.550	0.000
X_1X_2	0.021	0.0420	0.016	0.987
X_1X_3	−0.0096	0.2904	0.118	0.907
X_1X_4	−0.01400	0.0319	0.616	0.547
X_2X_3	−0.00256	0.0153	−0.109	0.915
X_2X_4	−0.00010	0.0017	0.007	0.994
X_3X_4	0.002045	0.0116	0.196	0.847

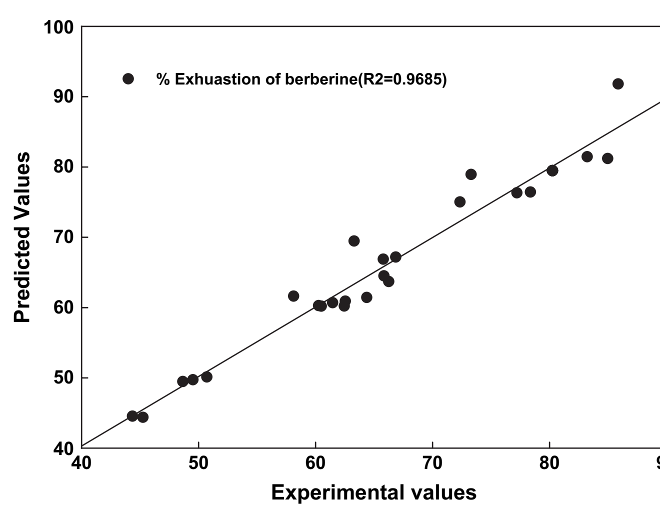

Fig. 4. Experimental and predicted values of % exhaustion of Berberine.

Table 4
Optimum conditions for maximum % exhaustion of Berberine onto polyamide

Parameter	Optimum value
PH	10.78
Temperature	85
Concentration	0.7274
Time	84.96

Table 5
ANOVA for % exhaustion of Berberine onto polyamide

Source	Degree of freedom (d.f.)	Sum of squares (SS)	Mean square (MS)	$F_{statistics}$	P
Regression	14	4420.07	315.720	30.94	0.000
Linear	4	2563.35	211.554	20.73	0.000
Square	4	1852.19	463.048	45.38	0.000
Interaction	6	4.53	0.755	0.07	0.998
Residual error	16	163.27	10.204		
Lack of fit	10	163.27	16.327		
Pure error	6	0.00	0.00		
Total	30	4583.34			

at which the slope of the contour is zero in all directions. The coordinates of the central point within the highest contour levels in each of these figures will correspond to the optimum values of the respective constituents. The maximum predicted yield is indicated by the surface confined in the smallest curve of the contour diagram [14].

The contour plots given in Fig. 5 show the relative effects of any two variables when the remaining variables are kept constant for all combinations. The response contour plots of mutual interactions among the variables were found to be elliptical. The similar types of trends were found in literature [14] for dye removal. The optimum values drawn from Fig. 5 are in close agreement with those obtained by optimizing the regression model Eq. (5) using Monte-Carlo technique. This confirms that the design of experiments could be effectively used to optimize the process variables using the statistical design of experiments concept. Experiments were carried out at the optimum conditions obtained by the theoretical analysis and highly feasible results were obtained that are being tabulated in Table 4.

The statistical significance of the ratio of mean square variation due to regression and mean square residual error was tested using Analysis of Variance (ANOVA). ANOVA is a statistical technique that subdivides the total variation in a set of data into component parts associated with specific sources of variation for the purpose of testing hypotheses on the parameters of the model [14]. According to the ANOVA, which is shown in Table 5 for Berberine, the $F_{statistics}$ values for all regressions were higher. The large value of $F_{statistics}$ indicates that most of the variation in the response can be explained by the regression model equation. The associated P-value is used to estimate whether $F_{statistics}$ is large enough to indicate statistical significance. A P-value less than 0.0001 (i.e., $\alpha = 0.0001$, or 99.99% confidence interval) indicates that the model is considered to be statistically significant [14]. The ANOVA table also shows a term for residual error, which measures the amount of variation in the response data left unexplained by the model. The form of the model chosen to explain the relationship between the factors and the response is correct.

The $F_{statistics}$ values of 90.94 for Berberine are greater than tabulated $F_{14,16}$ values which indicate that the fitted model exhibits no lack of fit (0.001 for Berberine) at the confidence level. ANOVA for Berberine indicated that the second-order polynomial model (Eq. (5)) was highly significant and adequate to represent the actual relationship between the response (% exhaustion) and the variables, with zero P-value for Berberine and a very high coefficient of determination ($R^2 = 0.9685$). This implies that 96.85% of the sample variation for Berberine is explained by the independent variables and this also means that the model did not explain only about 0.315% of sample variation for Berberine.

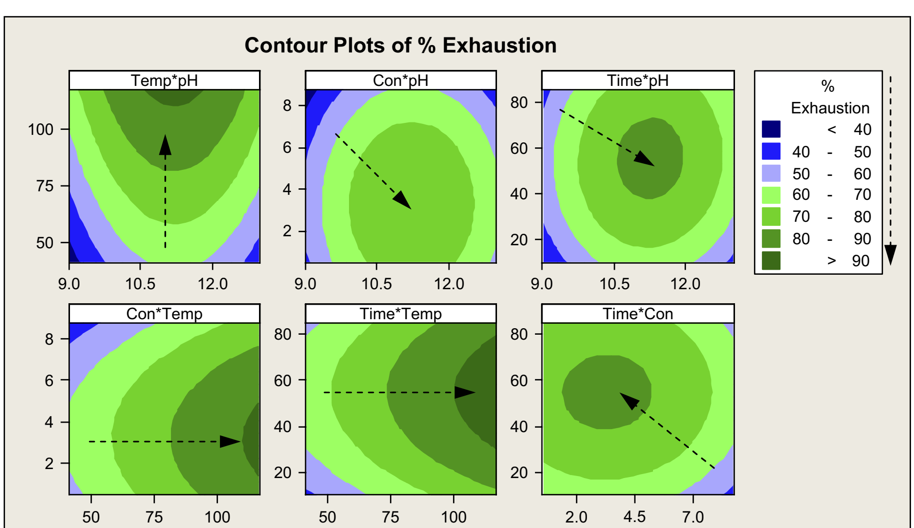

Fig. 5. Response contour plot for the % exhaustion of Berberine onto polyamide.

4. Conclusions

Berberine chloride, natural yellow 18 colorant, as well as antimicrobial agent was successfully applied to polyamide polymer and effects of four important variables namely pH, temperature, concentration and time were studied in detail. A new methodology, 2^4 response surface central composite design was successfully employed for experimental design and analysis of results i.e., to study the linear, quadratic and interaction effects of each of the variables and also to optimize those variables for maximum % exhaustion. Appropriate empirical model equations were developed for predicting the % exhaustion for Berberine using Excel solver functions. Graphical response contour plots were used to locate the optimum points.

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