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RESEARCH ARTICLE

Application of Face Centered Central Composite Design in Evolution of Optimized Drug Delivery System of Golden Nutraceutical with Frame Independent Variables

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Abstract: ***Background*:** Poor solubility of curcumin results in poor rate of absorption, rapid biotransformation as well as rapid rate of elimination. Due to this curcumin does not show better therapeutic effect through oral administration, to avoid poor rate of absorption, rapid biotransformation and topical drug delivery system plays a vital role.

***Objective*:** The objective of the present work was the successful implementation of face-centered central composite design (FCCCD) to study various independent variables to develop an optimized formulation.

***Methods*:** Carbopol 934 P and menthol were considered as independent variables and their impact was determined on dependent responses like drug content, viscosity, and percent drug release by developing topical gels using 3^2 central composite design. Thirteen curcumin loaded topical gels were prepared employing 3^2 CCD. Characterization of these formulations was done by drug content, viscosity, and percent drug release.

***Results*:** The effect of independent variable, *i.e.*, the concentration of Carbopol 934P and menthol was determined over the dependent variables by developing a response surface methodology. Optimized batch was investigated employing design expert software by overlay plot developed by statistical software with drug content, viscosity, and percent drug release 99.34%, 82.10 cps, and 65.576% respectively. Optimized data was evaluated by formulating four validation check batches. Promising results were observed by comparing the predicted values with experimental values, which proves the validity of the optimized data.

***Conclusion*:** Therefore, it can be concluded that the application of face-centered central composite design is a useful statistical tool to get the optimized formulation with the least manpower, material, and money.

ARTICLE HISTORY

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1. INTRODUCTION

Central composite design (CCD) is a popular statistical tool for optimization study determining the effect of independent variables on dependent variables by creating mathematical equations and graphs by response surface methodology [1, 2].

Response surface methodology (RSM) is a methodology used to determine functional relationship between variables and a set of independent variables to get optimized drug delivery systems. RSM helps in reducing the number of experimental batches that are necessary to generate mathematical equations in the design of an experiment to identify the optimum level of independent variables required for a desired response [3].

Curcumin (CUR), as golden drug, has its wide pharmacological applications. It is also referred to as a magical remedy for the treatment of chronic and nonchronic diseases. However, cardiac disease, carcinoma, and neurological disorders occur due to disturbances of multiple signaling pathways**.** Poor solubility of CUR results in poor rate of absorption, rapid biotransformation as well as rapid rate of elimination, due to this CUR does not show better therapeutic effect through oral administration, to avoid poor rate of absorption as well as rapid biotransformation topical drug delivery system plays a vital role [4-9].

Therefore, the aim of the present work is developing an optimized topical gel of curcumin by employing face-centered central composite design with the help of response surface methodology [10].

2. MATERIALS AND METHODS

CUR powder was purchased from Sigma Aldrich, Mumbai, India. Carbopol 934P was purchased from Sigma Aldrich Chemicals, Bangalore. Menthol was purchased from procured as gift sample from GenekaHealthcare, Haridwar, India. Triethanolamine was procured from Fischer Chemi-

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cals Ltd., Chennai. Analytical. All chemicals and solvents used were of analytical grade.

2.1. Experimental Design

A face-centered central composite design was applied as per the standard protocol for the formulation of topical gels of CUR, which was selected as a model drug. In the present work, the concentration of Carbopol 934 (CP 934) and methanol was selected as independent variables and drug content, viscosity, and percent drug release were determined as dependent variables. All experimental runs, coded and actual levels of independent variables are summarized in the below-mentioned table (Table **1**).

The function of independent variables was expressed by polynomial equations. The effect of the independent variables can be expressed by a common polynomial (Equation: 1)

$$Y1 = b_0 + b_1X_1 + b_2X_2 + b_3X_1X_2 + b_4X_1^2 + b_5X_2^2 + b_6\ X_1X_2^2 + b_7X^2{}_1X_2 \quad (1)$$

Here Y1 is used to express as a dependent variable while b0 is used to express the arithmetic mean response of all 13 runs.

2.2. Formulation of Topical Gel of Curcumin

CP 934P was added to the distilled water. CUR was taken and dissolved with some amount of ethanol. Prepared ethanolic solution of CUR was then added to the previously prepared aqueous solution of CP 934 and stirred mechanically (RemiMotorsLtd, Mumbai, India) and allowed to stand for 3 h. Triethanolamine was taken and added to this solution to formulate the gel formulation of CUR. The pH was the formulation was adjusted to 6.8. [11].

2.3. Evaluation of Formulated Batches

2.3.1. Drug Content Studies

Drug content study of the formulated batches was observed by dissolving the desired amount of gel (≈100 mg) in phosphate buffer pH 7.2 (50ml) containing 20% v/v ethanol. These solutions were transferred and filtered using membrane filter 9 (0.45 µm). Drug content was calculated using UV-Vis spectrophotometer at 425 nm wavelength maxima [12, 13].

2.3.2. Determination of Viscosity

The viscosity of the prepared gel (in cps) was measured by using Brookfield Viscometer (RVTDV II). The gels were allowed to settle for 30m while the spindle was rotated at 100 rotations per minute and the viscosity values were noted [14].

2.3.3. In-vitro Diffusion Studies

The *in vitro* drug release study was carried out using Franz-diffusion cell apparatus. The effective diffusion area of the Franz cells was 59.6 ± 3.1 mm^2. Formulated gel was allowed to spread over the cellophane membrane to mimic the diffusion process of skin; which was fixed between the donor and receptor compartment. Phosphate buffer pH 6.8 was used to fill the receptor compartment with continuous stirring using a magnetic bar at 50 rpm. Temperature of the system was maintained at about 37 ± 0.5°C. The aliquots were withdrawn continuously at 1, 2, 3, 4, 5, 6, 7, and 8 h while the volume of the chamber was replenished with the same volume of phosphate buffer. The samples withdrawn were analyzed spectrophotometrically by UV-Vis spectrophotometer (Shimadzu UV-1800, Japan) at 424 nm [15].

Table 1. Central composite design and level of independent variables.

Formulation Code	Coded Value		Actual Value (mg)	
-	Factor A	Factor B	Factor A	Factor B
TG1	1	0	3.0	5.0
TG2	0	-1	2.0	2.5
TG3	1	-1	3.0	2.5
TG4	-1	0	1.0	5.0
TG5	0	0	2.0	5.0
TG6	-1	1	1.0	7.5
TG7	0	0	2.0	5.0
TG8	0	0	2.0	5.0
TG9	-1	-1	1.0	2.5
TG10	0	0	2.0	5.0
TG11	0	1	2.0	7.5
TG12	1	1	3.0	7.5
TG13	0	0	2.0	5.0

2.3.4. Optimization and Data Validation

For the optimization study, thirteen batches were developed by employing 32 central composite designs by selecting nine possible combinations and the centre point was repeated four times. The impact of independent variables was observed using design expert software (DX8, trial version). Significance of the models was tested. Optimized batch selection was made for the desired results of dependent variables. Four formulations were (VTG1 –VTG4) developed and validated by employing the response surface methodology. Experimentally observed responses were compared with predicted responses by calculating the percent prediction error (% bias) [16].

2.3.5. Accelerated Stability Studies

Stability studies were carried out on the optimized formulation according to International Conference on Harmonization (ICH) guidelines. The formulation packed in aluminium tube was subjected to accelerated stability testing for 3 months as per ICH norms at a temperature (40 ± 2°C) and relative humidity 75 ± 5%. Samples were taken at regular time intervals of 1 month over a period of 3 months and analyzed for the change in drug content, viscosity, and *in vitro* drug release. Any changes in evaluation parameters, if observed, were noted. Tests were carried out in triplicate and the mean value of the observed values was noted along with the standard deviation.

3. RESULTS AND DISCUSSION

3.1. Drug Content and Viscosity

Drug content and viscosity of all formulations were found in ranges of 98.5- 99.6% and (74 $x10^3$-88 $x10^3$ cps (Table **2**). It was observed that the values of drug content increased with the increase in the concentration of CP 934 while a slight decrease was observed when concentration of menthol increased. Results also indicate that the viscosity increases with increase in the concentration of CP 934 while decreases as the concentration of menthol increases.

3.2. *In-vitro* Diffusion Study

In vitro diffusion studies of all batches were performed and it was observed between 24.65-75.17 % as shown in Table **2**. Hereby *in vitro* diffusion of formulation decreased by increase in the concentration of CP 934P but remarkably an increase in diffusion was observed by increasing the concentration of menthol (Fig. **1**).

3.3. Data Analysis

Statistically analyzed data clearly indicate that the drug content (Y_1), viscosity(Y2), and drug release (Y_3) values are heavily dependent on the selected independent variables. Equations 1-3, which relate the responses of drug content (Y_1), viscosity(Y2), and drug release (Y_3) as ANOVA response for various dependent variables, are like (equations 2-4):

$$Y1=99.26+0.35X1-0.20X2+0.075X1X2-0.11X12-0.060X22+0.025X12X2+0.025X1X22 \quad (2)$$

$$Y2 =79.80+4.00X1-2.00X2+0.001X1\ X2-0.80X12+0.200X22+0.50X12X2+1.50X1X22 \quad (3)$$

$$Y3 =56.27-3.06X1+21.27X2+0.91X1X2-3.80X12-5.77X22-0.59X12X2-1.52X1X22 \quad (4)$$

Table 2. Characterization of formulated Batches of curcumin loaded topical gels.

Formulation Code	Carbopol 934 (%)	Menthol (ml)	Drug Content (%) (n=3)	Viscosity (Cpsx1000) (n=3)	% Drug Release (%) (n=3)
TG1	1	0	99.500 ± 0.034	83.000 ± 0.1.110	49.410 ± 0.880
TG2	0	-1	99.400 ± 1.220	82.000 ± 1.020	29.230 ± 1.080
TG3	1	-1	99.600 ± 0.950	88.000 ± 0.660	24.650 ± 1.190
TG4	-1	0	98.800 ± 0.340	75.000 ± 0.850	55.530 ± 0.730
TG5	0	0	99.300 ± 0.260	80.000 ± 0.630	52.080 ± 0.540
TG6	-1	1	98.500 ± 0.370	74.000 ± 0.430	75.170 ± 0.190
TG7	0	0	99.200 ± 1.010	79.000 ± 0.210	51.780 ± 0.270
TG8	0	0	99.100 ± 0.470	80.000 ± 0.190	53.390 ± 0.650
TG9	-1	-1	99.000 ± 0.090	77.000 ± 0.670	35.630 ± 0.810
TG10	0	0	99.300 ± 0.760	80.000 ± 0.580	52.110 ± 0.730
TG11	0	1	99.000 ± 0.250	78.000 ± 0.070	71.770 ± 0.090
TG12	1	1	99.400 ± 0.120	85.000 ± 0.730	67.820 ± 0.260
TG13	0	0	99.400 ± 0510	80.000 ± 0.650	51.990 ± 0.590

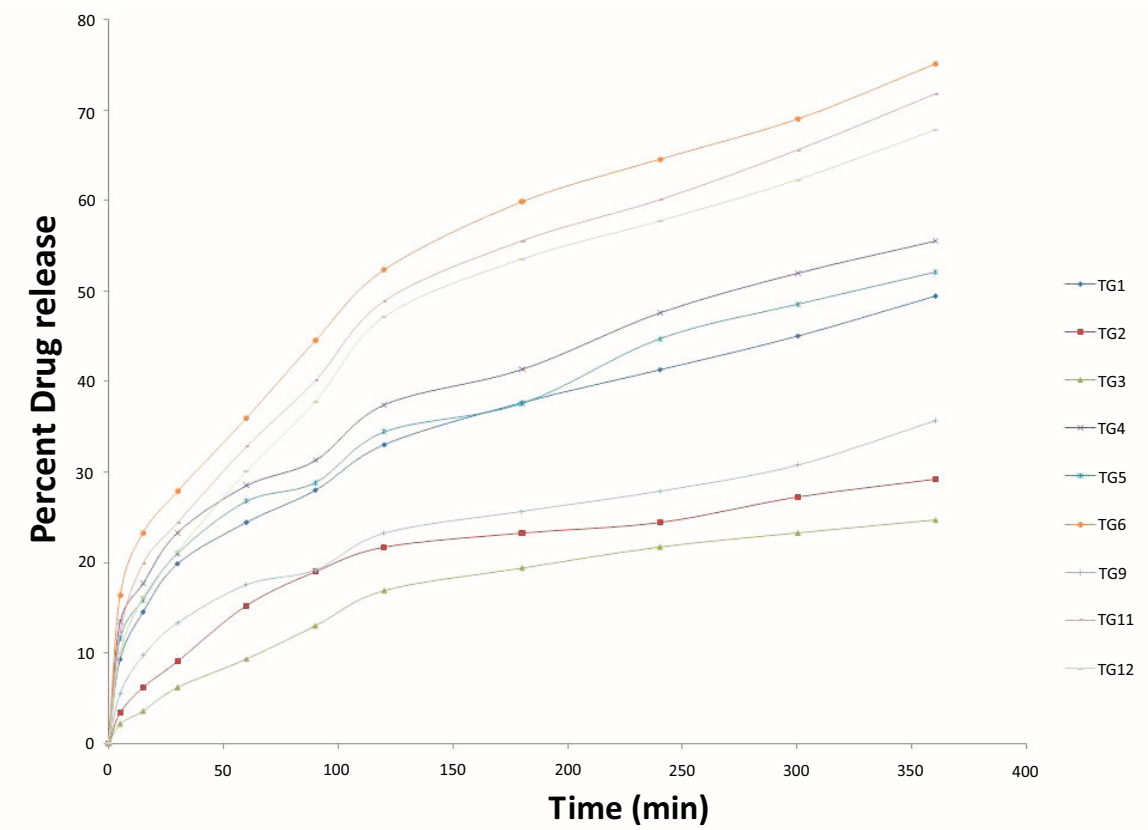

Fig. (1). *In-vitro* drug release profile of formulated batches. (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

Table 3. ANOVA statistical data for drug content.

Source	Sum of Squares	df	Mean Square	F-value	*p*-value	
Model	1.0800	8	0.1347	10.3600	0.0193	
A-Carbpol	0.2450	1	0.2450	18.8500	0.0122	
B-Menthol	0.0800	1	0.0800	6.1500	0.0682	
AB	0.0225	1	0.0225	1.7300	0.2587	
A^2	0.0173	1	0.0173	1.3300	0.3131	
B^2	0.0051	1	0.0051	0.3956	0.5635	significant
A^2B	0.0008	1	0.0008	0.0641	0.8126	
AB^2	0.0008	1	0.0008	0.0641	0.8126	
A^2B^2	0.0008	1	0.0008	0.0650	0.8114	
Pure Error	0.0520	4	0.0130	-	-	
Cor Total	1.1300	12	-	-	-	

In Table **3**, the sum of squares is Type III, which - partially, The Model F-value of 10.36 implies the model is significant. There is only a 1.93% chance that an F-value this large could occur due to noise. In Table **4**, the sum of squares is Type III *i.e*. partial. The Model F-value of 110.08 implies the model is significant. There is only a 0.02% chance that an F-value this large could occur due to noise. In Table **5**, Factor coding is Coded. Sum of squares is Type III – partial. The Model F-value of 3.83 implies the model is significant relative to the noise. There is a 10.52% chance that an F-value this large could occur due to noise.

Table 4. ANOVA statistical data for viscosity.

Source	Sum of Squares	df	Mean Square	F-value	*p*-value	
Model	176.1200	8	22.0200	110.0800	0.0002	
A-Carbpol	32.0000	1	32.0000	160.0000	0.0002	
B-Menthol	8.0000	1	8.0000	40.0000	0.0032	
AB	0.0000	1	0.0000	0.0000	1.0000	
A^2	0.9143	1	0.9143	4.5700	0.0993	
B^2	0.0571	1	0.0571	0.2857	0.6213	significant
A^2B	0.3333	1	0.3333	1.6700	0.2663	
AB^2	3.0000	1	3.0000	15.0000	0.0179	
A^2B^2	2.2300	1	2.2300	11.1700	0.0288	
Pure Error	0.8000	4	0.2000	-	-	
Cor Total	176.9200	12	-	-	-	

Table 5. ANOVA statistical data for percent drug release.

Source	Sum of Squares	df	Mean Square	F-value	*p*-value	
Model	2806.4700	8	350.810	3.830	0.0052	
A-Carbpol	18.7300	1	18.730	0.2044	0.6746	
B-Menthol	904.8300	1	904.830	9.880	0.0348	
AB	3.2900	1	3.290	0.0360	0.8588	
A^2	20.6300	1	20.630	0.2252	0.6599	
B^2	47.5600	1	47.560	0.5192	0.5111	significant
A^2B	0.4680	1	0.468	0.0051	0.9464	
AB^2	3.0900	1	3.090	0.0337	0.8632	
A^2B^2	11.6900	1	11.690	0.1276	0.7390	
Pure Error	366.4300	4	91.610	-	-	
Cor Total	3172.9100	12	-	-	-	

3.4. Response Surface Analysis

Response surface curve for drug content (Y_1), viscosity (Y2), and drug release (Y_3) were of both independent variables, *i.e*., concentration of CP 934P and menthol. There was an almost linear increase in the drug content with increase in the levels of CP 934 P while a slow decrement upon increasing the concentration of menthol. The counter plot also shows that the combined effect is almost increasing with drug content (Fig. **2**).

Fig. (**3**) illustrates the impact of CP 934P menthol and its combined impact on the viscosity of the formulated gel of CUR. Response surface curves indicate that the viscosity of the formulated gel of CUR increases with increase in the concentration of CP 934 while decreases as the concentration of menthol increases. The counter plot also shows that the combine effect although is increasing but not totally linear with viscosity.

Fig. (**4**)_ illustrates the effect of independent variable, *i.e*., CP 934P and menthol as well as their combined effect on drug release of the formulated gel of CUR. Response surface curves indicate that the drug release from the formulated gel of CUR decreases as the concentration of CP 934 increases while as the concentration of menthol increases.

It was observed that the values of drug content increase by increase in concentration of CP 934 while a slight decrease was observed when concentration of menthol increases. Results also indicate that the viscosity increases with increase in the concentration of CP 934 and increases with the increase in the concentration of menthol. Overlay plot (Fig. **5**) was also plotted with the help of design expert software in the darker area showing the desired values for the optimized formulation to confirm that the optimized product is within the desired area.

3.5. Data Validation

Data validation of the optimized formulation was done by formulating four different validation check batches VTG1-VTG4 which were evaluated for their dependent response. These experimentally observed responses were compared with their predicted values, which were found to be in close agreement as predicted by the model developed (Table **6**). Hence, one can say that this developed model was found to be significant as well as prove the validity of the predicted values were also very close to the experimental results (Figs. **6**-**8**).

3.6. Stability Study

Table **7** shows that there was no significant change in the dependent responses of the optimized formulation even after its exposure to accelerated conditions of temperature (40ºC) and humidity conditions (75 ± 5%RH). Hence, the optimized formulation was found to be stable after subjecting to accelerated stability conditions.

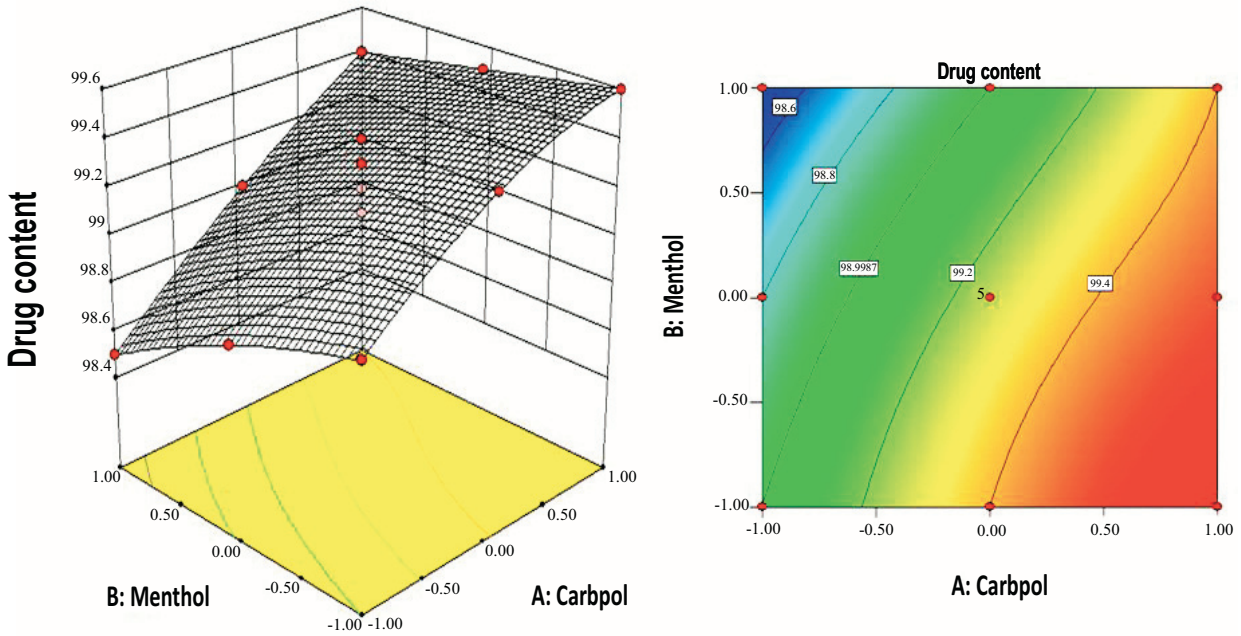

Fig. (2). Response surface and contour plot for drug content. (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

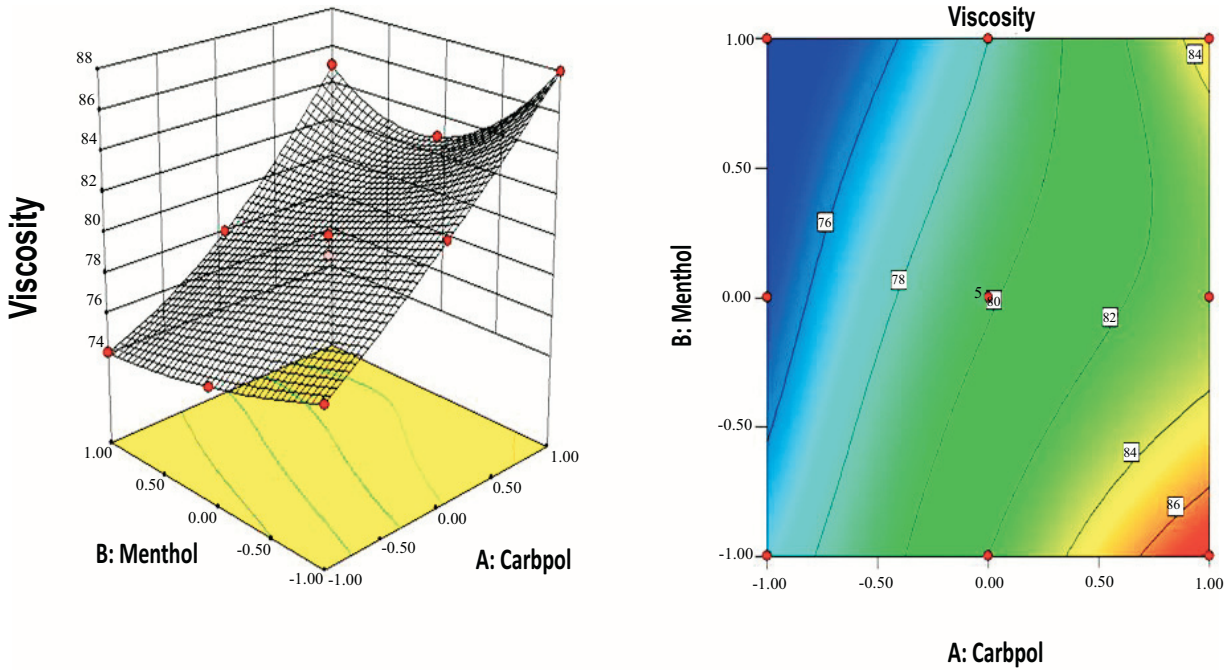

Fig. (3). Response surface and contour plot for viscosity. (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

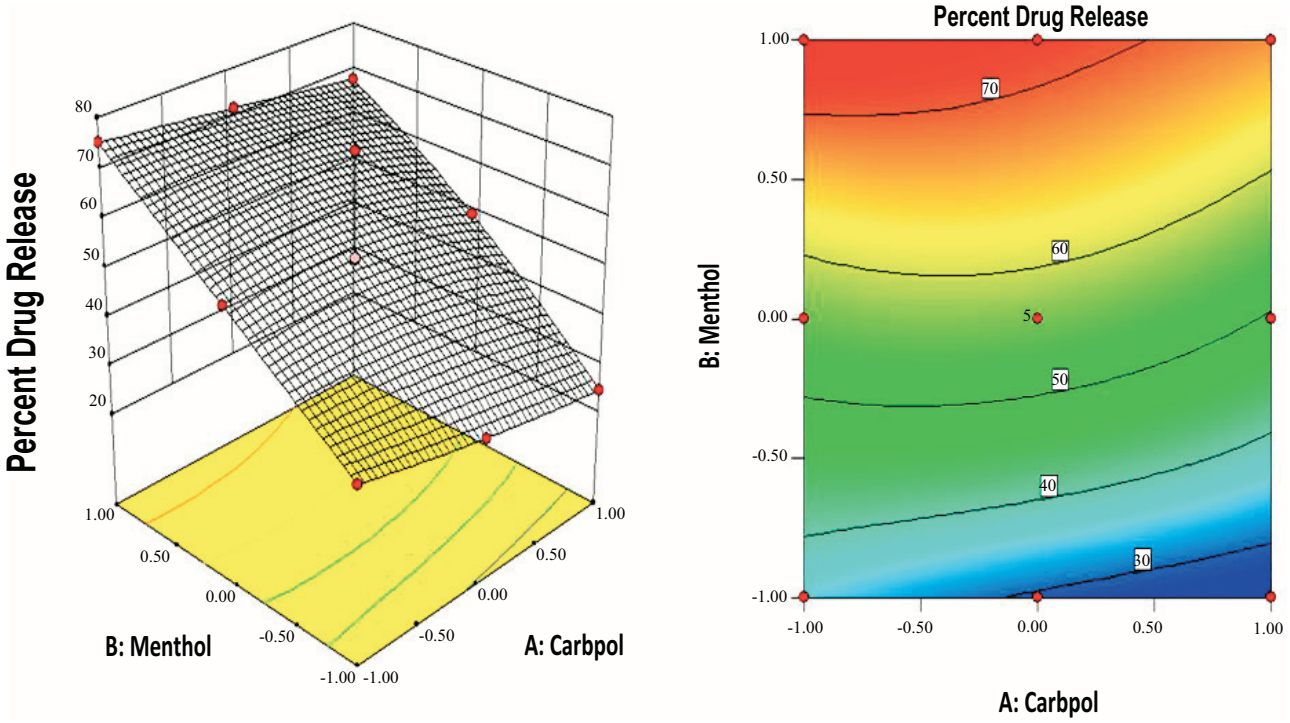

Fig. (4). Response surface and contour plot for percent drug release. (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

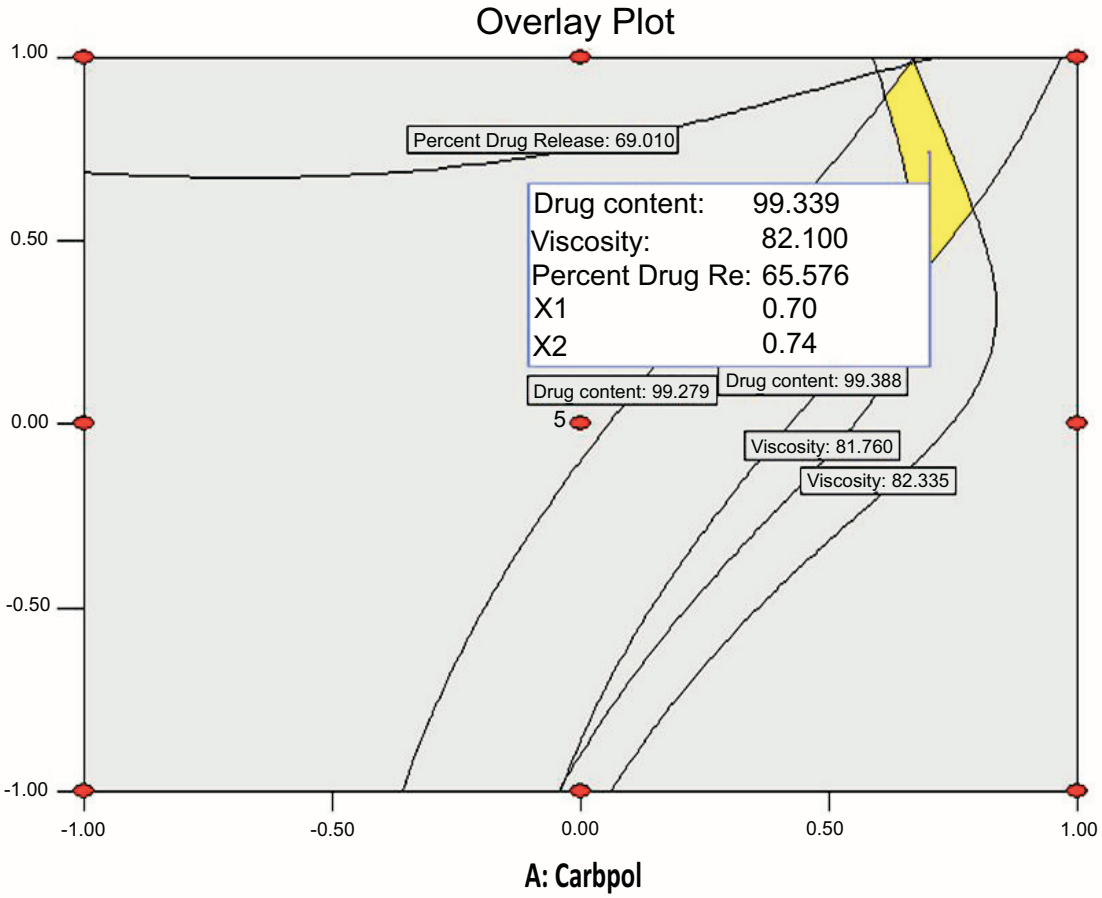

Fig. (5). Overlay plot of optimized batch as depicted by the flag. (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

Table 6. Validation of data for optimized batch.

Batch Code	CP 934P	Menthol	Response	Predicted Value	Observed Value	% Error
Optimized Batch	0.700	0.740	Drug content	99.339	99.335 ± 1.030	0.0040
			Viscosity	82.100	82.130 ± 0.034	-0.0365
			Percent drug release	65.576	65.410 ± 0.140	0.2531
VTG1	0.700	0.440	Drug content	99.385	99.388 ± 0.730	-0.0030
			Viscosity	81.830	81.850 ± 0.884	-0.0244
			Percent drug release	60.925	60.950 ± 0.090	-0.0410
VTG2	0.630	0.890	Drug content	99.286	99.289 ± 0.630	-0.0030
			Viscosity	81.881	81.876 ± 0.020	0.00610
			Percent drug release	68.014	68.601 ± 0170	-0.8630
VTG3	0.670	0.940	Drug content	99.290	99.280 ± 0.410	0.01007
			Viscosity	82.210	82.270 ± 0.330	-0.0729
			Percent drug release	68.420	68.320 ± 0.090	0.14616
VTG4	0.770	0.580	Drug content	99.380	99.390 ± 0.300	-0.0100
			Viscosity	82.240	82.220 ± 0.770	0.02431
			Percent drug release	62.730	62.630 ± 0.290	0.15941

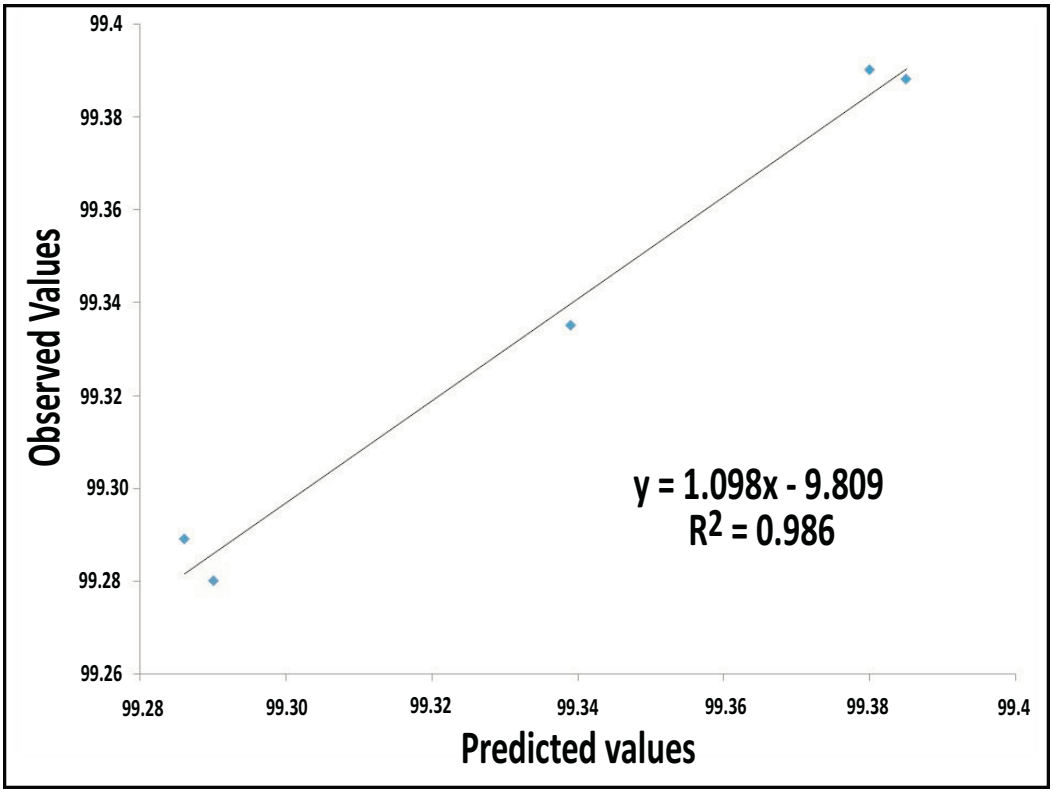

Fig. (6). Predicted *versus* observed response for drug content. (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

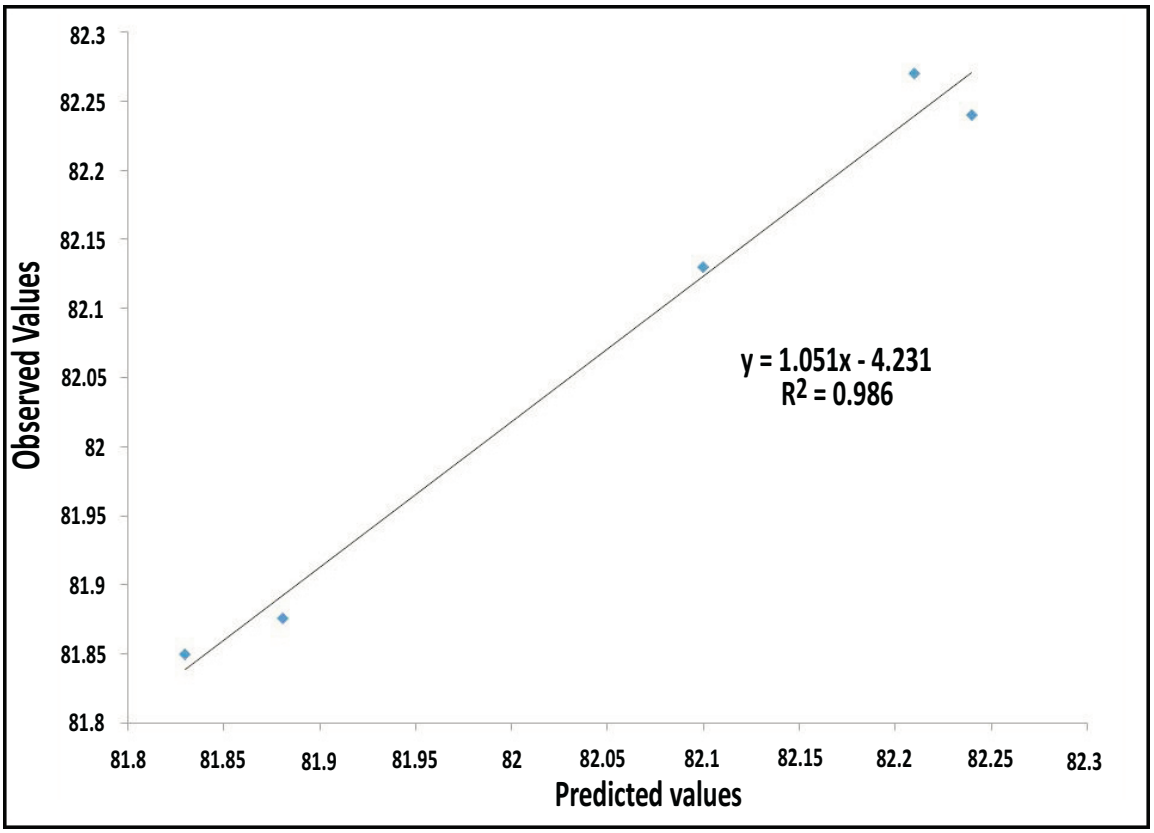

Fig. (7). Predicted *versus* observed response for viscosity. (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

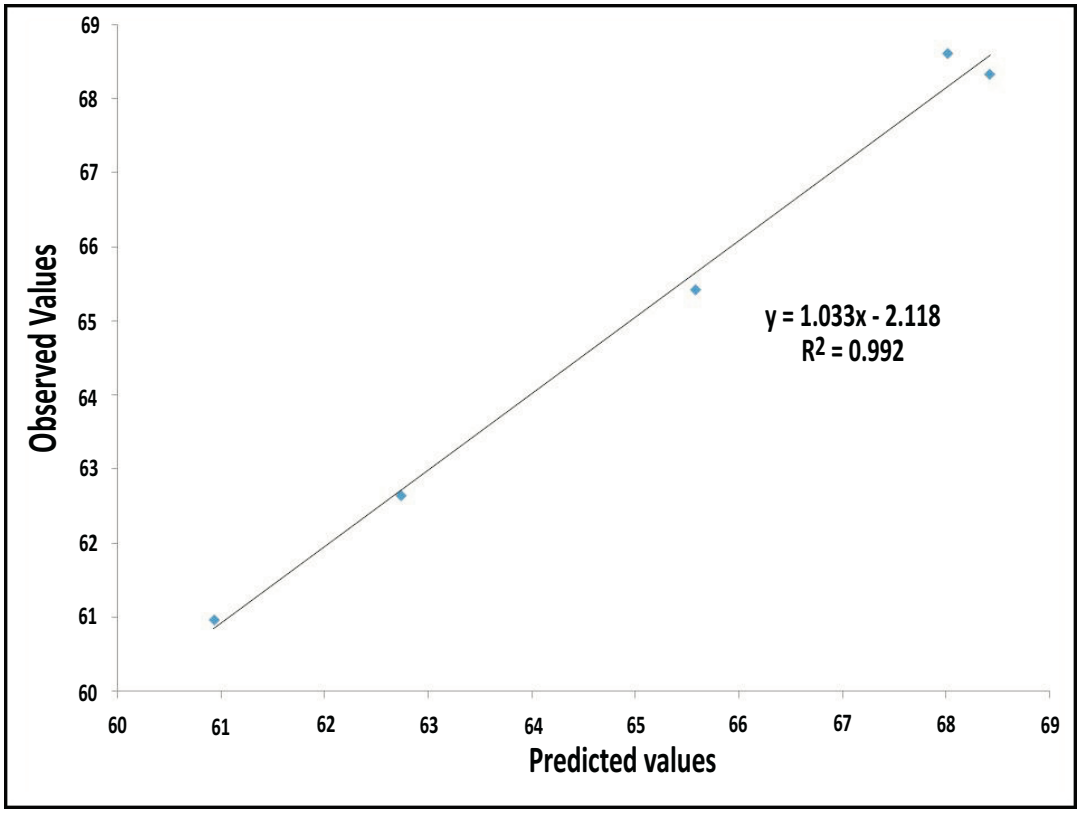

Fig. (8). Predicted *versus* observed response for percent drug release. (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

Table 7. Accelerated stability study of optimized formulation.

Characteristic Parameter	Temperature: 40 ± 2ºC; Relative Humidity (RH): 75 ± 5%RH			
	Initial	After 1 Month	After 2 Month	After 3 Month
Drug content	99.339	99.330	99.310	99.310
Viscosity	82.100	82.100	82.080	82.080
Percent drug release	65.576	65.570	65.550	65.550

Note: *(n=3).

CONCLUSION

The curcumin loaded topical gels were formulated using Carbopol 934P and menthol by applying face-centered central composite design. The impact of independent variables (Carbopol 934P and menthol) was observed on the dependent variables. Data optimization was done using design exert as a statistical tool to find the optimized batch using response surface methodology followed by data validation. Validation check was done by formulating different batches and evaluated experimentally. The experimental values were found to be close to the predicted values. The stability study for the optimized batch shows promising results. Hence, it can be concluded that the formulation optimization study of curcumin loaded topical gel employing face-centered central composite design is a powerful tool for the researcher with effective utilization of man, money, and material.

LIST OF ABBREVIATIONS

CCD = Central Composite Design

CUR = Curcumin

FCCCD = Face-centered Central Composite Design

RSM = Response Surface Methodology

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No animals/humans were used for studies that are the basis of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The author confirm that the data supporting the findings of the article is available within the article.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Declared none.

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