Response Surface Methodology for Optimization and Characterization of Limonene-based Coenzyme Q10 Self-Nanoemulsified Capsule Dosage Form

Submitted: January 7, 2004; Accepted: April 26, 2004

Anitha Palamakula,[1] Mohammad T.H. Nutan,[1] and Mansoor A. Khan[1,2]

[1]School of Pharmacy, Texas Tech University Health Sciences Center, 1300 Coulter, Amarillo, TX 79106
[2]Current Address: FDA/CDER/DPQR, White Oak, LS Building 64, HFD-940, 5600 Fishers Lane, Rockville, MD 20895

ABSTRACT

The aim of this study was to systematically obtain a model of factors that would yield an optimized self-nanoemulsified capsule dosage form (SNCDF) of a highly lipophilic model compound, Coenzyme Q10 (CoQ). Independent variables such as amount of R-(+)-limonene (X_1), surfactant (X_2), and cosurfactant (X_3), were optimized using a 3-factor, 3-level Box-Behnken statistical design. The dependent variables selected were cumulative percentage of drug released after 5 minutes (Y_1) with constraints on drug release in 15 minutes (Y_2), turbidity (Y_3), particle size (Y_4), and zeta potential (Y_5). A mathematical relationship obtained, $Y_1 = 78.503 + 6.058X_1 + 13.738X_2 + 5.986X_3 - 25.831X_1^2 + 9.12X_1X_2 - 26.03\ X_1X_3 - 38.67\ X_2^2 + 11.02X_2X_3 - 15.55\ X_3^3$ ($r^2 = 0.97$), explained the main and quadratic effects, and the interaction of factors that affected the drug release. Response surface methodology (RSM) predicted the levels of factors X_1, X_2, and X_3 (0.0344, 0.216, and 0.240, respectively), for a maximized response of Y_1 with constraints of >90% release on Y_2. The observed and predicted values of Y_1 were in close agreement. In conclusion, the Box-Behnken experimental design allowed us to obtain SNCDF with rapid (>90%) drug release within 5 minutes with desirable properties of low turbidity and particle size.

KEYWORDS: response surface methodology, Box-Behnken design, coenzyme Q10, R-limonene, statistical modeling.

INTRODUCTION

Many drugs, those currently available in the market and those under development, have poor aqueous solubility. This leads to poor dissolution and frequently results in variable bioavailabilities. Several approaches and formulation strategies designed to overcome this problem have been reported in the literature.[1,2] Self-emulsifying drug delivery systems (SEDDS) and self-nanoemulsified drug delivery systems (SNEDDS) are some of the most recent approaches.[1,2] In this case, isotropic mixtures of oils, surfactant, and cosurfactant are used to solubilize the lipophilic drug. These SEDDS and SNEDDS have a tendency to form fine oil-in-water emulsions when introduced into an aqueous media subjected to mild agitation.[3,4] After attaining these formulations, emulsions should exhibit predictable release profiles and stability of the liquid in capsule dosage form. Stability of emulsions is a major problem associated with SNEDDS liquid dosage forms, which include oils, surfactants, and cosurfactants. The present study deals with an optimization procedure for preparing a stable self-nanoemulsified capsule dosage form (SNCDF) of a highly lipophilic compound using the chiral essential oil component, R-(+)-limonene. Coenzyme Q10 (CoQ) was selected as a model drug for highly lipophilic compound with poor aqueous solubility and low oral bioavailability.[5,6] R-(+)-limonene was selected based on prior experiments for determining the stability of CoQ in essential oil components. The main objective of the study was to statistically determine the levels of factors by screening the process variables that yield CoQ SNCDF with fast drug release within 5 min. Many statistical experimental designs have been recognized as useful techniques to optimize the process variables. Different types of screening designs, such as fractional factorial and Plackett-Burman screening designs, have been used for preformulation evaluations.[7-10] Response surface methodology (RSM) is used when only a few significant factors are involved in optimization. Different types of RSM designs include 3-level factorial design, central composite design (CCD),[11,12] Box-Behnken design,[13] and D-optimal design.[14] A modified central composite experimental design, Box-Behnken design, is an independent, rotatable or nearly rotatable quadratic design (contains no embedded factorial or fractional factorial design), in which the treatment combinations are at the midpoints of the edges of the process space and at the center.[15] Among all the RSM designs, Box-Behnken design requires fewer runs (15 runs) in a 3-factor experimental design. A 3-factor, 3-level design would require a total of 27 unique runs without any repetitions and a total of 30 runs with 3 repetitions. Hence, the Box-Behnken design was applied to optimize the CoQ SNCDF with constraints on the release of drug after 15 min. The independent variables for the present study were the following: amount of R-(+)-limonene (X_1), Cremophor EL (X_2), and Capmul GMO-50 (X_3). The dependent variables included drug release profile, turbidity, particle size, and zeta potential.

Corresponding Author: Mansoor A. Khan, R.Ph., Ph.D., Director, FDA/CDER/DPQR, White Oak, LS Building 64, HFD-940, 5600 Fishers Lane, Rockville, MD 20895. Tel: (301) 796-0016. Fax: (301) 796-9816. Email: Khanm@cder.fda.gov.

Table 1. Variables in the Box-Behnken Design

Variables	Levels Used		
	Low	Medium	High
Independent Variables			
X_1 = R-limonene	18	49.5	81
X_2 = Cremophor EL	7.2	32.4	57.6
X_3 = Capmul GMO-50	1.8	7.2	12.6
Dependent Variables			
Y_1 = Dissolution after 5 min	1.6	82.06	Maximize
Y_2 = Dissolution after 15 min	1.3	99.69	>90
Y_3 = Turbidity			
Y_4 = Particle size			
Y_5 = Zeta potential			

MATERIALS AND METHODS

Materials

CoQ was a generous gift from Kyowa Hakko (New York, NY). R-(+)-limonene was purchased from Sigma Aldrich (Milwaukee, WI). Cremophor EL (polyoxyl 35 castor oil) was obtained from BASF Corp (Mount Olive, NJ). Capmul GMO-50 (glyceryl mono oleate) was obtained from Abitec Corp (Janesville, WI). High-performance liquid chromatography (HPLC)-grade methanol and n-hexane were purchased from VWR Scientific (Minneapolis, MN). Hydroxypropyl methylcellulose (HPMC) capsules were supplied by Capsugel (Greenwood, SC). All the chemicals were used as received.

Box-Behnken Statistical Design for Optimization of CoQ Self-Nanoemulsified Capsule Dosage Form

Box-Behnken statistical screening design was used to optimize and evaluate main effects, interaction effects, and quadratic effects of the formulation ingredients on the in vitro performance of SNCDF. A 3-factor, 3-level design used is suitable for exploring quadratic response surfaces and constructing second-order polynomial models. This cubic design is given by a set of points at the midpoint of each edge of a multidimensional cube and a center point replicate. The nonlinear computer-generated (Statgraphics, Manugistics Inc, Rockville, MD) quadratic model is given as

$$Y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 + b_{11}x_{12} + b_{22}x_2^2 + b_{33}x_3^2, \quad (1)$$

where Y is the measured response associated with each factor level combination; b_0 is an intercept; b_1 to b_{33} are the regression coefficients; and X_1, X_2, and X_3 are the independent variables.[16] The dependent and independent variables selected are shown in Table 1. These high, medium, and low levels were selected from the preliminary experimentation. The amounts of R-(+)-limonene (X_1), Cremophor EL (X_2), and Capmul GMO-50 (X_3) used to prepare each of the 15 formulations are given in Table 2.

Preparation of CoQ Self-Nanoemulsified Capsule Dosage Form

SNCDF of CoQ was prepared by varying the concentrations of R-(+)-limonene, Cremophor EL, and Capmul GMO-50.

Table 2. Box-Behnken Design: Independent (X) and Dependent Variables (Y)*

	X_1	X_2	X_3	Y_1	Y_2	Y_3	Y_4	Y_5
Form No.	R-(+)-Limonene (mg)	Cremophor EL (mg)	Capmul-GMO50 (mg)	% Dissolved in 5 Minutes ± SD	% Dissolved in 15 Minutes ± SD	Turbidity (NTUs) ± SD	Particle Size (nm) ± SD	Zeta Potential (ζ= mV) ± SD
1	81	57.6	7.2	44.4 ± 29.8	99.6 ± 13.5	63.2 ± 2.3	10.9 ± 2.5	12.1 ± 2.1
2	81	7.2	7.2	6 ± 9.82	1.34 ± 2.18	140 ± 4.9	49.8 ± 4.1	62.3 ± 11.1
3	18	57.6	7.2	3.75 ± 6.5	13.1 ± 7.61	9.73 ± 3.5	14.9 ± 5.2	70.4 ± 24.2
4	18	7.2	7.2	1.82 ± 1.07	1.44 ± 2.49	12.9 ± 3.8	>1000	58 ± 3.98
5	81	32.4	12.6	18.2 ± 8.73	36.1 ± 15.6	16.8 ± 2.3	32.6 ± 1.2	35.2 ± 3.02
6	81	32.4	1.8	57.8 ± 9.69	72.9 ± 12.1	9.37 ± 1.0	39.3 ± 8.1	27 ± 5.43
7	18	32.4	12.6	68.4 ± 1.65	89.9 ± 6.2	5.13 ± 0.9	38.6 ± 9.0	16.8 ± 6.78
8	18	32.4	1.8	3.95 ± 3.63	76.08 ± 16	5.13 ± 1.1	24.7 ± 2.4	12.9 ± 2.87
9	49.5	57.6	12.6	58.4 ± 2.56	87.9 ± 3.99	14.3 ± 2.4	16.0 ± 2.9	9.09 ± 1.90
10	49.5	57.6	1.8	24.8 ± 5.80	39.7 ± 29.5	4.53 ± 2.1	31.3 ± 3.1	16.8 ± 2.98
11	49.5	7.2	12.6	1.60 ± 1.49	2.97 ± 2.39	108 ± 11.2	26 ± 5.4	74.9 ± 4.01
12	49.5	7.2	1.8	12.1 ± 0.84	26.5 ± 3.3	41.7 ± 1.6	123 ± 12.3	49.7 ± 6.1
13	49.5	32.4	7.2	81.2 ± 9.90	94.6 ± 4.35	7.67 ± 3.9	13.3 ± 1.5	5.02 ± 1.80
14	49.5	32.4	7.2	72.1 ± 7.32	88.2 ± 3.56	7.70 ± 3.0	29.5 ± 3.9	4.11 ± 0.99
15	49.5	32.4	7.2	82.06 ± 10.2	95.4 ± 1.48	8.23 ± 2.4	14 ± 1.7	6.45 ± 0.05

*NTUs indicates nephlometric turbidity units.

CoQ was accurately weighed into a screw-capped glass vial and dissolved in R-(+)-limonene. The mixture was warmed in a water bath at 37°C. Cremophor EL and Capmul GMO-50 were added to the mix using a positive displacement pipette and stirred for 1 hour using a magnetic bar. Fifteen formulations with different concentrations of surfactant, cosurfactant, and R-(+)-limonene, each containing CoQ at a final loading of 30 mg, were filled into size 3 HPMC capsules. Filled capsules were stored at room temperature until used in subsequent studies.

In Vitro Evaluation of Designed Formulations of CoQ SNCDF

The designed formulations were evaluated by means of visual observations for spontaneity of emulsification, emulsion droplet size, zeta potential, turbidity, and dissolution profile of CoQ in each formulation.

Visual Observations for Spontaneity of Emulsification

To assess its self-emulsification properties, the CoQ SNCDF formulation in a 120-mg capsule corresponding to 30 mg of CoQ was introduced into 250 mL of prewarmed water in a glass Erlenmeyer flask at 25°C, and the contents were gently stirred manually. The tendency to spontaneously form a transparent emulsion was judged as good or bad based on the clarity of emulsion formed.[1,17]

Emulsion Droplet Size Analysis

The mean particle size diameter of the resultant nanoemulsions (triplicates) was determined by using a particle sizing system (Z380 PSS NiComp, Santa Barbara, CA). Samples, taken in a small glass tube (60 × 50 mm), were directly placed into the module, and the data were collected for 10 min. Particle size was calculated from the volume size distribution. All studies were repeated in duplicates for each sample from the above emulsion, with good agreement being found between the measurements.

Turbidity Measurements

Turbidity of the resultant emulsions given in nephlometric turbidity unit (NTU) was measured using an Orbeco-Hellige turbidimeter (model 966, Farmingdale, NY). The turbidimeter used in the study was carefully calibrated with formazin standards. Accuracy of the instrument is essential especially for small and diluted emulsions with high surfactant concentrations. Accuracy of the above turbidimeter was found to be approximately ±0.01 NTU with stray light less than or equal to 0.01 NTU.

Zeta Potential Measurement

The difference in potential between the surface of the electro-neutral region of the solution and the surface of tightly bound layer of ions on the particle is known as zeta potential. The zeta potential was measured by using a NiComp PSS ZW380 (Santa Barbara, CA). The nanoemulsions were taken in a cuvette, and the electrodes were attached and placed in the ZW380 for measurement. Each sample was analyzed in triplicate using automatic mode.

Dissolution Studies

In dissolution studies, the *United States Pharmacopeia* rotating paddle method was used. Dissolution profiles of the capsules filled with the self-emulsified formulations of CoQ in R-limonene were determined using rotating paddle apparatus (VK7000, VanKel, Cary, NC). The dissolution experiments in triplicate for each formulation were performed at 37°C ± 0.5°C, with a speed of rotation at 50 rpm in 900 mL of water. Capsules were held to the bottom of the vessel using aluminum sinkers. Samples (3 mL) withdrawn after 5, 10, and 15 minutes were filtered and analyzed using HPLC method. Details of the HPLC method can be found elsewhere.[2]

HPLC Analysis

HPLC analysis of aqueous CoQ samples was performed by a method previously described by Nazzal et al.[2] Briefly, CoQ was analyzed using a C18, 3.9 × 150 mm reverse phase column (Nova-Pak; Waters, Milford, MA) at ambient temperature. The mobile phase composition was methanol:*n*-hexane (9:1), at a flow rate of 1.5 mL/minutes to elute CoQ at a wavelength of 275 nm. The samples were loaded into the autosampler (712 WISP, Waters) and analyzed using Waters HPLC instrument attached to a 510 pump and a 490E UV detector. The counts for area under the peak were determined using STAR 5.3 software (Varian, Walnut Creek, CA).

Optimization of Formulation Ingredients

After generating the polynomial equations relating the dependent and independent variables presented in Table 1, the process was optimized for the response Y_1. Optimization was performed to obtain the levels of X_1, X_2, and X_3, which maximized Y_1 with constraints on Y_2.

Results and Discussion

The RSM using Box-Behnken screening design for 3 factors offers an advantage of fewer experimental runs (15 runs) as compared with that of central composite models, circumscribed (CCC) or inscribed (CCI), which require 20 runs.[12,16,18,19] The independent and dependent variables for

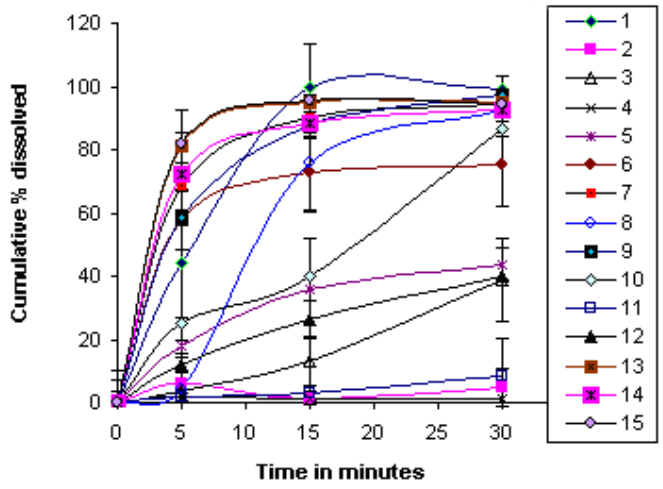

Figure 1. Dissolution profiles of CoQ SNCDF formulations that are listed in Table 2.

design-generated experimental runs are given in Tables 1 and 2. The dissolution profile for the 15 formulations is presented in Figure 1. Based on the experimental design generated by X-Stat (version 2.01, John Wiley and Sons, New York, NY), the factor combinations resulted in different responses. Table 2 indicates the effect of these factors on turbidity, particle size, and zeta potential of the 15 formulations. From these results, it can be concluded that all these formulations resulted in acceptable turbidity (<150 NTUs) and particle size range (< 150 nm) for nanoemulsions, and no particular pattern was found. Zeta potential indicates the stability of emulsions by measuring the difference in potential between the tightly bound layer of ions on the particle surface and the electro-neutral region of the solution. An absolute value, less than or greater than 25 mV is indicative of flocculated and deflocculated emulsions, respectively.[20] All the zeta potential values obtained are expressed in Table 2, indicating that the formulations 1, 7, 9, 13, 14, and 15 might have aggregated droplets similar to flocculated systems. From Figure 1 and Table 2 it can be inferred that these 3 factors have a profound effect on the drug release profiles. Formulations numbered 1, 7, 9, 13, 14, and 15 showed higher drug release of >85% after 15 minutes of dissolution. However, the percentage of drug released after 5 minutes from formulations 1, 7, and 9 was <70%, and only formulations 13, 14, and 15 showed rapid drug release of >70% (Figure 1). In order to obtain a formulation having rapid drug release of >85% within 5 minutes, RSM optimization was used to determine the levels of these factors. The mathematical relationship in the form of a polynomial equation for the measured response, Y_1, obtained with the statistical package Statgraphics (version 4, Manugistics Inc) is listed below.

$$Y_1 = 78.503 + 6.058 \times X_1 + 13.738 \times X_2 + 5.986 \times X_3 - 25.831 \times X_1^2 + 9.12 \times X_1X_2 - 26.03\, X_1X_3 - 38.67\, X_2^2 + 11.02 \times X_2X_3 - 15.55\, X_3^3 \quad (2)$$

The confidence that the regression equations would predict the observed values better than the mean for Y_1 and Y_2 were 97.02 and 86.18, respectively. The polynomial equation represents the quantitative effect of process variable (X_1, X_2, and X_3) and their interactions on the response Y_1. The values of the coefficients X_1, X_2, and X_3 are related to the effect of these variables on the response Y_1. Coefficients with more than 1 factor term and those with higher order terms represent interaction terms and quadratic relationship, respectively. A positive value represents an effect that favors the optimization, while a negative value indicates an antagonistic effect. The values of X_1, X_2, and X_3 were substituted in the equation to obtain the theoretical values of Y_1. The predicted values and the observed values were found to be in good agreement.

As illustrated in Table 3, a P value of ≤ .05 for any factor in analysis of variance (ANOVA) indicates significant effect of the corresponding factors on the response, ie, dissolution after 5 minutes (Y_1). From the F ratios given in ANOVA for

Table 3. Analysis of Variance for Y_1*

Source	Sum of Squares	*Df*	Mean Square	F Ratio	*P* Value
X_1	293.668	1	293.668	3.87	.1064
X_2	1510.03	1	1510.03	19.88	.0066
X_3	286.562	1	286.562	3.77	.1098
X_1X_1	2463.78	1	2463.78	32.43	.0023
X_1X_2	332.698	1	332.698	4.38	.0906
X_1X_3	2711.81	1	2711.81	35.70	.0019
X_2X_2	5523.27	1	5523.27	72.71	.0004
X_2X_3	486.423	1	486.423	6.40	.0525
X_3X_3	893.288	1	893.288	11.76	.0187
Total error	379.822	5	75.9644		
Total (corr)	13912.0	14			

*R-squared = 97.26%; R-squared (adjusted for *df*) = 92.35%; standard error of estimate = 8.71; mean absolute error = 4.56; Durbin-Watson statistic = 2.27.

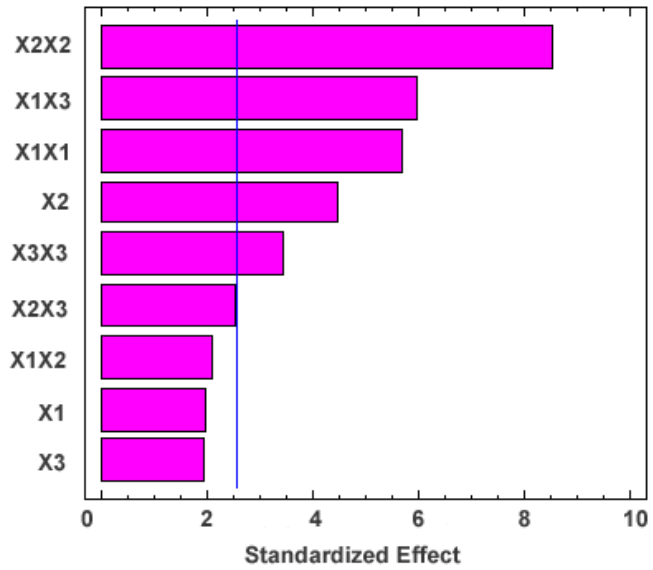

Figure 2. Standard pareto chart showing the effects of independent variables X_1 (R-(+)-limonene); X_2 (Cremophor EL); and X_3 (Capmul GMO-50) and their combined effects on the drug release profiles of CoQ SNCDF formulation.

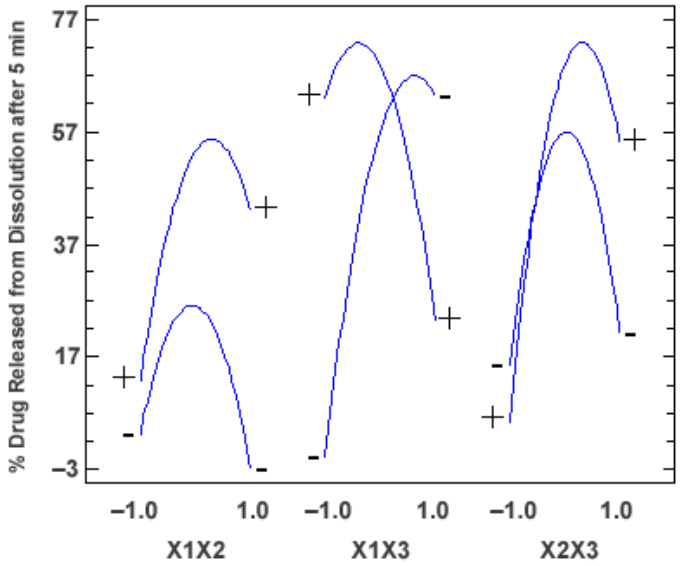

Figure 3. Interaction plot showing the quadratic effects of interactions between factors on drug release profiles of CoQ SNCDF formulation.

Y_1, it can be concluded that the effect of Cremophor EL, and the ratio of R-(+)-limonene to Capmul GMO-50 have significant effects on the release profile formulation.

The relationship between the dependent and independent variables was further elucidated using response surface plots and contour plots. Also, the main effect of the independent variables on the dependent variables was further investigated using a pareto chart and interaction plot. Figures 2, 3, 4, and 5 show the effect of factors X_1, X_2, and X_3 on the response Y_1. From these figures, the following observations can be made.

Effect of X_1, X_2 and X_3 on Y_1 (Drug release from formulation dissolution for 5-minutes dissolution): Figure 2 (standardized pareto chart for Y_1) depicts the main effect of the independent variables on the dissolution of the formulations. The length of each bar in the graph indicates the effect of these factors and the level of their effects on responses. The length of the bar extending behind the reference line indicates the extent of corresponding factor effects on Y_1. From Figures 2 and 3, it can be inferred that the factor B (Cremophor EL, X_2), AA (quadratic effect of R-(+)-limonene, X_1^2), BB (quadratic effect of Cremophor EL, X_2^2), CC (quadratic effect of cosurfactant, Campul GMO-50, X_3^2), and AC (interaction effect of R-(+)-limonene, X_1 and Capmul GMO-50, X_3) have a significant effect on the drug release from formulation dissolution for 5 min.

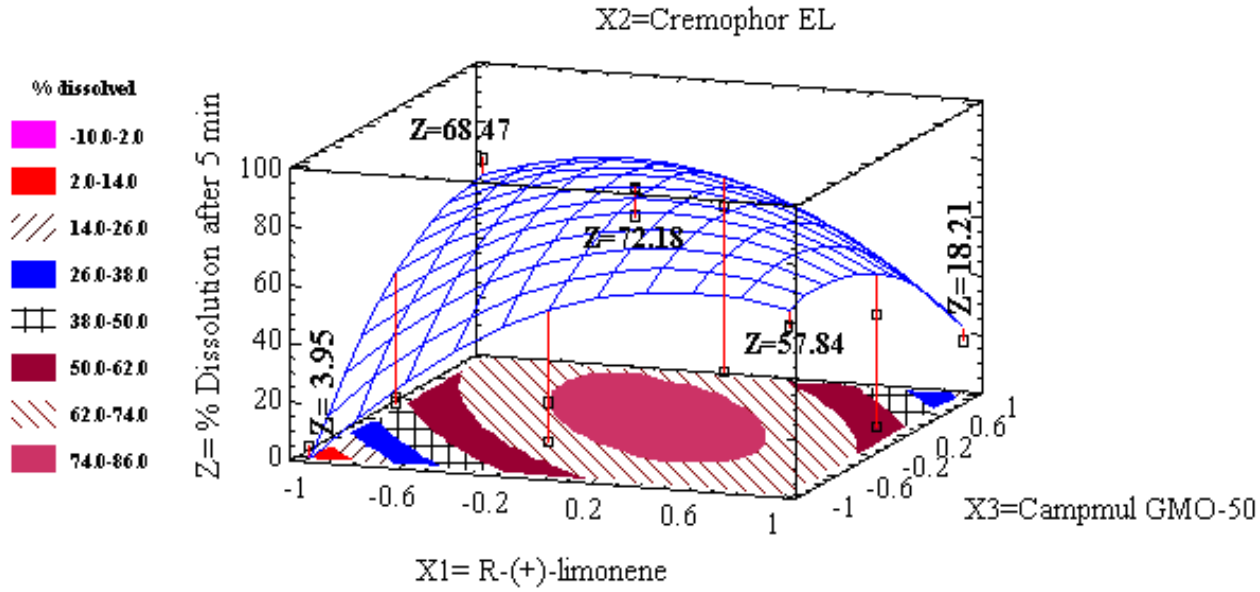

Figure 4. Response surface plots showing the effect of independent variables, varying ratio of R-(+)-limonene (X_1) and Capmul GMO-50 (X_3) on response Y_1 (ie, the drug release profiles of CoQ SNCDF formulation).

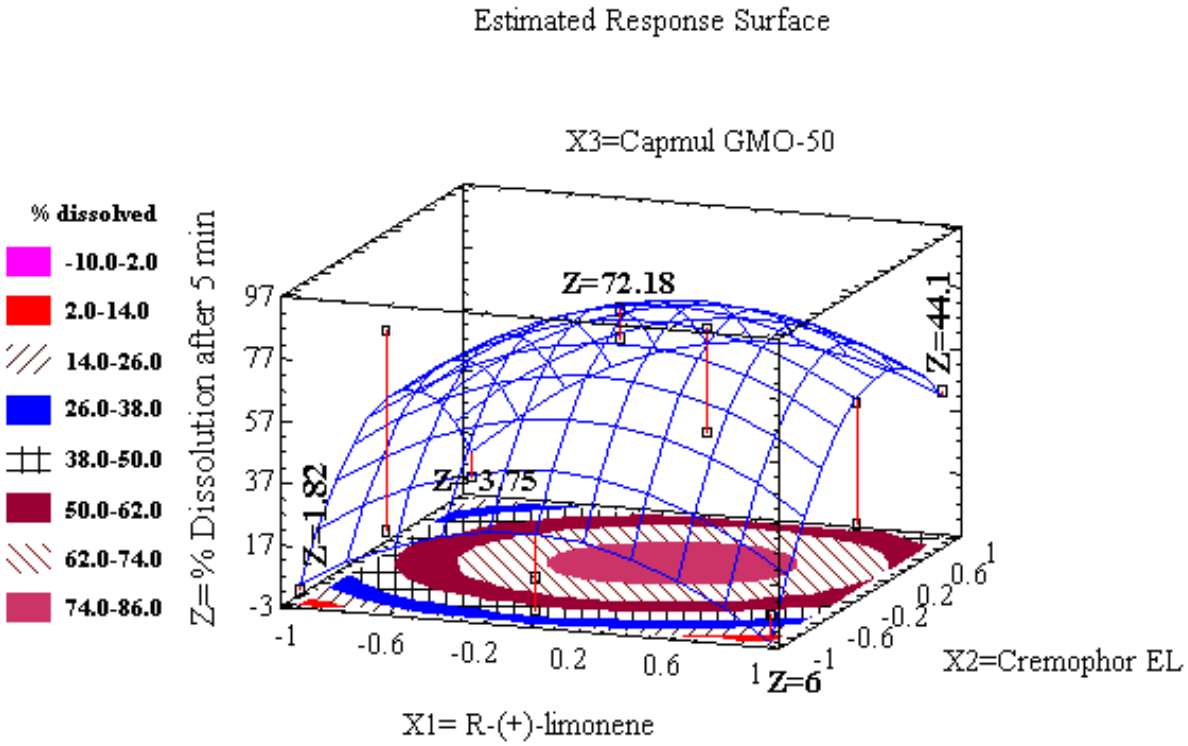

Figure 5. Response surface plots showing the effect of independent variables, varying ratio of R-(+)-limonene (X_1) and Cremophor EL (X_2) on the drug release profiles, response Y_1 of CoQ SNCDF formulation.

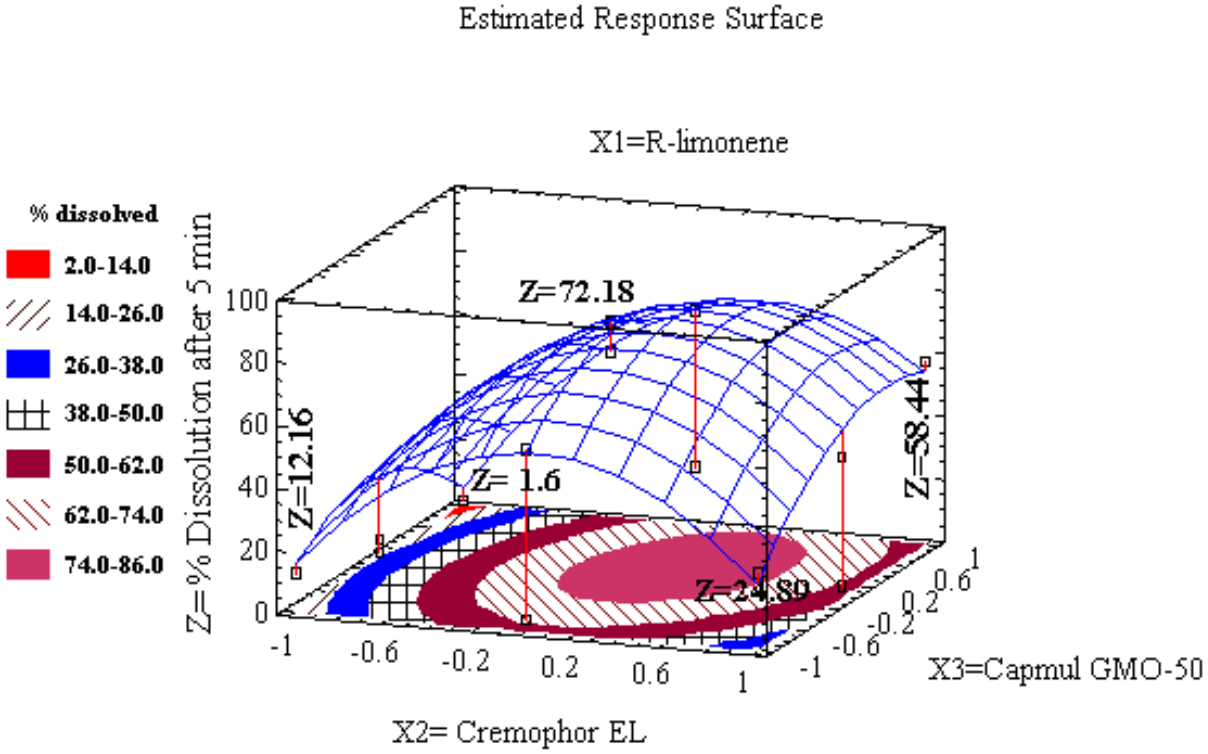

Figure 6. Response surface plots showing the effect of independent variables, varying ratio of Cremophor EL (X_2) and Capmul GMO-50 (X_3) on the drug release profiles, response Y_1 of CoQ SNCDF formulation.

Figures 4, 5, and 6 further explain the effect of X_1, X_2, and X_3 ratios on the response Y_1. From the 3D plots, it is clear that the ratio of R-(+)-limonene and Capmul GMO-50 has a major effect on determining drug release within 5 minutes from formulations (Figure 4). This figure shows that at a lower concentration of R-(+)-limonene, the percentage dissolved increased with an increase in the concentration of Capmul GMO-50 (from 3.95% to 68.47%). However, at a higher concentration of R-(+)-limonene, the percentage dissolved from formulation decreased with an increase in Capmul GMO-50 concentration

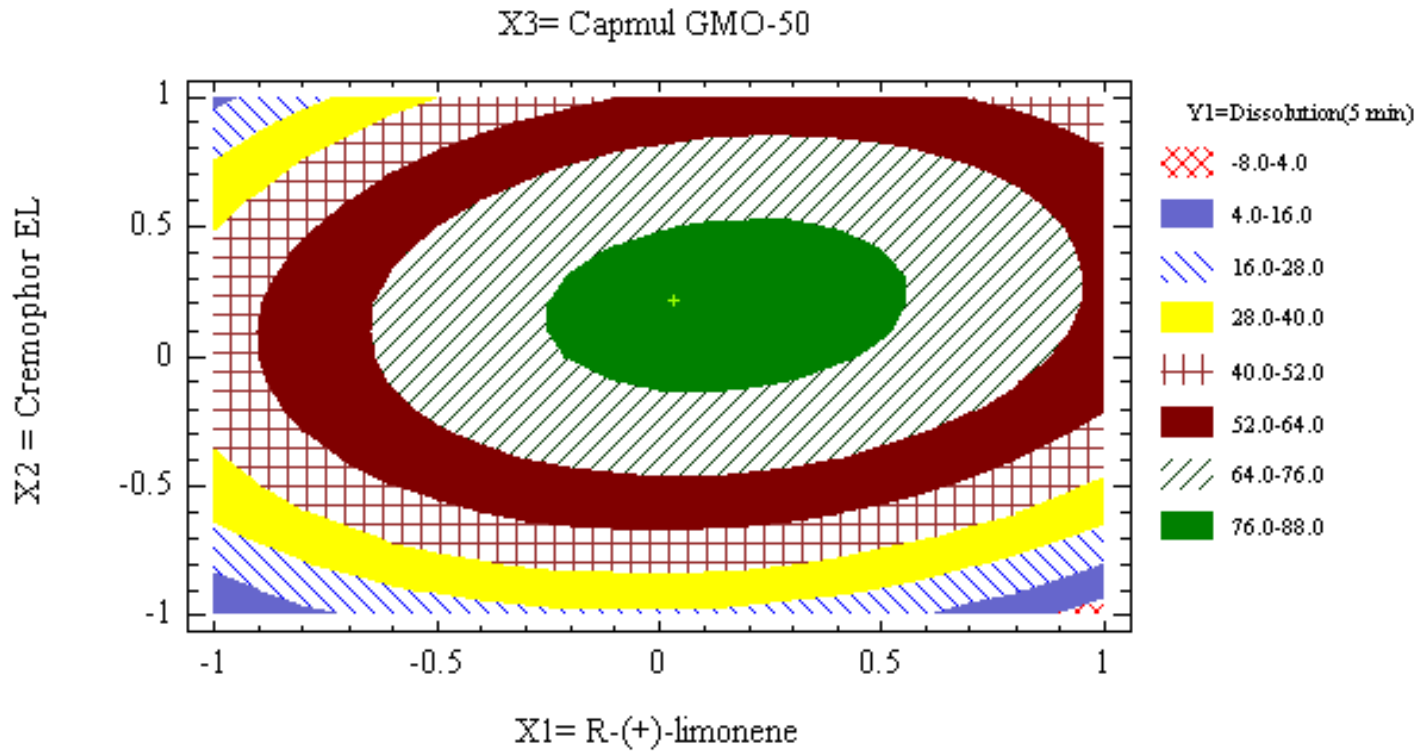

Figure 7. Contour plot showing the effects of varying ratios of R-(+)-limonene (X_1) and Cremophor EL (X_2) on the drug release profiles, response Y_1 of CoQ SNCDF formulation.

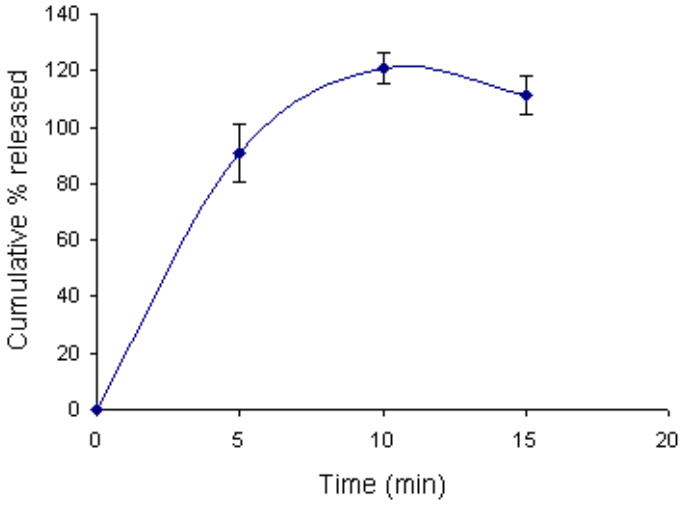

Figure 8. Dissolution of new optimized CoQ SNCDF formulation.

(57.84% to 18.21%). This finding can be explained by the fact that cosurfactant by itself does not emulsify the oil, rather the cosurfactant acts by enhancing the emulsifying capability of surfactants. Hence, optimum ratio of surfactant and cosurfactant is a key factor in achieving an emulsion.

Figure 5 indicates that an optimum ratio of R-(+)-limonene and Cremophor EL is required to yield a formulation with higher percentage of drug release. As shown in Figure 5, the surfactant showed minimal effect on the dissolution of formulation after 5 minutes when the concentration of R-(+)-limonene was low. However, at higher levels of R-(+)-limonene the percentage dissolved from formulation increased with an increase in the concentration of Cremophor EL (from 6% to 44.1%). This percentage can be further improved by the addition of cosurfactant, Capmul GMO-50, in the formulation.

The importance of a surfactant and cosurfactant ratio in an emulsion formulation and the drug release from emulsions is shown in Figure 6. The figure indicates that sufficient concentration of cosurfactant is needed for maximal effect of surfactant on emulsification of lipophilic substance with solvent. Figure 7 is a representative contour plot, which further elucidates the effects of varying ratio of R-(+)-limonene and Cremophor EL with a fixed amount of Capmul GMO-50 on response Y_1. Figure 7 illustrates that the emulsification of R-(+)-limonene increases as the concentration of Cremophor EL is increased. Maximum dissolution of the drug was found at limonene levels from -0.25 to 0.5 with lower levels of Cremophor EL from 0 to 0.5 as indicated by the central solid (black) portion of the plot.

Having studied the effect of independent variables on the responses, the levels of these factors were determined by using a computer optimization process, RSM. The predicted values of Y_1 and Y_2 were 81.6% and 95.9%, respectively, at X_1, X_2, and X_3 levels of 0.0344, 0.216, and 0.240, respectively. As a confirmation process, a fresh formulation of CoQ SNCDF was prepared with CoQ (30 mg), R-(+)-limonene (45 mg), Cremophor EL (38 mg), and Capmul GMO-50 (10 mg). The optimized levels of factors yielded a formulation with rapid drug release of >90% within 5 minutes and complete drug release within 15 minutes (Figure 8). The observed and

predicted values were in very close agreement. Further, the optimized formulation had turbidity value of 16.1 ± 1.08 NTUs, droplet size of 23.1 ± 2.11 nm, and zeta potential of 11.9 ± 1.83 mV.

CONCLUSION

Optimization of limonene-based CoQ SNCDF using RSM, Box-Behnken design, was performed. The ratio of independent variables, R-(+)-limonene, Cremophor EL, and Capmul GMO-50, showed a significant effect on the drug release characteristics of the formulation. The optimum ratio of these factors at 3 levels was chosen based on the quantitative effect and the polynomial equations generated by RSM. The optimized formulation prepared by using these predicted levels of factors provided desired observed responses forming nanoemulsions with >97% drug release in 5 min, and complete drug release within 15 minutes of dissolution.

REFERENCES

1. Nazzal S, Khan MA. Response surface methodology for the optimization of ubiquinone self-nanoemulsified drug delivery system. *AAPS PharmSciTech*. 2002;3(1):E3.

2. Nazzal S, Zaghloul AA, Reddy IK, Khan MA. Analysis of ubidecarenone (CoQ10) aqueous samples using reversed phase liquid chromatography. *Pharmazie*. 2001;56:394-396.

3. Pouton CW. Lipid formulations for oral administration of drugs: non-emulsifying, self-emulsifying and 'self-microemulsifying' drug delivery systems. *Eur J Pharm Sci*. 2000;11(suppl 2):S93-S98.

4. Charman SA, Charman WN, Rogge MC, Wilson TD, Dutko FJ, Pouton CW. Self-emulsifying drug delivery systems: formulation and biopharmaceutic evaluation of an investigational lipophilic compound. *Pharm Res*. 1992;9:87-93.

5. Li S, Lin S, Chien YW, Daggy BP, Mirchandani HL. Statistical optimization of gastric floating system for oral controlled delivery of calcium. *AAPS PharmSciTech*. 2001;2(1):E1.

6. Chopra RK, Goldman R, Sinatra ST, Bhagavan HN. Relative bioavailability of coenzyme Q10 formulations in human subjects. *Int J Vitam Nutr Res*. 1998;68:109-113.

7. Sastry SV, Khan MA. Aqueous based polymeric dispersion: Plackett-Burman design for screening of formulation variables of atenolol gastrointestinal therapeutic system. *Pharm Acta Helv*. 1998;73:105-112.

8. Korakianiti ES, Rekkas DM, Dallas PP, Choulis NH. Optimization of the pelletization process in a fluid-bed rotor granulator using experimental design. *AAPS PharmSciTech*. 2000;1(4):E35.

9. Abu-Izza K, Garcia-Contreras L, Lu DR. Preparation and evaluation of zidovudine-loaded sustained-release microspheres. 2. Optimization of multiple response variables. *J Pharm Sci*. 1996;85:572-576.

10. Hamed E, Sakr A. Application of multiple response optimization technique to extended release formulations design. *J Control Release*. 2001;73:329-338.

11. Boza A, De la Cruz Y, Jordan G, Jauregui-Haza U, Aleman A, Caraballo I. Statistical optimization of a sustained-release matrix tablet of lobenzarit disodium. *Drug Dev Ind Pharm*. 2000;26:1303-1307.

12. Box GEP, Wilson KB. On the experimental attainment of optimum multifactorial conditions. *Royal Statistics Society*. 1951;13:1-12.

13. Singh SK, Dodge J, Durrani MJ, Khan MA. Optimization and characterization of controlled release pellets coated with experimental latex: I. Anionic drug. *Int J Pharm*. 1995;125:243-255.

14. Sanchez-Lafuente C, Furlanetto S, Fernandez-Arevalo M, et al. Didanosine extended-release matrix tablets: optimization of formulation variables using statistical experimental design. *Int J Pharm*. 2002;237:107-118.

15. Ragonese R, Macka M, Hughes J, Petocz P. The use of the Box-Behnken experimental design in the optimisation and robustness testing of a capillary electrophoresis method for the analysis of ethambutol hydrochloride in a pharmaceutical formulation. *J Pharm Biomed Anal*. 2002;27:995-1007.

16. Box GEP, Behnken DW. Some new three level designs for the study of quantitative variables. *Technometrics*. 1960;2:455-475.

17. Craig DQM, Barker SA, Banning D, Booth SW. An investigation into the mechanism of self-emulsification using particle size analysis and low frequency dielectric spectroscopy. *Int J Pharm*. 1995;114:103-110.

18. Khan MA, Dib J, Reddy IK. Statistical optimization of Ketoprofen-Eudragit S100 coprecipitates to obtain controlled-release tablets. *Drug Dev Ind Pharm*. 1996;22:135-141.

19. Box GEP, Hunter WG, Hunter JS. *Statistics for Experimenters: An Introduction to Design, Data Analysis, and Model Building*. New York, NY: John Wiley & Sons; 1976.

20. Leiberman HA, Reiger MM, Banker GS. *Pharmaceutical Dosage Forms: Disperse Systems*. Vol 2. New York, NY: Mercel Dekker; 1989.