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Response Surface Methodology for the Optimization of Ubiquinone Self-Nanoemulsified Drug Delivery System

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ABSTRACT The aim of the present study was to prepare and evaluate an optimized, self-nanoemulsified drug delivery system of ubiquinone. A 3-factor, 3-level Box-Behnken design was used for the optimization procedure with the amounts of Polyoxyl 35 castor oil (X_1), medium-chain mono- and diglyceride (X_2), and lemon oil (X_3) as the independent variables. The response variable was the cumulative percentage of ubiquinone emulsified in 10 minutes. Different ubiquinone release rates were obtained. The amount released ranged from 11% to 102.3%. Turbidity profile revealed 3 regions that were used to describe the progress of emulsion formation: lag phase, pseudolinear phase, and plateau turbidity. An increase in the amount of surfactant decreased turbidity values and caused a delay in lag time. Addition of cosurfactant enhanced the release rates. Increasing the amount of the eutectic agent was necessary to overcome drug precipitation especially at higher loading of surfactants and cosurfactants. Mathematical equations and response surface plots were used to relate the dependent and independent variables. The regression equation generated for the cumulative percentage emulsified in 10 minutes was $Y1 = 90.9 - 22.1X_1 + 5.03X_2 + 13.95X_3 + 12.13X_1X_2 + 15.13X_1X_3 - 14.40X_1^2 - 6.25X_3^2$. The optimization model predicted a 93.4% release with X_1, X_2, and X_3 levels of 35, 35, and 30 respectively. The observed responses were in close agreement with the predicted values of the optimized formulation. This demonstrated the reliability of the optimization procedure in predicting the dissolution behavior of a self-emulsified drug delivery system.

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INTRODUCTION

Large proportions of new drug candidates have poor water solubility. To overcome this problem, various formulation strategies were reported in the literature including complexation with cyclodextrins, solid dispersions, and coprecipitates [1,2]. In recent years, however, much attention has been focused on lipid-based formulations with particular emphasis on self-emulsifying drug delivery systems [3]. Self-emulsifying drug delivery systems (SEDDS) are isotropic mixtures of oil, surfactant, cosurfactant, and drugs, which form a fine oil-in-water emulsion when introduced into aqueous medium under gentle agitation [4-6].

A literature search reveals an exhaustive number of publications characterizing the self-emulsified drug delivery systems [7-14]. Reported studies often use conventional methods (such as droplet-size analysis, ternary phase diagrams describing the efficient self-emulsification region and the liquid crystalline phases associated with the emulsion disruption process, low-frequency dielectric spectroscopy, zeta potentiometry, and surface tensiometry) to evaluate the in vitro performance of the emulsion-based drug delivery systems. These methods, however, require a large number of experiments to describe the effect of excipients and excipient selection on the physical properties of the formulations. Besides, no established

dissolution method was introduced to describe the efficiency of self-emulsification that could be used to distinguish between different preparations. The emulsion disruption process and the initial release rate from capsules are often visually evaluated and described in terms of the ease of emulsification and the color of the resultant emulsion (ie, milky or transparent). Two criteria are proposed in this study to describe the physical properties of a SNEDDS formulation: (a) the dissolution-time profile, which provides the amount of active ingredient emulsified over time, and (b) the turbidity-time profile, which provides lag time and rate of emulsion formation as well as relative droplet size.

Simultaneous monitoring of dissolution and turbidity studies reduces the overall number of experiments required to describe the self-emulsified preparations and reduces the amount of the active ingredient required to prepare these formulations. Screening and optimizing self-emulsified drug delivery systems could be further simplified by the use of a statistical design that requires only a small number of experiments, thereby eliminating the need for time-consuming and detailed ternary phase diagrams. The statistical optimization designs have been documented for the formulation of pharmaceutical solid dosage forms [15-17]. Wehrle et al [18] have reported the use of a sequential statistical design for optimizing the droplet size of a miconazole emulsion. Nevertheless, no such information was found in the literature for optimizing the dissolution properties of liquid self-emulsified drug delivery systems, which are profoundly influenced by several formulation variables.

Ubiquinone, also known as CoenzymeQ$_{10}$ (CoQ$_{10}$) was used as the model drug for its poor water solubility, which presented a challenge in the development of a formulation with improved aqueous dissolution [2].

The objective of the present work was to apply a response surface methodology for the optimization of a CoQ$_{10}$ self-nanoemulsified drug delivery system. As part of the optimization process, the main effects, interaction effects and quadratic effects of the formulation ingredients were investigated. Excipients and their interactions were evaluated for their effect on the cumulative percent of CoQ$_{10}$ emulsified into aqueous medium in 10 minutes. This allows the validation of turbidimetry in conjugation with dissolution studies as valuable tools in characterizing a self-nanoemulsified formulation using a minimal number of experiments within the statistical design. Dissolution is particularly important since release rates are greatly influenced by formulation ingredients as well as the liquid crystalline phases during the initial stages of the disruption process.

MATERIALS AND METHODS

Experimental design

A 3-factor, 3-level Box-Behnken design was used for the optimization procedure. This design is suitable for exploring quadratic response surfaces and constructing second-order polynomial models. The design consists of replicated center points and the set of points lying at the midpoint of each edge of the multidimensional cube that defines the region of interest. The non-linear quadratic model generated by the design is of the form: $Y = A_o + A_1X_1 + A_2X_2 + A_3X_3 + A_4X_1X_2 + A_5X_2X_3 + A_6X_1X_3 + A_7X_1^2 + A_8X_2^2 + A_9X_3^2 + E$, in which Y is the measured response associated with each factor-level combination; A_o is an intercept; A_1-A_9 are the regression coefficients; X_1, X_2 and X_3 are the factors studied; and E is the error term [19]. The independent factors and the dependent variables used in the design are listed in Table 1.

Table 1 - Variables in the Box-Behnken design

Independent Variables		Levels		
		Low	Middle	High
X_1 =	Amount of Cremophor EL added (mg)	10	40	70
X_2 =	Amount of Capmul MCM-C8 added (mg)	20	55	90
X_3 =	Amount of Lemon oil added (mg)	25	30	35
Dependent Variables		Constraints		
		Low	High	Goal
Y_1 =	Cumulative percent of CoQ10 emulsified after 10 minutes (%)	90	95	maximize
Y_2 =	Cumulative percent of CoQ10 emulsified after 15 minutes (%)	100	100	100
Y_3 =	Lag time (minute)	3.25	6	minimize
Y_4 =	Emulsification rate (%/min)	30	35	maximize
Y_5 =	Turbidity of the resultant emulsion (NTU)	2.25	6	minimize

Materials

Coenzyme Q10 was a generous gift from Kyowa Hakko USA (New York, NY). Polyoxyl 35 castor oil (Cremophor EL) was obtained from BASF Corp (Mount Olive, NJ).

Medium-chain mono- and diglycerides (Capmul MCM-C8) were obtained from Abitec Corp (Janesville, WI). Cold-pressed lemon oil was purchased from Young Living (Payson, UT). Hydroxypropyl methylcellulose (HPMC) capsules were supplied by Shionogi Qualicaps (Whitsett, NC). High-performance liquid chromatography (HPLC)-grade methanol and n-hexane were purchased from VWR Scientific (Minneapolis, MN). All the chemicals were used as received.

Preparation of the self-nanoemulsified formulations

Accurately weighed 30 mg of CoQ_{10} were mixed with lemon oil in a screw-capped glass vial and melted in a water bath (Ikamag® Ret-G, Terochem Scientific, Toronto, Canada) at 37°C. Cremophor EL and Capmul MCM-C8 were added to the oily mixture using a positive displacement pipette (Microman®, Gilson Inc, Middleton, WI) and stirred with a magnetic bar. Prepared formulations, while molten, were poured into size 4 HPMC capsules. Each capsule represents 30 mg of CoQ_{10} in addition to the specified amounts of lemon oil, Cremophor EL, and Capmul MCM-C8 given in Table 2. Filled capsules were stored at room temperature for 24 hours to allow complete solidification of the systems before use in subsequent studies. Droplet size of the formulations was measured and revealed an average diameter of 100 nm. Detailed characterization and particle size analysis of the eutectic based self-nanoemulsified drug delivery system of CoQ_{10} was performed and can be found elsewhere [20].

Table 2 - Observed responses for the Box-Behnken design

Run	X_1	X_2	X_3	Y_1	Y_2	Y_3	Y_4	Y_5
1	10	55	25	95.1	104.3	5.5	28.7	129.3
2	70	90	30	78.2	108.0	7.0	17.1	6.6
3	10	90	30	88.5	99.4	4.5	27.3	174.1
4	40	55	30	91.1	103.6	6.3	35.2	5.6
5	40	90	35	97.3	101.2	3.3	18.3	15.1
6	10	55	35	102.3	105.7	4.0	31.4	145.7
7	40	20	35	87.3	99.1	5.0	6.1	6.9
8	70	55	25	11.0	64.5	12.5	4.8	11.6
9	10	20	30	96.0	105.5	5.0	29.7	70.8
10	70	55	35	78.7	96.7	7.0	20.7	5.5
11	40	55	30	90.6	104.4	6.3	38.2	5.6
12	40	55	30	97.1	102.7	6.3	31.7	5.4
13	40	20	25	75.6	100.5	6.8	28.2	3.2
14	70	20	30	37.2	63.5	8.5	9.7	2.3
15	40	90	25	72.3	94.5	5.0	12.1	16.6

Dissolution studies

Dissolution profiles of the capsules filled with the self-nanoemulsified formulations were determined using USP XXIII rotating paddle apparatus (VanKel, model VK7000, Cary, NC) at 37°C and at a rotating speed of 50 rpm in 900 mL of water. Capsules were held to the bottom of the vessel using copper sinkers. Samples (3 mL) withdrawn at fixed time intervals were filtered using a 10-mm VanKel filter and assayed for CoQ_{10} by HPLC. The dissolution experiments were carried out in triplicate.

Turbidimetry

Theory

A beam of light passing through a cloud of small particles will be scattered by the particles in all directions. Measuring the light received at an angle normal to the concentric light beam provides indications on size and number of scattered particles [21]. Light scattering by colloids conforms to Rayleigh theory, which predicts that light scattering or measured turbidity t in a simplified equation can be given by

$$t = K.n.v^2$$

in which K is a machine constant, n is the number of particles, and v is particle volume [21,22]. Turbidity given in nephlometric turbidity units (NTU) could be used as an indirect measure of the emulsion droplet size. Pouton [22] has reported a direct correlation between the intensity of the scattered light and the volume of the dispersed droplets. To assess spontaneity and efficacy of emulsification, the methods reported by Groves and Mustafa [21] and Pouton [22] were modified and adapted in the present study. Turbidity of the dispersion and the relative intensity of the scattered light were correlated with time during the emulsification process.

A cumulative percentage of the formulation released from the capsules and emulsified into the medium could be obtained by plotting NTU_t x 100 / $NTU_{plateau}$ with time.

NTU_t is the turbidity reading at any time t, and $NTU_{plateau}$ is the turbidity reading when the emulsion reaches the plateau. This is based on the assumption that a plateau reading reflects 100% of the formulation emulsified regardless of the actual amount of CoQ_{10} dissolved. Plots of the cumulative percentage of the formulation released with time have the characteristic lag phase, pseudolinear phase, and a gradual tailing toward a plateau [22]. The lag phase reflects the time elapsed before the contents of the capsules are released into the medium, whereas the slope of the pseudolinear phase could be considered as the emulsification rate given as a percentage of the formulation emulsified per minute.

Experimental method

Turbidity profiles of the capsules filled with the self-nanoemulsified formulations were determined using HACH turbidimeter (Model 2100AN, Loveland, CO). A low-pressure flow cell was used to read sample turbidity of the capsules subjected to the same dissolution conditions as described above. Two 1/8-inch tygon tubings were connected to the pump attached to the dissolution autosampler (VanKel, model VK8000, Cary, NC). One tube connected the pump and the inlet of the flow cell, and the other connected the pump to the dissolution vessel. The inlet of the tube connecting the pump to the dissolution vessel was covered with a 40-mm nylon screen and was immersed into the medium, allowing the sample to be continuously withdrawn from a zone midway between the surface of the medium and the top of the rotating blade. A third tube was installed to the outlet of the flow cell leading back to the dissolution vessel. Before the start of the experiments, deionized water was pumped through the flow cell until a reading below 0.150 NTU was maintained. Throughout the study, dissolution medium was continuously pumped into the flow cell and back to the dissolution vessel. The turbidimeter was set so that a reading was recorded on the attached printer every 15 seconds. Turbidimetry experiments were carried out in triplicate. Turbidimetry validation and the correlation of turbidity with droplet-size analysis were performed in a separate study and can be found elsewhere [20].

HPLC analysis

Detailed HPLC method for the analysis of aqueous CoQ_{10} samples was described by Nazzal et al [23]. Briefly, CoQ_{10} was analyzed at ambient temperature utilizing a C18, 3.9 mm × 150 mm reverse phase chromatography column (Nova-Pak; Waters, Milford, MA). The mobile phase consisted of methanol : n-hexane (9:1) and was pumped at a flow rate of 1.5 mL/min^{-1}. The HPLC instrument consisted of a 510 pump (Waters), 712 WISP autosampler (Waters), and a 490E UV detector (Waters) set at a wavelength of 275 nm.

RESULTS AND DISCUSSION

Fifteen experiments were required for the response surface methodology based on the Box-Behnken design. The experimental runs and the observed responses for the 15 formulations are given in Table 2. Based on the experimental design, the factor combinations resulted in different CoQ_{10} release rates. The range of the responses was 102.3% in formulation No. 6 (maximum) and 11% in formulation No. 8 (minimum). Dissolution and turbidity profiles of all the 15 formulations are shown in Figures 1, 2, 3, and 4. The modified turbidity/time profile correlating the cumulative amount of the formulation released with time is given in Figures 5 and 6. The mathematical relationship in the form of a polynomial equation for the measured responses obtained with the statistical package Statgraphics plus (Version 4, Manugistics Inc, Rockville, MD) is listed in Table 3. The confidence that the regression equation would predict the observed values better than the mean for Y_2, Y_3, Y_4, and Y_5 was 86.3%, 93.9%, 72.8%, and 98.0%, respectively. The polynomial equation relating the response Y_1 and independent variables was: $Y_1 = 90.9 - 22.1X_1 + 5.03X_2 + 13.95X_3 + 12.13X_1X_2 + 15.13X_1X_3 - 14.40X_1^2 - 6.25X_3^2$.

The above equation represents the quantitative effect of process variables (X_1, X_2, and X_3) and their interactions on the response (Y1). The values of the coefficients X_1-X_3 are related to the effect of these variables on the response (Y1). Coefficients with more than one factor term and those with higher order terms represent interaction terms and quadratic relationships respectively. A positive sign represents a synergistic effect, while a negative sign indicates an antagonistic effect. The values of X_1-X_3 were substituted in the equation to obtain the theoretical values of Y_3. The theoretical (predicted) values and the observed values were in reasonably good agreement as seen from Table 4. The significance of the ratio of mean square variation due to regression and residual error was tested using analysis of variance (ANOVA). The ANOVA indicated a significant ($P < .05$) effect of factors on response (F cal [14.13] > F crit [8.30]).

The relationship between the dependent and independent variables was further elucidated using contour and response surface plots. The effect of X_1 and X_2 and their interaction on Y_1 at a fixed level of X_3 (30mg) are given in Figures 7 and 8. At low levels of X_2 (amount of Capmul MCM-C8 added), Y_1 increases from 35.73% to 104.18% when the amount of Cremophor EL (X_1) decreases from 70 to 10 mg. Similarly, at high levels of X_2, Y_1 increases from 70.03% to 89.98% when X_1 decreases from 10 to 70mg. The possible explanation for this is that Cremophor EL (surfactant) strongly localized to the surface of the emulsion droplet reduces interface free energy and provides a mechanical barrier to coalescence resulting in a thermodynamically spontaneous dispersion [24]. However, at high Cremophor EL concentration (X_1), progress of emulsification might be compromised by viscous liquid

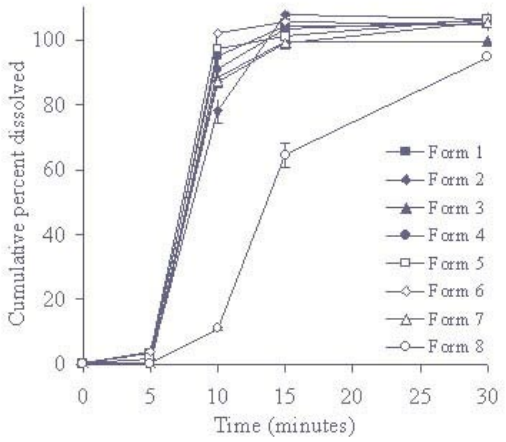

Figure 1 - Dissolution profiles of ubiquinone (Coenzyme Q10) from the self- emulsified formulations (Form) 1 to 8.

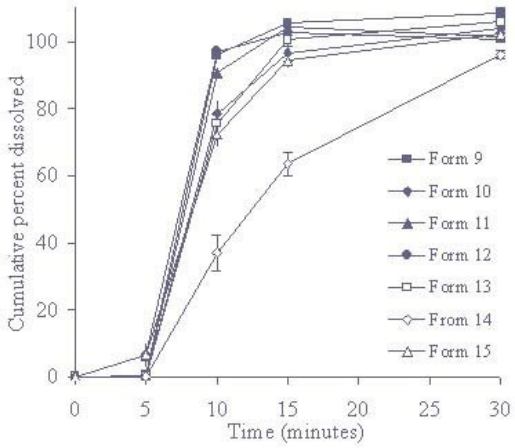

Figure 2 - Dissolution profiles of ubiquinone (Coenzyme Q10) from the self- emulsified formulations (Form) 9 to 15.

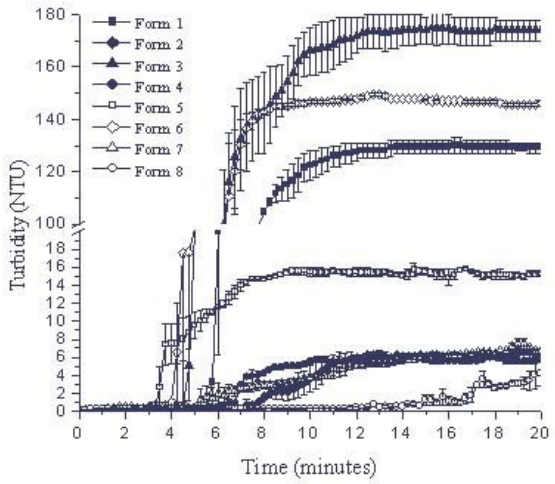

Figure 3 - Turbidity profiles of ubiquinone (Coenzyme Q10) SEDDS from Form 1 to 8.

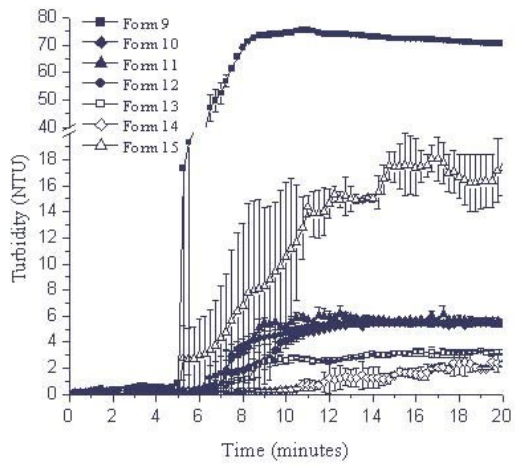

Figure 4 - Turbidity profiles of ubiquinone (Coenzyme Q10) SEDDS from Form 9 to 15.

crystalline gel formed at the surfactant-water interface. It was reported that when a self-emulsified system is diluted by the aqueous phase various mesomorphic phases are observed between the formulation and the water [25]. A delay in the progress of emulsion formation may be due to the time required for the transformation from one liquid crystalline structure to another during the first stages of the disruption process [14]. Thus, the cumulative amount of CoQ_{10} emulsified after 10 minutes decreased with an increase in X_1. On the other hand, addition of Capmul MCM-C8 (X_2) as a cosurfactant increases the interfacial fluidity by penetrating into the surfactant film. This creates void space among surfactant molecules and facilitates the progress of emulsion formation [26]. As shown in the figure, at high X_1, Y_1 increases from 35.73% to 70.03% as X_2 (amount of added Capmul MCM-C8) increases from 20 to 90 mg. The role of added lemon oil (X_3) and

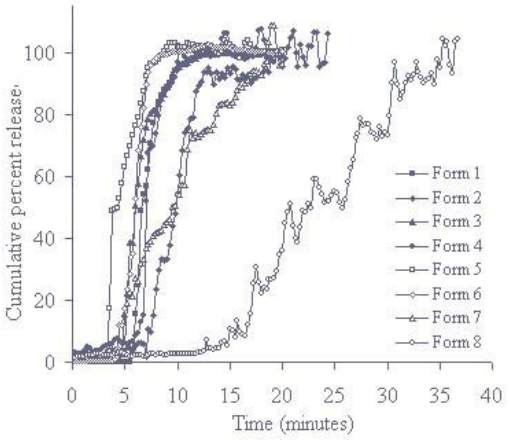

Figure 5 - Cumulative percentage of the formulation released (emulsified) with time from Form 1 to 8.

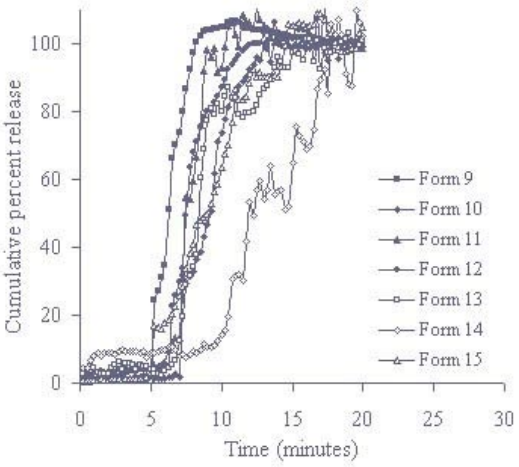

Figure 6 - Cumulative percentage of the formulation released (emulsified) with time from Form 9 to 15.

Table 3 - Regression equations for the responses*

$Y_2 = 102.51 - 10.28X_1 + 4.31X_2 + 4.86X_3 + 12.65X_1X_2 + 7.70X_1X_3 + 2.03X_2X_3 - 7.61X_1^2 - 2.89X_3^2$

$Y_3 = 6.17 + 2.00X_1 - 0.6875X_2 - 1.31X_3 - 1.00X_1X_3 + 1.13X_1^2 - 1.12X_2^2$

$Y_4 = 35.05 - 8.11X_1 - 75.73X_2 + 76.21X_3 - 144.66X_2X_3 - 80.29X_1^2 + 66.19X_2^2 + 66.64X_3^2$

$Y_5 = 5.53 - 61.74X_1 + 16.16X_2 + 1.56X_3 - 24.74X_1X_2 - 5.63X_1X_3 - 1.30X_2X_3 + 60.24X_1^2 - 2.34X_2^2 + 7.25X_3^2$

*The terms with small magnitude of coefficient are deleted from the equation

its interaction with X_1 (amount of Cremophor EL added) on the cumulative percentage of CoQ_{10} emulsified (Y1) can be discussed with the help of Figures 9 and 10. As shown in the figures, with a low amount of added lemon oil, Y_1 levels decreased from 95.05% to 20.6% when X_1 increased from 10 to 70 mg. The cumulative percentage of CoQ_{10} emulsified decreased from 92.7% to 78.78% at high X3 using the same increase in X_1 levels from 10 to 70 mg. This is because the eutectic effect of lemon oil on CoQ_{10} is decreased with the addition of liquid excipients. CoQ_{10} was found to form a eutectic mixture with essential oils. When formulating a semisolid self-nanoemulsified formulation based on a eutectic mixture, the amount of added lemon oil as a eutectic agent should be sufficient to melt CoQ_{10} at or below body temperature. However, diluting the binary system with emulsifiers induces supersaturation and decreases the efficacy of the added lemon oil on melting CoQ_{10}. This causes a slower melting and delayed progress in emulsion formation resulting in a lower overall amount of the drug emulsified into the medium. This could be overcome by increasing the amount of added lemon oil in the formulation. As shown in the figure, the cumulative percentage of CoQ_{10} emulsified, when the amount of added Cremophor EL (X_1) was maintained at

Table 4 - Observed and predicted values of the response Y1

Run	Observed	Predicted	Residuals
1	95.1	95.1	0.0
2	78.2	70.0	8.2
3	88.5	90.0	-1.5
4	91.1	92.9	-1.8
5	97.3	105.4	-8.1
6	102.3	92.7	9.6
7	87.3	88.7	-1.4
8	11.0	20.6	-9.6
9	96.0	104.2	-8.2
10	78.7	78.8	0.0
11	90.6	92.9	-2.3
12	97.1	92.9	4.2
13	75.6	67.5	8.1
14	37.2	35.7	1.5
15	72.3	70.9	1.4

ANOVA for Y_1

Source	DF	SS	MS	F-ratio
Total (corrected	14	8610		
Regression	7	8041	1149	
Residual	7	569	81.3	14.13

Confidence that the regression equation predicts the observed values better than the mean = 93.4%

70 mg, increased from 20.6% to 78.78% when X_3 increased from 25 to 35 mg. Figure 11 is a contour plot that shows the effect of X2 and X3 on Y1. The role of the added Capmul MCM-C8 (X2) and its interaction with the amount of lemon oil added (X_3) on the variable

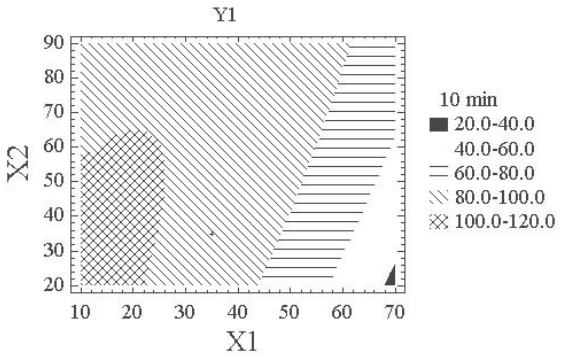

Figure 7- Contour plot showing the effect of the amount of Cremophor EL (X1) and Capmul MCM-C8 (X2) added on the response Y1.

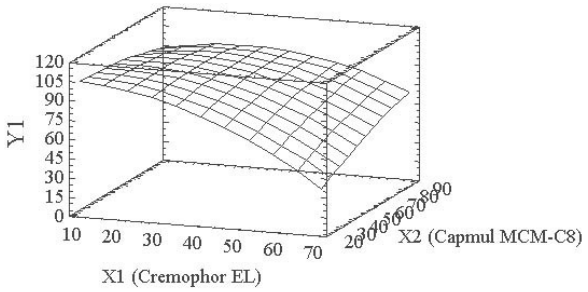

Figure 8- Response surface plot (3D) showing the effect of the amount of Cremophor EL (X1) and Capmul MCM-C8 (X2) added on the response Y1.

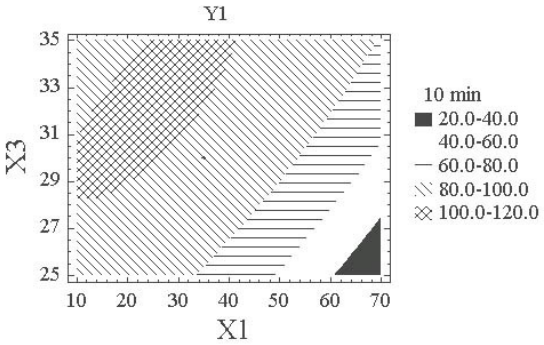

Figure 9 - Contour plot showing the effect of the amount of Cremophor EL (X1) and lemon oil (X3) added on the response Y1.

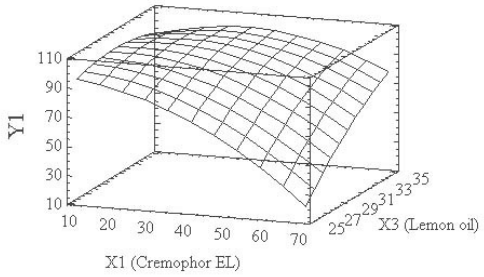

Figure 10 - Response surface plot (3D) showing the effect of the amount of Cremophor EL (X1) and Lemonlemon oil (X3) added on the response Y1.

Y_1 is shown in Figure 12. Conversely, at high levels of X_3, Y_1 increases from 88.73% to 105.43% when X_2 increases from 20 to 90 mg. At low levels of X_3, added Capmul MCM-C8 has little effect on Y_1 in which the cumulative percentage of CoQ_{10} emulsified only increases from 67.48% to 70.88% using the same increase in X_2 levels from 20 to 90 mg. A possible explanation for this behavior might be that at higher concentrations of the oily phase and with a low amount of added Cremophor EL and Capmul MCM-C8, the proportion of the surfactant mix that facilitates water penetration decreases, and the mixture becomes more lipophilic causing increased difficulty of emulsification [27]. This behavior, however, was not observed (Figure 10) when an increase in the amount of Cremophor EL added caused a decrease in the cumulative percentage of CoQ_{10} emulsified. This discrepancy might be due to the differences in the HLB[[define?]] of Cremophor EL (X_1) and Capmul MCM-C8 (X_2) mixtures. Bachynsky et al [28] showed that the HLB of the surfactant mixtures has a significant effect on the performance of the self-emulsifying system. However, optimum surfactant mixture should be obtained at an appropriate combination with the oily phase [29] given as lemon oil (X_3) in this study.

After generating the polynomial equations relating the dependent and independent variables, the process was optimized for the response Y_1. Optimization was performed to obtain the levels of X_1-X_3 which maximize Y1 at constrained conditions of Y_2 through Y_4. The optimized levels and predicted values of Y_1-Y_5 are shown in Table 5. To verify these values, a new formulation was prepared according to the predicted levels of X_1, X_2, and X_3. Obtained Y_1 was in a close

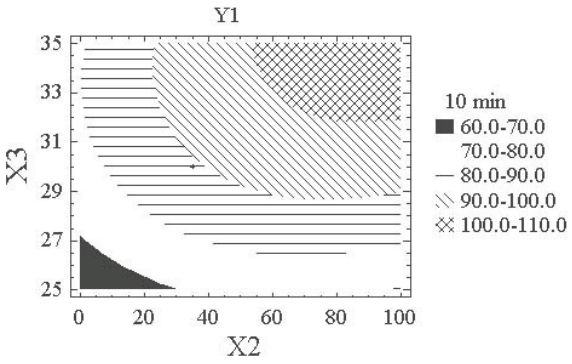

Figure 11 - Contour plot showing the effect of the amount of Capmul MCM-C8 (X_2) and lemon oil (X_3) added on the response Y_1.

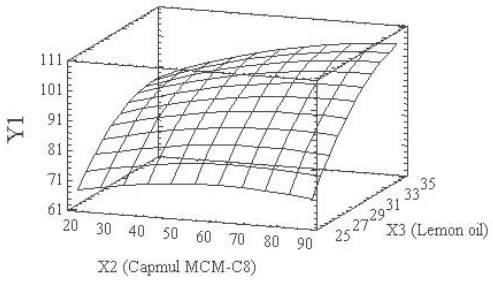

Figure 12 - Response surface plot (3D) showing the effect of the amount of Capmul MCM-C8 (X_2) and lemon oil (X_3) added on the response Y_1.

agreement with the predicted value. The predicted and observed values are shown in Table 5. This demonstrated the reliability of the optimization procedure in predicting the dissolution behavior of self-emulsified drug delivery systems.

Table 5 - Optimized values obtained by the constraints applies on Y1-Y5

Variable	Nominal values	Response	Expected values	Observed values
X_1	35	Y_1	93.4	96.0
X_2	35	Y_2	103.2	103.7
X_3	30	Y_3	6.0	6.1
		Y_4	33.3	42.9
		Y_5	5.2	4.3

CONCLUSION

Optimization of the self-nanoemulsified formulation of CoQ_{10} was performed using Box-Behnken design. The amount of added lemon oil (X_3) and the surfactant mix of Cremophor EL (X_1) and Capmul MCM-C8 (X_2) showed a significant effect on the emulsification rates as well as the physical properties of the resultant emulsion. The quantitative effect of these factors at different levels was predicted by using polynomial equations. Response surface methodology was then used to predict the levels of the factors X_1, X_2, and X_3 required to obtain an optimum formulation with minimum turbidity and lag time and with a maximum emulsification rate. A new formulation was prepared according to these levels. Observed responses were in close agreement with the predicted values of the optimized formulation, thereby demonstrating the feasibility of the optimization procedure in developing self-emulsified formulations.

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