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Quality by design: Understanding the formulation variables of a cyclosporine A self-nanoemulsified drug delivery systems by Box–Behnken design and desirability function

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Abstract

Quality by design (QBD) refers to the achievement of certain predictable quality with desired and predetermined specifications. A very useful component of the QBD is the understanding of factors and their interaction effects by a desired set of experiments. The present project deals with a case study to understand the effect of formulation variables of nanoemulsified particles of a model drug, cyclosporine A (CyA). A three-factor, three-level design of experiment (DOE) with response surface methodology (RSM) was run to evaluate the main and interaction effect of several independent formulation variables that included amounts of Emulphor El-620 (X_1), Capmul MCM-C8 (X_2) and 20% (w/w) CyA in sweet orange oil (X_3). The dependent variables included nanodroplets size (Y_1), nanoemulsions turbidity (Y_2), amounts released after 5 and 10 min (Y_3, Y_4), emulsification rate (Y_5) and lag time (Y_6). A desirability function was used to minimize lag time and to maximize the other dependent variables. A mathematical relationship, $Y_5 = 9.09 - 0.37X_1 + 0.37X_2 - 0.45X_3 + 0.732X_1X_2 - 0.62X_1X_3 + 0.3X_2X_3 + 0.02X_1^2 - 0.28X_2^2 + 0.471X_3^2$ ($r^2 = 0.92$), was obtained to explain the effect of all factors and their colinearities on the emulsification rate. The optimized nanodroplets were predicted to yield Y_1, Y_2, Y_3, Y_4, Y_5 and Y_6 values of 42.1 nm, 50.6 NTU, 56.7, 107.2, 9.3%/min and 3.5 min, respectively, when X_1, X_2, and X_3 values were 36.4, 70 and 10 mg, respectively. A new batch was prepared with these levels of the independent variables to yield Y_1–Y_6 values that were remarkably close to the predicted values. In conclusion, this investigation demonstrated the potential of QBD in understanding the effect of the formulation variables on the quality of CyA self-nanoemulsified formulations.

Keywords: Quality by design; Cyclosporine; Self-nanoemulsification; Box–Behnken; Optimization

1. Introduction

Quality by design (QBD) refers to the achievement of certain predictable quality with desired and predetermined specifications (FDA guidance of industry, 2006). QBD is a broad term that encompasses predefined target quality, physicochemical, physiological, pharmacological and clinical considerations to obtain desired products that are safe and effective. For practical consideration, it is expected that variables associated with raw materials characteristics, product design, process and scale-up issues will be thoroughly investigated. Therefore, a very useful component of the QBD is the understanding of factors and their interaction effects by a desired set of experiments. To understand the variables and their interactions, many statistical experimental designs have been recognized as useful techniques. Response surface methodology (RSM) is used when only a few significant factors are involved in optimization (Ragonese et al., 2002). 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 (Box and Behnken, 1960; Govender et al., 2005).

Cyclosporine A (CyA) is a cyclic undecapeptide used as an oral immunosuppressor for organ transplantation (Noble and

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Markham, 1995). In spite of the great therapeutic interest of this drug, the bioavailability after oral dosing is low (10–60%) with a higher variability (Lindholm et al., 1988). The incomplete and variable bioavailability of CyA has been attributed to its high molecular weight, high lipophilicity, low intestinal permeability (Dai et al., 2004). In order to improve the therapeutic efficacy of CyA and decrease its side effects, many research efforts have been made (Ruxandra et al., 2001; Varela et al., 2001; Dai et al., 2004). In recent years, much attention has been focused on self-emulsifying drug delivery systems for the bioavailability improvement of water insoluble drugs (Pouton, 2000). Self-nanoemulsified drug delivery systems (SNEDDS) are isotropic mixtures of oil, surfactant, co-surfactant, and drugs, which form a fine oil in water emulsion when introduced into aqueous medium under gentle agitation (Nazzal et al., 2002). Different methods are used to characterize the self-emulsified drug delivery systems (Gullapalli and Sheth, 1999; Kommuru et al., 2001). Reported studies often use droplet size analysis, ternary phase diagrams describing the efficient self-emulsification region, low frequency dielectric spectroscopy, zeta potentiometry, and surface tensiometry to evaluate the in vitro performance of the emulsion-based drug delivery systems. Turbidimetry method was also used to describe the efficiency of self-emulsification, to determine lag time and to distinguish between different preparations (Nazzal et al., 2002). These methods, however, require a large number of experiments to describe the effect of excipients and excipient selection on the formulations characteristics. In this regard, a statistical design that requires only a small number of experiments and eliminates the need for time-consuming and detailed ternary phase diagrams was desired. The objective of the present work was to apply Box–Behnken design for understanding the quality and optimization of CyA SNEDDS. The independent variables for the present study were the following: amount of surfactant (SAA) namely Emulphor El-620 (X_1), co-surfactant (CoSAA) namely Capmul MCM-C8 (X_2) and 20% CyA solution in sweet orange oil (X_3). 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 emulsification rate of formulations into aqueous medium with constraints on nanodroplet size, nanoemulsion turbidity, percent CyA released after 5 and 10 min and lag time. This allows the validation of turbidimetry in conjugation with dissolution studies as valuable tools in characterizing a SNEDDS using a minimal number of experiments within the design space.

2. Materials and methods

Cyclosporine (Purity 99%) (Cas no.: CYC140175) was obtained from Poli Industria Chemica S.P.A, Rozzano, Milano, Italy. Mono and diglycerides of caprylic acid (Capmul MCM-C8) (Lot no.: 50130-6) was supplied by Abitec Corp., Janesville, WI, USA. Emulphor: Ethoxylated castor oil (Alkamuls El-620) (Lot no.: 347653) was obtained from Rhodia Inc., Cranbury, NJ, USA. Sweet orange oil (Cas no.: 8016-38-4) was obtained from Sciencelab Inc., Houston, TX, USA. HPLC grade methanol, acetonitrile and phosphoric acid were purchased from VWR scientific, Minneapolis, MN, USA. Hydroxypropyl methylcellulose (HPMC) capsules (size 0, Lot no.: E0401467) were obtained from Shionogi Qualicaps, Whitest, NC, USA. All chemicals were used as received. Reagents were of analytical grade, preparation of HPLC mobile phase was done with Millie-Q demineralized double-distilled water.

Table 1
Variables in Box–Behnken design

Independent variables	Levels		
	Low	Middle	High
X_1: amount of Emulphor El-620 added (mg)	20	50	80
X_2: amount of Capmul MCM (C8) added (mg)	30	50	70
X_3: amount of oily phase added	10	30	50

Dependent variables	Constraints		
	Low	High	Goal
Y_1: particle size (nm)		90	Maximize
Y_2: turbidity (NTU)		100	Maximize
Y_3: cumulative percent of CyA released after 5 min	40		Maximize
Y_4: cumulative percent of CyA released after 10 min	80	100	100
Y_5: emulsification rate (%/min)	7		Maximize
Y_6: lag time (min)	3		Minimize

Amounts (mg) of each independent variable used to prepare the 15 formulations

Run	X_1	X_2	X_3
1	20	30	30
2	20	50	10
3	20	50	50
4	20	70	30
5	50	30	10
6	50	30	50
7	50	50	30
8	50	50	30
9	50	50	30
10	50	70	10
11	50	70	50
12	80	30	30
13	80	50	10
14	80	50	50
15	80	70	30

2.1. Box–Behnken experimental design

A three-factor, three-level Box–Behnken design was applied for the optimization procedure using JMP 6 software (SAS, SAS Institute, Cary, NC, USA). The independent factors and the dependent variables used in this design are listed in Table 1. The amounts of emulphor, capmul and oily phase (20% (w/w) CyA in sweet orange oil) used to prepare each of the 15 formulations are given in Table 1. These high, medium, and low levels were selected from the preliminary experimentation. After generating the polynomial equations relating the dependent and independent variables, the process was optimized for the emulsification rate, Y_5. Optimization was performed using a desirability func-

tion to obtain the levels of X_1, X_2, and X_3, which maximized Y_1–Y_5 while minimizing Y_6.

2.2. Preparation of CyA loaded self emulsified systems

The oily phase comprising 20% CyA solution in sweet orange oil was accurately weighed into screwcapped glass vial. The amount of Emulphor EL-620 and Capmul MCM-C8 were added to the oily mix using a positive displacement pipette and stirred with a magnetic bar. Fifteen formulations with different concentrations of SAA, CoSAA, and oily phase, each containing CyA at a final loading of 25 mg, were filled into size 0 HPMC capsules. Filled capsules were stored at room temperature until used in subsequent studies.

2.3. Emulsion droplet size and turbidity analysis

Formulation (60 mg) was diluted with water, pre-equilibrated at 37 °C, to 100 ml in an Erlenmeyer flask and gently mixed with hand. The droplet size distribution of the resultant emulsions was determined by photon correlation spectroscopy using a Nicomp particle sizing system (Nicomp PSS ZW 380, Santa Barbara, CA, USA). The particle size of emulsions was determined in a small volume module. Samples were directly placed into the module and the data were collected for 10 min. Particle size was calculated from the Nicomp number weighted size distribution. For the same samples, turbidity of the emulsions given in nephlometric turbidity units (NTU) was measured using HACH turbidimeter (2100AN IS Turbidimeter, HACH, Loveland, CO, USA). Turbidity measurements were performed on a clear screwcapped bottle filled with 30 ml of the emulsion. The instrument was carefully calibrated with formazin standards. Accuracy of the instrument, as specified by the manufacturer and based on instrument calibration, is approximately ±0.01 NTU with stray light less than or equal to 0.01 NTU. All studies were repeated as duplicates, with good agreement being found among measurements.

2.4. Dissolution studies

Dissolution studies were performed according to the method described in USP 29/NF 24 for the dissolution of CyA capsules. Dissolution profiles of the capsules filled with the 15 self-nanoemulsified formulations were determined using USP XXIII rotating paddle apparatus at 37 °C and a rotating speed of 50 rpm in a 500 ml of water. Capsules were held to the bottom of the vessel using Teflon plated copper sinkers. Aliquots (3 ml) were withdrawn after 5, 10, 15, 20 and 30 min, filtered using a 0.45 μm millipore nylon filter and assayed for CyA by a validated HPLC method. The HPLC system was composed of a C18, 4.6 mm × 250 mm (5 μm packing) reverse phase chromatography column (Maxsil RP2, Phenomenex, Torrance, CA, USA). The mobile phase consisted of acetonitrile:water:methanol:phosphoric acid (63:30:7:0.5) and was pumped isocratically at a flow rate of 1.25 ml/min. The HPLC instrument (Hewlett Packard, CA, USA) consisted of a quaternary HP 1050 pump, HP 1050 autosampler and 1050 HP UV detector set at a wavelength of 210 nm. The built in HP thermostatted column compartment was set at 60 °C. The dissolution experiments were carried out in triplicates.

2.5. Turbidimetry studies

Turbidimetry was used to monitor the process of self-emulsification by measuring the turbidity of the solution during dissolution as the emulsification process takes place. Turbidity profiles of the capsules filled with the fifteen formulations were determined using HACH turbidimeter. Low pressure flow through cell (Lot no.: 4745000, HACH, Loveland, CO, USA) was used to allow direct reading of sample turbidity associated with capsules subjected to the same dissolution conditions as described above. Two 1/16 in. tygon tubes were connected to a peristaltic pump. First tubing was installed between the pump and the inlet of the flow cell while the other connected the pump to the dissolution vessel. Inlet of the tube connecting the pump to the dissolution vessel was covered with a 40 μm nylon screen and immersed into the medium so that the sample can be continuously withdrawn from a zone midway between the surface of the medium and the top of the rotating blade. 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 0.1 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 s. Turbidimetry experiments were carried out in triplicates.

3. Results and discussion

Box–Behnken design was applied in this study to optimize the CyA SNEDDS with constraints on the particle size, turbidity, amounts released after 5 and 10 min and lag time. The constraints applied were to minimize the lag time and to maximize particle size, turbidity, amounts of drug released after 5 and 10 min and emulsification rate. From the preliminary experimentation, higher variability was found for the amounts of drug released from the smaller particle size than from the larger ones. Accordingly, in order to reduce this variation, maximize constraints were used for particle size and turbidity up to 90 nm and 100 NTU, respectively.

The observed responses for the 15 formulations are given in Table 2. The USP dissolution profile for the 15 formulations is presented in Fig. 1A–C. To assess spontaneity and efficacy of emulsification, the method reported by Nazzal et al. (2002) were adapted in the present study. Turbidity of the 15 formulations during dissolution expressed as the relative intensity of the scattered light was correlated with time during the emulsification process (Fig. 2A–C). The current design was confined to the standard compendial requirements for conducting dissolution experiments. The plots of turbidity against emulsification time have the characteristic lag phase, pseudo linear phase and a gradual tailing toward a plateau as the emulsion systems approached equilibrium. NTU values obtained for the samples placed in the

Table 2
Observed responses[a] for the 15 formulations of Box–Behnken design

Run #	Y_1 (nm)	Y_2 (NTU)	Y_3 (%)	Y_4 (%)	Y_5 (%/min)	Y_6 (min)
1	40.5	73.5	19.92	98.2	9.57	3.25
2	44.5	38.8	42.29	102.3	9.98	4.25
3	88.3	278	29.7	83.83	9.8	4.25
4	87	235	44.42	102.9	9	4.5
5	10.3	28.7	18.23	95.73	9.47	3.25
6	9.8	72.1	32.12	82.29	9.32	4.75
7	32.6	15.1	40.2	99.26	9	3.65
8	35.1	15.5	32.49	96.37	9.34	3.25
9	37.8	17.3	42.3	102	8.93	3.25
10	34.2	10.8	53.9	107	9.46	6.75
11	49.2	35.2	39.8	100.3	9.69	5.5
12	16.8	28.3	25.6	71.46	7.21	3.25
13	7.2	23	18.13	87.78	10.63	2.75
14	14.5	24.4	43.18	95.32	7.95	3
15	11.3	14.4	47.68	100.8	9.57	5.25

Y_1: nanodroplet size; Y_2: nanoemulsion turbidity; Y_3 and Y_4: amounts of CyA released after 5 and 10 min; Y_5: emulsification rate; Y_6: lag time.
[a] Standard deviation of the responses did not exceed 3% of the measured value.

dissolution medium at 37 °C after reaching equilibrium could be termed $NTU_{plateau}$. Lag phase of the turbidity–time profile reflects the time elapsed before the formula is released from the capsule into the dissolution medium. A cumulative percent of the formulation emulsified with time could be obtained by plotting cumulative turbidity given by $(NTU_t \times 100)/NTU_{plateau}$ as a function of time, assuming that $NTU_{plateau}$ reflect 100% of the formula released from the capsules regardless of the actual amount of CyA dissolved in the medium (Fig. 3A–C). NTU_t is the turbidity reading at any time *t*. As seen in Fig. 3, plots of cumulative percent of the formulation released with time are identical to the original profiles correlating turbidity with time where the curve characteristics, lag time, pseudo linear and plateau phases, are preserved. The slope of the pseudo linear phase for the line correlating cumulative percent emulsified with time could be regarded as the emulsification rate or the emulsification efficacy.

Based on the experimental design generated, the factor combinations resulted in different responses. From these results, it can be concluded that all these formulations yielded acceptable turbidity (<80 NTUs) and particle size range (<90 nm) for nanoemulsions, except for formulation nos. 3 and 4 that showed higher turbidity (>200 NTUs). Similarly, it can be inferred from Fig. 1 that these three factors have a profound effect on the drug release profiles. For example, the amounts of the drug released after 5 and 10 min ranged from 13.13 to 47.68% and 71.46 to 107%, respectively. Based on the experimental design, the factor combinations provided different emulsification rates. The range of the emulsification rate was 7.21%/min in formulation no. 12 (minimum) and 10.63%/min in formulation no. 13 (maximum).

In order to obtain a formulation having higher emulsification rate, RSM optimization was used to determine the levels of these factors. The mathematical relationship in the form of factors' coefficients and its corresponding *P*-values for the measured responses is listed in Table 3. Coefficients with *P*-value less than 0.05 had a significant effect on the prediction efficacy of the model for the measured response. The confidences that the regression equation would predict the observed values better than the mean for Y_1, Y_2, Y_3, Y_4, Y_5 and Y_6 were 92, 73.7, 80.3, 75.6, 78.7, and 72.2%, respectively. The polynomial equation relating the response Y_5 and the independent

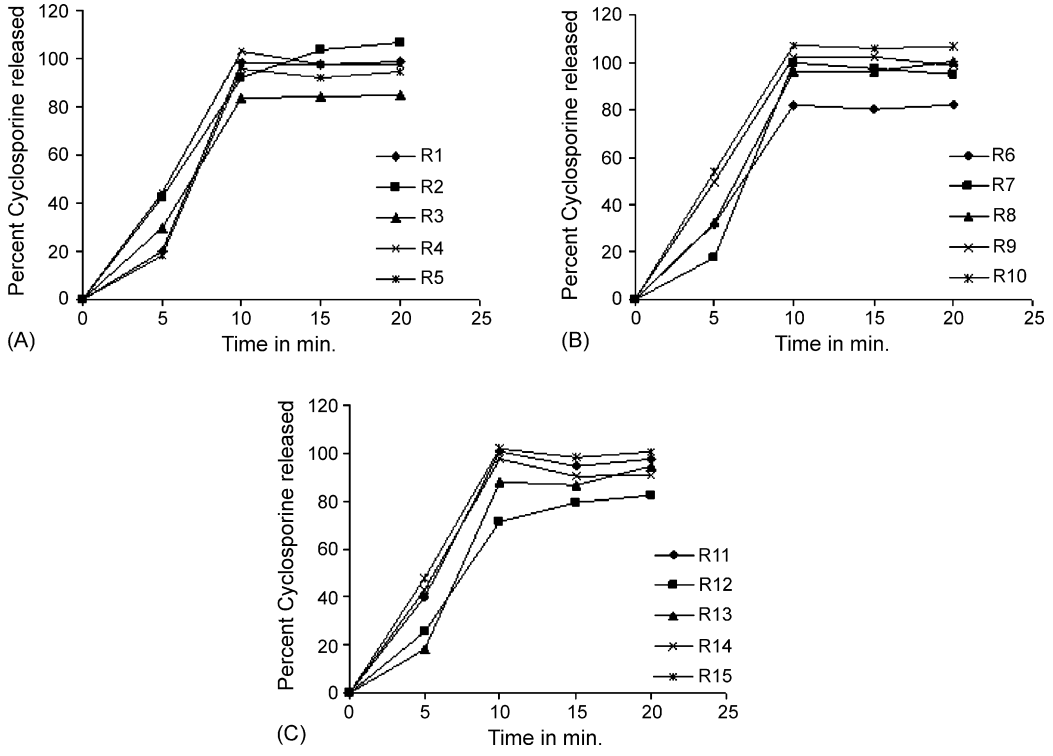

Fig. 1. (A–C) Dissolution profiles of CyA SNEDDS that are listed in Table 1.

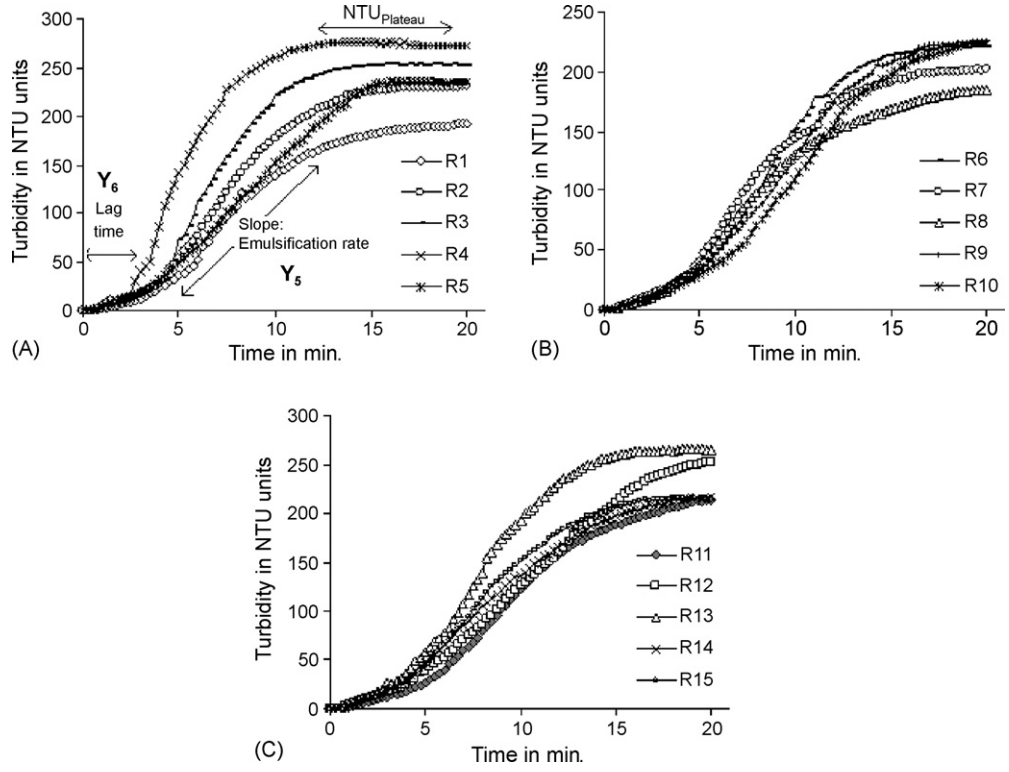

Fig. 2. (A–C) Turbidity profiles of the dissolution of CyA SNEDDS that are listed in Table 1.

variables was:

$$Y_5 = 9.09 - 0.37X_1 + 0.37X_2 - 0.45X_3 + 0.73X_1X_2 - 0.62X_1X_3 + 0.3X_2X_3 + 0.02X_1^2 - 0.28X_2^2 + 0.471X_3^2$$

The above equation represents the quantitative effect of process variables (X_1, X_2, and X_3) and their interactions on the response (Y_5). The values of the coefficients X_1–X_3 are related to the effect of these variables on the response (Y_5). Coeffi-

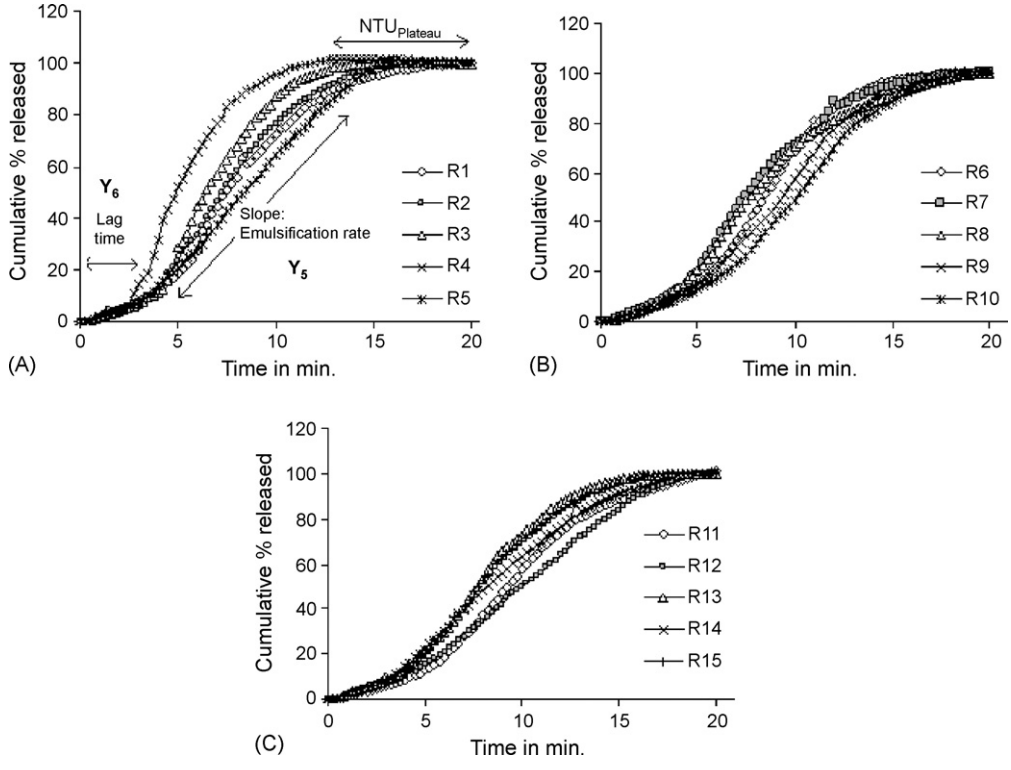

Fig. 3. (A–C) Normalized turbidity time profile showing the cumulative percent of CyA released with time from SNEDDS stated in Table 3.

Table 3
Regression equations for the responses

	A	X_1	X_2	X_3	X_1X_2	X_1X_3	X_2X_3	X_1^2	X_2^2	X_3^2
Y_1	35.16	−26.31	13.03	8.2	−13	−9.12	3.875	8.241	−4.5	−4.7
P-value	0.0004	0.0001	0.0037	0.0236	0.0155	0.0526	0.3318	0.0796	0.2836	0.2587
Y_2	15.96	−66.9	11.6	38.55	−43.85	−59.45	−4.75	63.09	8.74	11.99
P-value	0.5402	0.0006	0.4709	0.0488	0.0916	0.0369	0.8304	0.0346	0.7063	0.6076
Y_3	38.33	−0.217	11.24	1.53	−0.605	9.41	−6.99	−3.306	−0.618	−1.698
P-value	0.0001	0.9061	0.0014	0.4222	0.8168	0.0127	0.037	0.2562	0.8200	0.5393
Y_4	99.21	−3.983	7.915	−3.883	6.16	6.5025	1.685	−4.946	−0.923	−1.956
P-value	0.0001	0.0636	0.0053	0.0685	0.0484	0.0407	0.5093	0.1015	0.7237	0.4641
Y_5	9.09	−0.373	0.3712	−0.45	0.7325	−0.625	0.3	0.028	−0.281	0.471
P-value	0.0001	0.0411	0.0420	0.0217	0.0128	0.0232	0.1815	0.8920	0.2212	0.0663
Y_6	3.38	−0.25	0.9375	0.0625	0.1875	0.0625	−0.687	−0.41	1.089	0.589
P-value	0.0002	0.2872	0.0066	0.7779	0.5554	0.8416	0.0684	0.2415	0.0168	0.1147

Y_1: nanodroplet size; Y_2: nanoemulsion turbidity; Y_3 and Y_4; amounts of CyA released after 5 and 10 min; Y_5: emulsification rate Y_6: lag time; X_1: amount of oily phase; X_2: SAA amount and X_3: CoSAA amount.

cients with more than one factor term and those with higher order terms represent interaction terms and quadratic relationships, respectively. Concerning the P-value of the coefficients, X_1, X_2, X_3, X_1X_2 and X_1X_3 were found to have significant effects on the performance of the model for the prediction of the emulsification rate. A coefficient with positive sign represents a synergistic effect of the factor on the response, 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_5. 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). In ANOVA, the "Prob > F" parameter is the observed significance probability (P-value) of obtaining a greater F-value by chance alone if the specified model fits no better than the overall response mean. Observed significance probabilities of 0.05 or less are often considered evidence of a regression effect. A Prob > F of 0.0244 indicated a significant effect of the independent factors on the response (Y_5).

Table 4
Observed and predicted values and analysis of variance parameters for the response Y_5

Run #	Observed Y_5	Predicted Y_5	Residuals
1	9.57	9.57	0.00
2	9.98	9.79	0.19
3	9.8	10.14	−0.34
4	9	8.85	0.15
5	9.47	9.66	−0.19
6	8.5	8.16	0.34
7	9	9.09	−0.09
8	9.34	9.09	0.25
9	8.93	9.09	−0.16
10	9.46	9.80	−0.34
11	9.69	9.50	0.19
12	7.21	7.36	−0.15
13	10.63	10.29	0.34
14	7.95	8.14	−0.19
15	9.57	9.57	0.00

Source	d.f.	Sum of squares	Mean square	F ratio	Prob > F
ANOVA for Y_5					
Model	9	9.10	1.01	6.7616	0.0244
Error	5	0.74	0.14		
Cumulative total	14	9.85			

Y_5: emulsification rate and d.f.: degree of freedom.

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_5 at a middle level of X_3 is given in Fig. 4. At low levels of X_2 (amount of capmul added), Y_5 decreased from 9.6 to 7.43%/min when the amount of emulphor EL (X_1) increases from 20 to 80 mg. Conversely, at high levels of X_2, Y_5 increases from 8.8 to 9.5%/min when X_1 increases from 20 to 80 mg. The possible explanation for this is that emulphor EL (surfactant) is strongly localized to the surface of the emulsion droplet which reduces interface free energy and provides a mechanical barrier

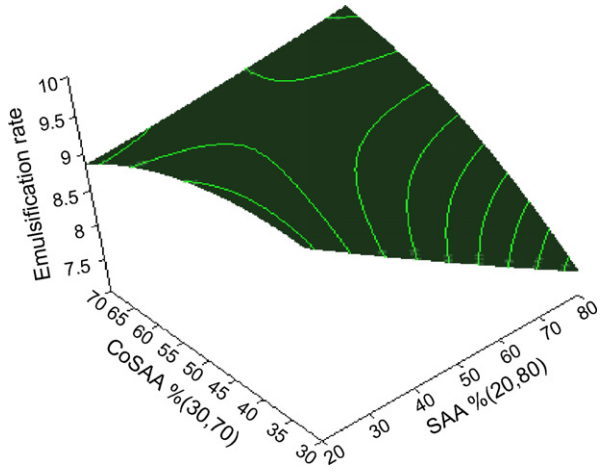

Fig. 4. Response surface (3D) and contour plots showing the effect of the amounts of Emulphor EL-620 (X_1) and Capmul MCM-C8 (X_2) added on the response Y_5.

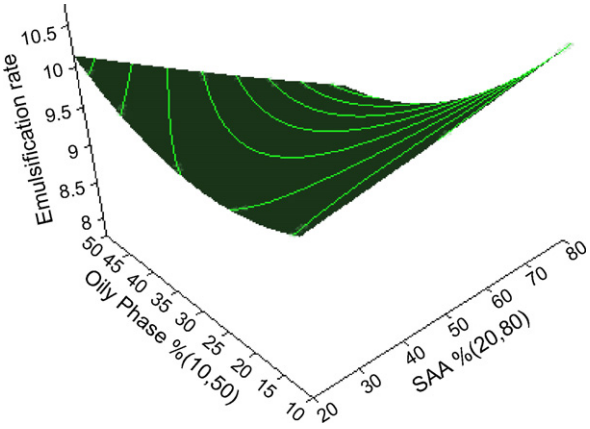

Fig. 5. Response surface (3D) and contour plots showing the effect of the amounts of Emulphor EL-620 (X_1) and sweet orange oil (X_3) added on the response Y_5.

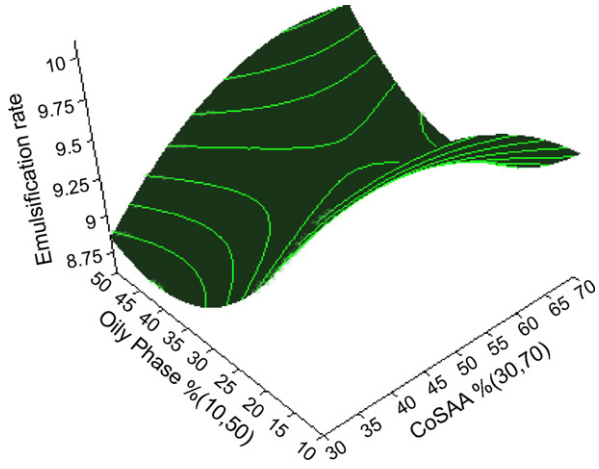

Fig. 6. Response surface (3D) and contour plots showing the effect of the amounts of Capmul MCM C8 (X_2) and sweet orange oil (X_3) added on the response Y_5.

to coalescence resulting in a thermodynamically spontaneous dispersion (Reiss, 1975). However, at high emulphor EL concentration (X_1), progress of emulsification might be compromised by viscous liquid 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 were observed between the formulation and the water (Iranloye et al., 1983). 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 (Kommuru et al., 2001). Thus, the emulsification rate decreased with an increase in X_1. On the other hand, addition of Capmul MCM C8 (X_2) as a CoSAA increases the interfacial fluidity by penetrating into the surfactant film. This creates void space among surfactant molecules and facilitates the progress of emulsion formation (Constantinides and Scalart, 1997). As shown in the figure, at high X_1, Y_5 increases from 7.42 to 9.54%/min as X_2 (amount of added Capmul MCM C8) increases from 30 to 70 mg.

The role of added sweet orange oil (X_3) and its interaction with X_1 (amount of emulphor EL added) on the rate of emulsification (Y_5) can be discussed with the help of Fig. 5. As shown in the figure, with a low level of the oil added, Y_5 levels increased from 9.71 to 10.2%/min when X_1 increased from 20 to 80 mg. The emulsification rate decreased from 10.1 to 8.2%/min at high X_3 using the same increase in X_1 levels. This result explained the efficacy of emulphor alone as a SAA to emulsify low oil concentrations and the need of SAA–CoSAA mixture to emulsify higher concentrations. As a result, an optimum ratio of sweet orange oil and emulphor EL is required to yield a formulation with higher emulsification rate.

From the 3D plots in Fig. 6, it is clear that, at a middle level of the SAA, the amounts of sweet orange oil and Capmul MCM C8 have major effects on determining the CyA SNEDDS emulsification rate. This figure shows that at a lower oil concentration, a small increase in the emulsification rate was observed by an increase in the concentration of Capmul MCM C8 from 30 to 70 mg. However, at a higher concentration of sweet orange oil, the emulsification rate increased from 8.15 to 9.45%/min with the same increase in capmul concentration. This finding can be explained by the fact that CoSAA by itself does not emulsify the oil, rather the CoSAA acts by enhancing the emulsifying capability of surfactants. Moreover, this behavior might be due to the fact that at higher concentrations of the oily phase and with a low amount of added emulphor and capmul, the proportion of the surfactant mixture that facilitates water penetration decreases and the mixture becomes more lipophilic causing increased difficulty of emulsification (Halbaut et al., 1996). This behavior, however, was not observed when an increase in the amount of emulphor added, at high oil levels, caused a decrease in the emulsification rate. This discrepancy might be due to the differences in the hydrophilic lipophilic balance (HLB) of emulphor (X_1) and capmul (X_2) mixtures. Bachynsky et al. (1997) 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 (Shah et al., 1994).

The main effect of the independent variables on the dependent variables was further investigated using a Pareto chart and interaction plot (Fig. 7). Regarding the interaction plots, it is shown that at low level of the SAA, the emulsification rate remained constant with changes in the CoSAA and the oily phase percentages. At high CoSAA concentration, the emulsification rate showed no change by changing the percentage incorporated of the SAA and the oily phase. Moreover, the effect of changing the amounts incorporated of the SAA and the CoSAA on the emulsification can only be observed at low oily phase concentration. The standardized Pareto chart for Y_5 depicts the main effect of the independent variables on the emulsification rate 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. From Fig. 7, it can be inferred that the factors X_1X_2 (interaction effect of emulphor and capmul), X_3 (level of the oily phase), X_1X_3 (interaction effect of emulphor and oily phase), X_1 (emulphor level) and X_2 (capmul level) have significant effects on the emulsification rate. The highest effect was observed for the interaction term X_1X_2 which confirms the results of the 3D

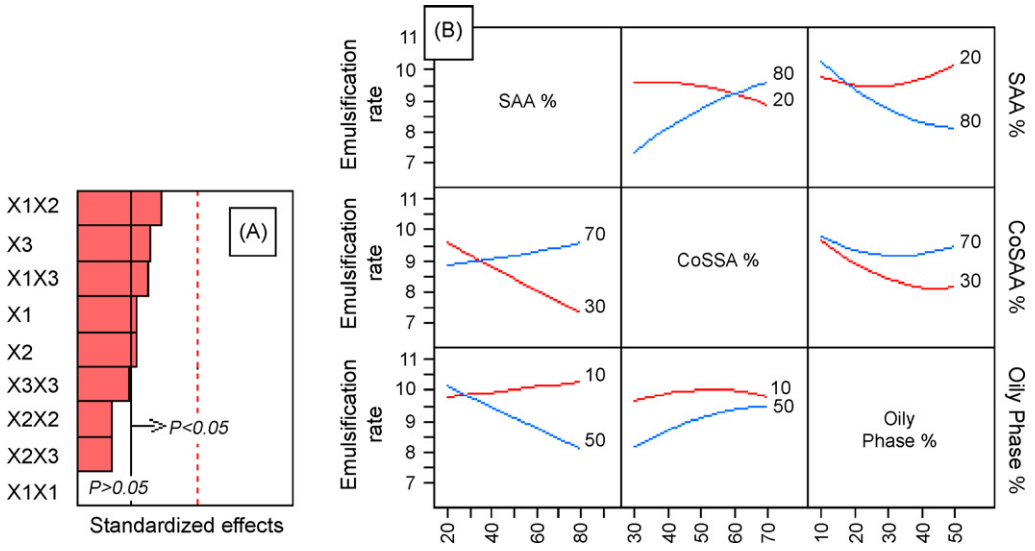

Fig. 7. Standard Pareto chart (A) showing the effects of independent variables and their combined effects on the emulsification rate of CyA SNEDD formulation. Interaction plot (B) showing the quadratic effects of interactions between factors on the emulsification rate of CyA SNEDD formulation.

plots that the SAA to CoSAA ratio is the most important factor to achieve a higher emulsification rate. With the traditional one variable at a time approach, this interaction variable could have easily been missed.

Having studied the effect of independent variables on the responses, the levels of these factors were determined by using a computer optimization process and a desirability function, RSM. The predicted values of Y_1, Y_2, Y_3, Y_4, Y_5 and Y_6 were 42.1 nm, 50.6 NTU, 56.7%, 107.2%, 9.3%/min and 3.5 min, respectively, at X_1, X_2, and X_3 levels of 36.4, 70 and 10 mg, respectively. As a confirmation process, a fresh formulation of CyA SNEDDS was prepared with 20% CyA in sweet orange oil (10 mg), Capmul MCM C8 (70 mg) and emulphor EL 620 (36.4 mg). The optimized levels of factors yielded a formulation with rapid drug release of 54% within 5 min and complete drug release within 10 min. The nanodroplets size, turbidity, emulsification rate and lag time of the optimized formula were 40 nm, 55.2 NTU, 9.5%/min and 3.8 min, respectively. The observed and predicted values were in close agreement. This demonstrated the reliability of the optimization procedure in predicting the emulsification rate of self-nanoemulsified drug delivery systems.

4. Conclusion

The quality of CyA SNEDDS was presented using Box–Behnken design. All the independent variables, namely amount of added CyA solution in sweet orange oil (X_3) and the surfactant mixture of emulphor EL620 (X_1) and Capmul MCM-C8 (X_2), were found to affect the emulsification rates as well as the physical properties of the resultant emulsion either through linear, quadratic or interaction effects. The highest effect was observed for the interaction term X_1X_2 which confirmed that the SAA to CoSAA ratio is the most important factor to achieve a higher emulsification rate. The optimized formulation prepared using the predicted levels of factors provided the desired observed responses with Y_1, Y_2, Y_3, Y_4, Y_5 and Y_6 values of 42.1 nm, 50.6 NTU, 56.7%, 107.2%, 9.3%/min and 3.5 min, respectively. Consequently, through the rigorous analysis of the three independent variables and its effects on the investigated responses, this study demonstrated the potential of QBD in developing self-emulsified formulations.

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