Original Research Article

In vitro characterization of statistically optimized quetiapine-loaded self-nanoemulsified systems with quality by design

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Abstract

Objective: To optimize and characterize quetiapine (QP)-loaded self-nanoemulsified drug delivery systems (SNEDDS) by 3-factorial 3-level Box–Behnken design (BBD) to improve the dissolution.

Methods: Amounts of olive oil (X_1), tween 80 (X_2), and PEG 400 (X_3) as independent factors, whereas % limpidity (Y1), self-emulsification time (SET) (Y2), and drug released at 15 min (T_{15}) (Y3) as responses were employed in BBD. Three-dimensional response surface plots were run to understand the main interaction and quadratic effects of independent variables. Preliminary screening was carried out by equilibrium solubility and emulsification efficiency studies. Nanoemulsification region was recognized by pseudoternary plot.

Results: Mean droplet size of optimized nanodispersion was 89.68 nm, electrokinetic potential was −27.2 mV and polydispersity index <1 and represented 94% of limpidity, 69 s of SET, and 93.4% of T_{15}. Software-generated model graphs (predicted versus actual and residual versus predicted) for all three responses were produced without outliers and thus indicated the adequacy of selected statistical model.

Conclusion: This study explained the effectiveness of BBD in insights of formulation variables and quality of QP-loaded nanoemulsified systems so as to enhance dissolution. As a result, BBD was a well-suitable experimental design in predicting the responses of QP-loaded liquid SNEDDS.

Keywords: Independent-variables, interaction-effect, limpidity, model-adequacy, response surface plot, self-emulsification time

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INTRODUCTION

Systemic drug delivery in schizophrenia is a major confront due to the presence of hindrances like, blood–brain barrier and P-glycoprotein, which forbid the entry of drugs into the brain.[1] Colloidal drug transporters resembling micelles, emulsions, liposomes, and nanoparticles have been largely accounted for brain drug delivery owing to the simple and easy scale-up methods of preparation.[2] Most of the hydrophobic antipsychotic moieties were prepared as micro and nanoemulsions to overcome the intrinsic demerits such as poor solubility, precipitation, degradation, and first-pass metabolism.

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Since the approval by Food and Drug Administration in 2008, quetiapine (QP) a dibenzothiazepine antipsychotic has been shown its promise in the monotherapy and maintenance of acute depressive and acute manic or mixed episodes of bipolar disorder. Frequently, lower doses of QP are also employed as an add-on medication in psychosis patients. Significant side effects of QP such as weight gain, medication-induced diabetes, and extrapyramidal symptoms are reported to be at sensible levels over other antipsychotics. Probably, these advantages have an account for 45% of QP use within the category of atypical drug moieties such as aripiprazole, ziprasidone, olanzapine, and risperidone.[3] However, QP belongs to Biopharmaceutical Classification System (BCS) Class II category shows extremely poor bioavailability (9%) with 6 h of half-life and 2.8 of log *P* value. The above solubility-based issues were addressed by cyclodextrin inclusion complexes,[4] solid dispersions,[5] nanoparticles,[6] solid lipid nanoparticles (SLN's),[7,8] microemulsions,[9] lipid-core nanocapsules,[10] microemulsions,[1] intranasal nanoemulsions,[11] and *in situ* nasal SLN's[6] at various instances.

Smart oral nanosized lipid systems like self-nanoemulsifying drug delivery systems (SNEDDS) are become superior in addressing solubility based issues in the recent past. The characteristic increase in interfacial area and decrease in surface free energy of internal and external phase are the core principles involved in improving the solubility, dissolution, and imparting the thermodynamic stability in SNEDDS as explained by Noyes–Whitney and Ostwald–Freundlich equations.[12,13] Moreover, prominent use of long-chain and medium-chain triglycerides as carriers in SNEDDS facilitates effective dual absorption mechanisms (portal and lymph).[14]

Pragmatically, SNEDDS are prepared by altering the carrier ratio and envisaging pseudoternary phase diagram with extended number of trails. Of late, application of statistical experimental tool in development of drug products is often practiced to optimize the quality of novel delivery systems. Central composite designs or Box–Behnken designs (BBDs) are popular among the statistical experimental tools by utilizing response surface (RS) mechanism to evaluate the relationship between formulation factors and response variables. A second-order RS model proposed by Box and Behnken is more economical since it requires fewer design runs (in three factorial design) with good prediction variance properties.[15] Further, each factor in the design requires only three levels which are experimentally convenient to conduct.

Considering the entire facts, the present study was focused to develop an optimized formulation of QP SNEDDS by employing an accurate, robust, and significant tool of quality by design (QbD), 3-factorial 3-level BBD, and to evaluate main and interaction effects of SNEDDS formulation variables such as amounts of olive oil (X_1), tween 80 (X_2) as surfactant, and PEG 400 (X_3) as cosurfactant on three responses: transmittance (Y1), self-emulsification time (SET) (Y2), and percentage drug released at 15 min (Y3). Three-dimensional RS plots were also aimed to envisage the effects of X1, X2, and X3 on QP release.

MATERIALS

QP was a generous gift of M/s Aurobindo Pharma Ltd (Hyderabad, India). Oils such as canola, gingelly, olive, rice bran, soyabean, and sunflower were purchased from the local supermarket with double-refined grade. Ethanol, glycerin, isopropyl myristate, oleic acid, PEG 400, propylene glycol, span 20, span 80, tween 20, and tween 80 were obtained from M/s. Merck Specialties Ltd., Mumbai, India. Deionised double-distilled water was used throughout the study. Methanol used in the study was of analytical grade. All other chemicals and materials used in the study were generally recognized as safe with the pharmaceutical grade.

Methods

Solubility of carriers/vehicles

Solubility of QP in various liquid vehicles such as oils, surfactants, and cosurfactants was assessed by shake flask method. An excess of QP was introduced into calibrated glass vial containing 5 ml of each vehicle. Mixtures in glass vial were sonicated for 5 min and equilibrated at 25°C ± 1.0°C in an isothermal shaker for 72 h. Then, contents of each vial were centrifuged at 3000 rpm for 15 min followed by filtering off undissolved QP through 0.45 μm nylon filter. Filtered samples were suitably diluted with methanol and drug concentration was assayed at 254 nm. Experiment was repeated for three independent observations and mean value was considered. Surfactants were selected based on their major characteristics properties such as emulsification efficiency, transparency (%), and dilution ability. Span 20, span 80, tween 20, and tween 80 were selected to screen the efficiency of emulsification with optimized oil phase. A measured quantity (10 μl) of optimized oil phase was added to 10 ml of aqueous surfactant (10%w/w) continuous stirring. The experiment was continued with increments of oil phase until mixtures turn into turbid/cloudy.[16,17]

Selection of cosurfactants (by emulsification)

Ethanol, glycerin, propylene glycol, and PEG 400 were selected to screen their efficiencies in assisting the surfactants in emulsification of oils. Similar to above, the oil phase was

added to aqueous solution of cosurfactant and surfactant mixture (1:1) and repeated until turbidity appears. Percentage transparency of above systems was also computed.[18,19]

Construction of ternary phase diagram

Nanoemulsion region of preconcentrates was identified by aqueous titration method. In this method, isotropic mixtures were prepared with blends of surfactant and cosurfactant (S_{mix}) (at 1:1, 1:2, 1:3, 2:1, and 3:1 volume ratios) and oil phase at 1:9 ratio and vice versa were titrated with double-distilled water. Data obtained on spontaneous emulsification of preconcentrates were used to develop the pseudoternary phase diagrams.[20]

Preparation of quetiapine-loaded self-nanoemulsified drug delivery systems

Weighed quantity (50 mg) of QP was thermostatically admixed with olive oil in a glass vial. Contents of glass vial were equilibrated with S_{mix} at predetermined ratios to obtain liquid SNEDDS. The composition of liquid SNEDDS was given in Table 1.

Box–Behnken Design

Box–Behnken statistical rotatable screening design was employed to evaluate the main interaction and quadratic effects of selected lipid/oil, surfactant, and cosurfactants on the performance of the formulation and subsequent optimization. Design expert software version 10.0.1 was used to produce nonlinear quadratic model in BBD. A 3-level 3-factorial design was applied and amounts of oil, surfactant, and cosurfactant were selected as factors and % limpidity, (SET in seconds) and T_{15}% were treated as responses in all 15 formulations (runs) as given in [Table 1]. Coded and actual values of high, medium, and low levels were comprehended from the pseudoternary illustrations.[21,22] The following polynomial equation (1) was used to fit the mean values of experimental data as:

$$Y = \beta0 + \beta1X1 + \beta2X2 + \beta3X3 + \beta4X1X2 + \beta5X1X3 + \beta6X2X3 + \beta7X12 + \beta8X22 + \beta9X32 + E \quad (1)$$

where Y is the analyzed response, β_0 is intercept; β_1 to β_9 are the regression coefficients, X1, X2, and X3 are the independent variables, whereas E is error term. Generated model adequacy was supported by plots of predicted versus actual values and also residual versus predicted runs and were estimated accordingly. On the basis of polynomial or model equation, 3D-RS plots were aimed to examine the relationship between responses, mixture components, and numeric factors.

Evaluation of self-nanoemulsified drug delivery systems

Physical stability

SNEDDS were subjected to heating, cooling (4°C and 45°C), and freeze-thaw cycles (−21°C and + 25°C) for 48 h. Formulations were then centrifuged at 3500 rpm for 15 min to observe extent of phase separation, creaming, and coalescence.

Self-emulsification time

Emulsification time of QP-loaded SNEDDS was recorded using USP type II dissolution apparatus. Each SNEDDS formulation was added dropwise to 500 ml of distilled water under continuous stirring (50 rpm) at 37°C. The emulsification time was sequentially graded on visual inspection [Table 1]. The study was repeated for similar observations in triplicate.

Limpidity measurement (percent transmittance)

The QP-loaded SNEDDS were reconstituted with deionized double-distilled water and the resulted

Table 1: Amount of each independent variable and observed responses of 15 formulations

Runs	Factors			Response[a]				
	X1	X2	X3	Y1 (%)	Y2 (s)	Y3 (%)	Thermodynamic stability	Dilution study with water*
1	27.5	75	33	89	73	89	Stable	B
2	27.5	65	39	91	79	87	Milky	A
3	20	55	39	95	67	95	Stable	A
4	27.5	75	45	74	82	88	Unstable	C
5	35	55	39	82	89	84	Unstable	B
6	35	65	33	87	93	81	Stable	B
7	20	65	45	82	75	89	Stable	B
8	35	75	39	78	102	78	Unstable	C
9	20	75	39	85	71	90	Stable	B
10	27.5	65	39	93	87	85	Stable	B
11	27.5	55	33	97	72	89	Stable	B
12	27.5	55	45	96	77	87	Stable	B
13	27.5	65	39	94	79	86	Stable	B
14	35	65	45	78	112	77	Milky	C
15	20	65	33	98	69	94	Stable	A

[a]Standard deviation did not exceed 2% of the measured value. Y1: Percentage limpidity, Y2: Self-emulsification time, Y3: *In vitro* drug release of QP at 15 min. *Dilution study - A. Rapidly forming clear or slightly bluish nanoemulsion within 2 min; B. Rapidly forming less clear emulsion that had a bluish-white appearance; C. Bright milky emulsion

nanoemulsions were examined for turbidity if any. Thereafter, its % transmittance was measured at 650 nm using UV-visible spectrophotometer against distilled water as blank. The studies were conducted after 100 times dilution of preconcentrates.

In vitro drug release

Dialysis bag method was employed to study the release of QP from SNEDDS. In this study, about 5 ml of each SNEDDS formulation was enclosed in a dialysis bag of molecular weight 12,000–14,000 g/mole and pore size of 2.4 nm. Aliquot of 5 ml samples were withdrawn at different time intervals by maintaining the sink condition. Further, the samples were filtered through a 0.22 μm membrane filter disc and assayed at 254 nm. Measurements were carried out in triplicate from three independent samples.[16]

Identification of optimized formulation

Numerical optimization technique was adapted for optimizing the formulation variables to obtain desired responses. Further experiments were repeated in triplicate to determine the dependability of optimized conditions. Mean values of experimental data were compared against predicted values and thus percent error was determined. Data of the results were treated to assess the accuracy and suitability of the conditions employed in preparation of SNEDDS.

Evaluation of optimized formulation

Zeta potential and droplet size analysis

Zeta potential and droplet size distribution patterns of optimized QP-loaded SNEDDS were assessed using dynamic light scattering technique with Zeta sizer (HAS 3000, Horiba Scientific, Singapore). SNEDDS were placed in clear disposable cuvette of Zeta sizer and results were noted. The polydispersity index (PDI) values of SNEDDS were calculated using $D_m = M_w/M_n$ equation, in which M_w is weight-average molar mass and M_n is number-average molar mass.[23]

RESULTS

Selection of carriers

Solubility parameter is obligatory since only specific combinations of excipient led to efficient nanoemulsion formation as shown in Figure 1. Both olive oil and isopropyl myristate had high solvent effect on QP as 3.61 and 2.57 mg/ml, respectively. Other liquid vehicles such as sunflower (0.173 mg/ml) and soyabean (0.163 mg/ml) oils had shown poor solubility. Solubility of QP was moderate in rice bran and canola oils. On the other hand, oil solubility of surfactants follows the rank order as span 80> tween 80> tween 20> span 20. Nonionic span 80 has characteristically high emulsification efficiency, transparency (97.3%), and five times flask inversions. Hence, span 80 was selected as amphiphilic carrier in the design of SNEDDS.

Selection of cosurfactants

Isotropic mixtures were produced on complete miscibility of cosurfactant with blend of olive oil and amphiphile. Maximum emulsification efficiency, high transmittance (99.43%), and 14 flask inversions were resulted with PEG 400 as shown in Table 2 when compared to that of other short-chain alcohols.

Ternary phase diagram

Nanoemulsification region was as broad as for the preconcentrate system with S_{mix} at 1:2 (w/w) ratio over other preconcentrates, demonstrated as colored region in pseudoternary phase diagram [Figure 2]. Existence of nanoemulsion region was identified between 20 and 35 mg range for the coordinate of olive oil; 55–75 mg for coordinate of span 80 and between 33 and 45 mg range for PEG 400.

Box–Behnken design

BBD was applied to optimize QP-loaded SNEDDS. As given in Tables 1 and 3, coded formulation factors with

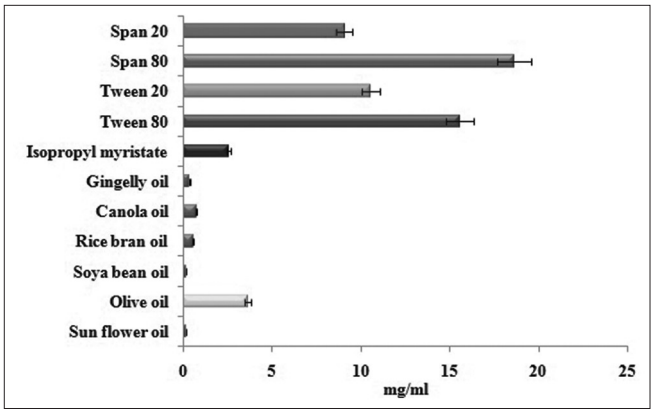

Figure 1: Selection of quetiapine in various oils (solubility studies)

Table 2: Emulsification efficiency of selected surfactants and cosurfactants

	Percentage transmittance	Number of flask inversions*
Surfactants		
Tween 80	93.8	7
Tween 20	56.1	10
Span 80	97.3	5
Span 20	61.7	13
Cosurfactants		
PEG 400	99.43	14
Propylene glycol	89.21	19
Ethanol	97.82	26
Glycerin	51.7	31

*All the data expressed as mean (n=3). PEG: Polyethylene glycol

responses such as percent limpidity, SET in seconds, and amount released after 15 min ($T_{15\ min}$) were prepared with three center points to predict the design's precision and capability. As 3D-RS plots given in [Figure 3a-i], response Y1 was significantly influenced by content of X1 (amount of olive oil added) (at $P = 0.0006$), whereas Y2 was influenced by X1 (olive oil) (at $P = 0.0002$), X3 (PEG 400) (at $P = 0.0187$), quadratic terms $X1^2$ (at $P = 0.0368$), $X2^2$ (at $P = 0.0514$) and Y3 were affected by X1 (at $P = 0.0112$), X2 (at $P = 0.0044$), X3 (at $P = 0.0059$) and $X1^2$ (at $P = 0.0249$), respectively. Software-generated model graphs (predicted vs. actual and residual vs. predicted) for all three responses were produced without outliers and thus indicated the adequacy of selected statistical model to fit well as depicted in Figure 4a-f. Levels of factors significantly influenced the responses of Y1, Y2, and Y3 differently.

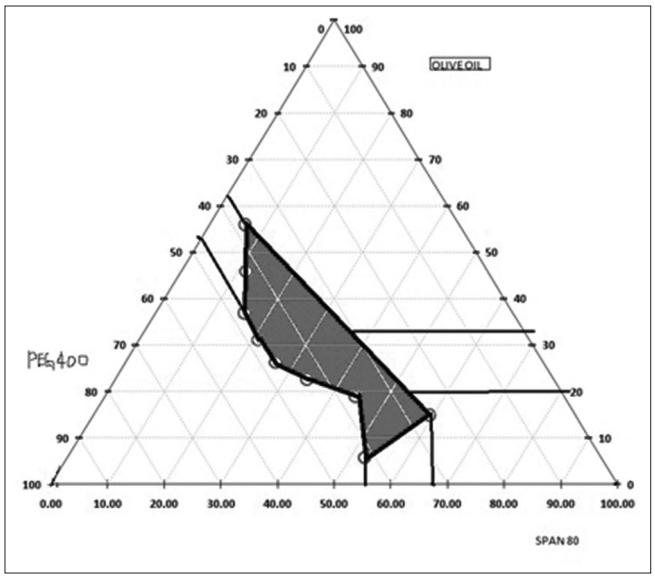

Figure 2: Ternary phase diagram of olive oil, span 80, PEG 400

Table 3: Formulation variables in Box-Behnken design used to prepare 15 formulations

Independent variables[a] (mg)	Levels					
	Low		Middle		High	
	Coded	Actual	Coded	Actual	Coded	Actual
X1: Amount of oil added	−1	20	0	27.5	+1	35
X2: Amount of surfactant added	−1	55	0	65	+1	75
X3: Amount of cosurfactant added	−1	33	0	39	+1	45
Dependent variable	**Constraints**					
	Low	**High**	**Goal**			
Y1: % Limpidity	80	95	Maximize			
Y2: Self-emulsification time (s)	2	120	Minimize			
Y3: Percentage drug released at (T1 5 min)	60	85	Maximize			

[a](mg) Oil - Olive oil, surfactant - Tween 80, Cosurfactant - PEG 400. PEG: Polyethylene glycol

Physical stability

Thermodynamic stability studies of SNEDDS were resulted with phase separation, creaming, precipitation, and coalescence of oil droplets for 4, 5, and 8 formulation runs as demonstrated in Table 1. Isotropic systems of 4, 8, and 14 runs were turned to bright milky. Most of the SNEDDS runs were thermodynamically stable and homogeneous over a period.

Percentage limpidity

Being characteristic property, transmittance affects the droplet size of stable emulsion. The percent limpidity was low in slightly turbid nanoemulsion of run 4 (74%) as shown in Table 1 owing to higher surfactant (75 mg) and cosurfactant (45 mg) concentrations. Whereas 98% limpidity response was shown by SNEDDS of run 15. Limpidity of nanoemulsion with uniformly distributed nanodroplets was as close to 100%. Quality characteristics of SNEDDS were obtained by BBD through polynomial equation in relation to response Y1 (% limpidity) and independent variables. The values of factors were substituted in equation (1), to obtain actual values of responses as:

$$Y1 = 117.58 + 1.69X1 + 4.37X2 + 4.48X3 + 0.02X1X2 + 0.038X1X3 - 0.05X2X3 - 0.09X12 - 0.024X22\ 0.03X32 \quad (2)$$

Self-emulsification time

With no time or in less than couple of minutes, the preconcentrates were emulsified on dilution with water at mild/gentle agitation. Above all, run 3 formulation was emulsified rapidly and produced a very clear and homogeneous nanoemulsion within 67 s with due concentrations of carriers [Table 1]. Whereas, run 14 was emulsified in 112 s owing to coalescence of concentrated lipid vehicle. Polynomial equation representing the response Y2 as SET and independent variables in terms of actual values was:

$$Y2 = -7.49 - 8.65X1 + 5.74X2\text{-}1.53X3 + 0.03X1X2 + 0.072X1X3 + 0.016X2X3 + 0.10X12\text{-}0.05X22\ 9.25E-00X32 \quad (3)$$

***In vitro* dissolution**

Dissolution profiles of 15 formulations are displayed in [Figure 5a–c]. More than 50% of QP was released from SNEDDS from first 5 min of study. In this study, T_{15}% was taken as a response variable (Y3) to optimize the dissolution profile of QP-loaded SNEDDS. T_{15}% was observed as 77% in run 14 (minimum) and 95% in run 3 (maximum) as shown in Table 1. Entire dialysis bag contents were

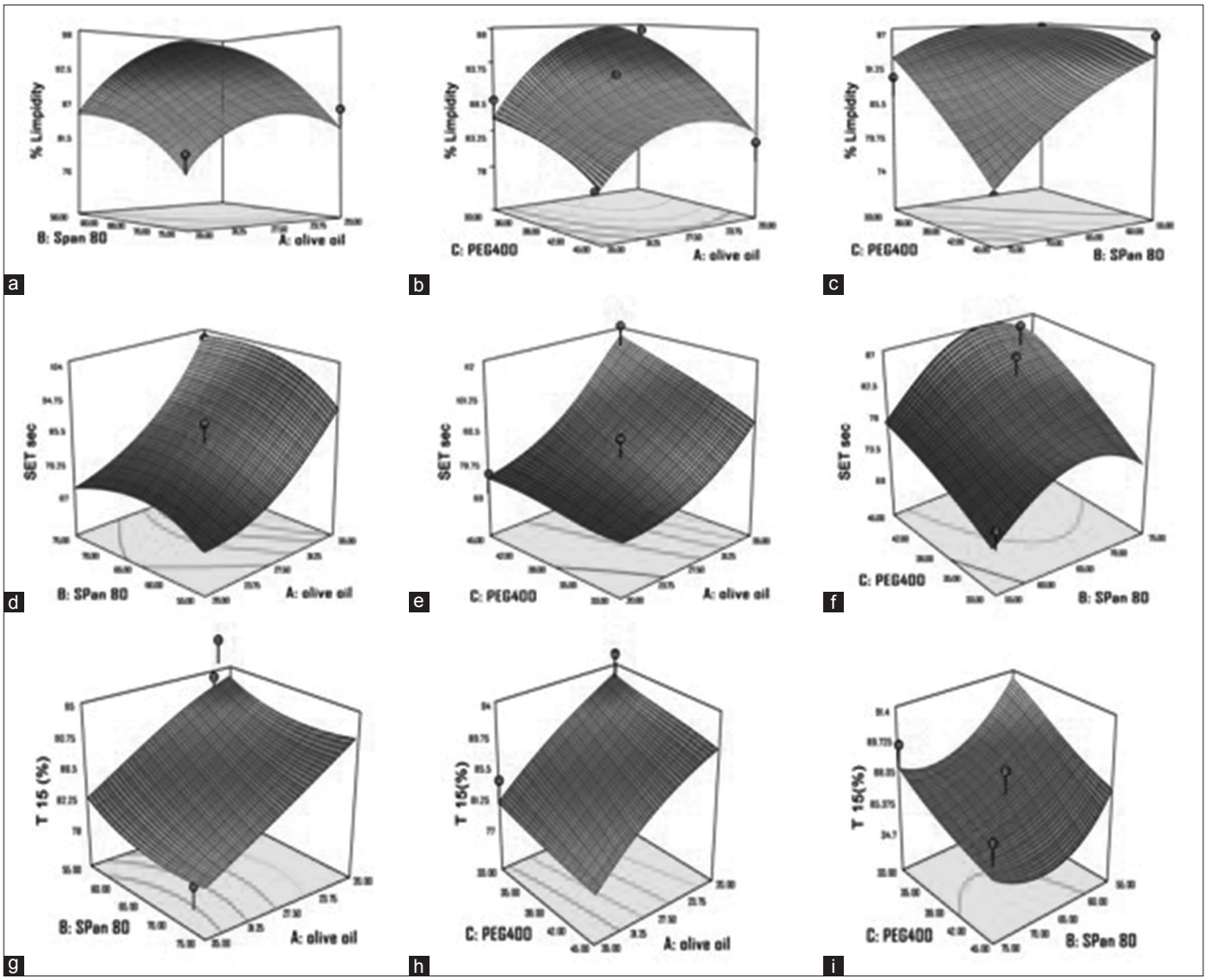

Figure 3: (a-i) 3D response surface plots of Y1, Y2, and Y3 at different levels of X1, X2, and X3

emptied within 30 min of the study. Factors such as % limpidity and SET had profound effect on drug release profiles and thus polynomial equation of Y3, a dependent variable was become:

$$Y3 = +216.37 + 0.30X1 - 2.63X2 - 1.48X3 - 3.33E003X1X2 + 5.55E003X1X3 + 4.16E - 003X2X3 - 0.02X12 + 0.018X22 + 0.01X3 \quad (4)$$

Identification of optimized formulation

As shown in Table 3, the constraints applied for numerical optimization technique in BBD were to minimize SET, to maximize both percent limpidity and release of drug after 15 min. Actual values of optimal factors for olive oil, span 80, and PEG 400 were found as 20, 55.07, and 34.78 mg, respectively, with experimental responses of limpidity, SET, and T_{15} as 94%, 69 s, and 93.4%.

Zeta potential and droplet size analysis

Mean droplet size of optimized nanodispersion was found to be 89.68 nm, electrokinetic potential was −27.2 mV, and PDI was <1 and thus indicated the formation of stable nanodispersion of QP.

DISCUSSION

Recent times, natural edible oils have exploited their limit of use over stability, owing to their sound safety profiles in terms of digestion and absorption. Being BCS Class II moiety, QP was more soluble in olive oil (3.607 mg/ml) owing to its long-chain fatty acid composition and thus facilitates lymphatic absorption. SNEDDs containing nonionic span 80 and PEG 400 are capable of resisting the pH changes during absorption process, reduce interfacial fluidity, and also free energy. With due higher emulsification efficiencies and limited number of flask inversions, selected nonionic

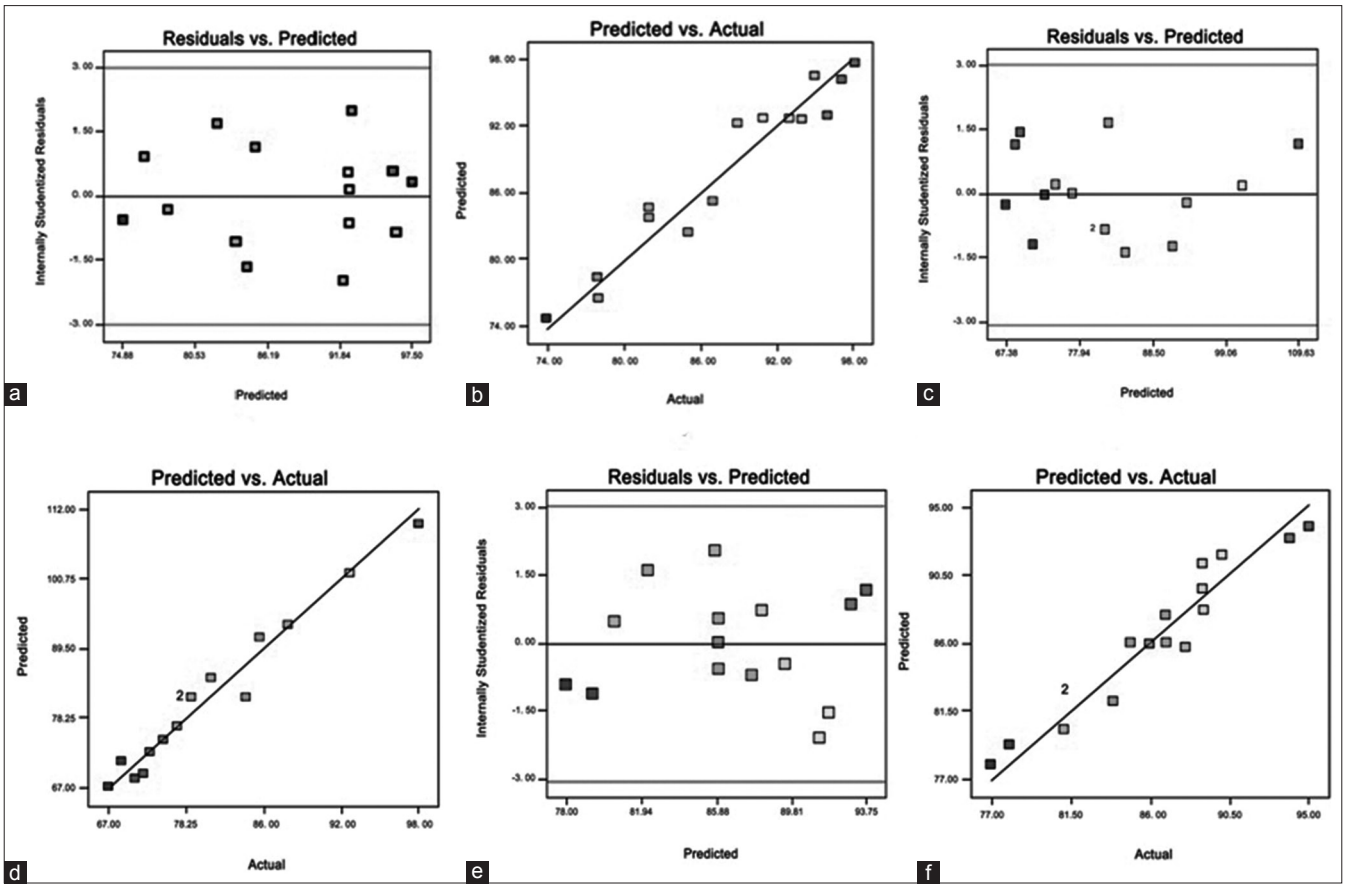

Figure 4: (a-f) Diagnostic plots of adequacy of model derived for Y1, Y2, Y3 responses

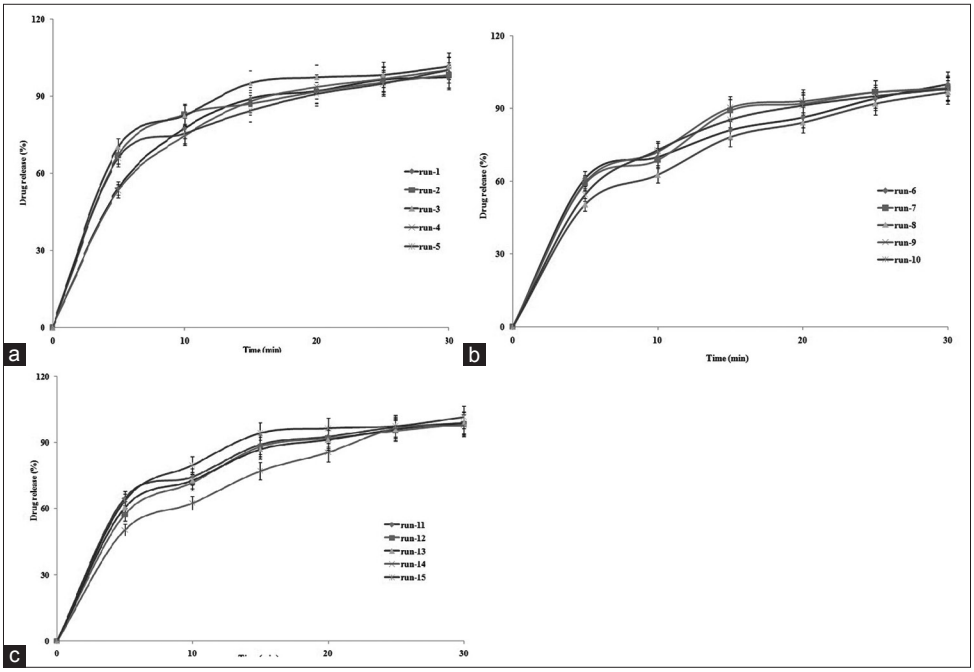

Figure 5: (a-c): Drug release profiles of quetiapine self-nanoemulsified drug delivery systems

amphiphiles enhance the dispersibility of SNEDDS, thereby increase QP absorption by utilizing aqueous pathways for permeation across the intestinal epithelium.[24]

Characteristically, SNEDDS undergo several mesomorphic transformations which in turn led to loss of solvent capacity, narrow down of nanoemulsification zone, and precipitation

of drug. However, addition of moderate amounts of PEG 400 was successfully retained nanoemulsification zone in the ternary phase diagram. Beyond the colored region, lipid vehicle concentration was high and led to heterogeneous micro/macrodispersions.

Thermodynamic stability and centrifugation had little or no effect on mesomorphic transformations of SNEDDS as they were not evident any phase separation, creaming, and coalescence. SNEDDS of runs 4, 5, and 8 stored at −21°C were slightly shifted to microemulsion region due to mesomorphic conversions and led coalescence as evidenced with cloudy/slight turbidity. However, interfacial tension in SNEDDS was sufficiently low, interfacial energy become comparable or even lower than its entropy and thereby free energy of entire nanodispersion became either zero or negative for thermodynamic stability of QP-loaded SNEDDS.[25]

Limpidity of SNEDDS was critically affected with interfacial fluidity between olive oil and water phase. As shown in Figure 3a, the effect of Y1 at middle level of X3 and at low levels of X1 at 20 mg, limpidity was increased from 85% to 97% and X2 at 55 mg; it was further decreased from 95% to 85%. Proportionate dynamic viscosity of span 80 with its concentration in SNEDDS was also a major reason for transmittance decrease. Statistical effect on limpidity was observed to be decreased from 98% to 87% at low levels of olive oil (20 mg) and PEG 400 (33 mg) as shown in Figure 3b, with due contribution of cosurfactant, a polar moiety was led to faster dispersion and firm binding with increased interfacial fluidity in SNEDDS. On the other hand, Y1 was analyzed as 97% at low levels of X3 (55 mg) and X2 (33 mg) from RS plot as shown [Figure 3c]. As RS plots depicted in Figure 3a-c and analyzed, low levels of both span 80 (X2) and PEG 400 (X3) and intermediate level of olive oil (X1) are required to produce optimized SNEDDS. It was also clearly evident with intense dark bluish-green area of 3D plots.

The influence of X1 and X2 and their interaction on SET (Y2) at middle level of X3 are shown in Figure 3d. At low levels of X2 (span 80), Y2 was decreased from 100 to 72 s when X1 increased from 20 to 35 mg. It was therefore attributed that the spontaneous dispersion of surfactant and thus binds the emulsion droplets strongly, thereby reduction in interfacial free energy. QP-loaded SNEDDS resulted with certain mesomorphic changes in preconcentrates like disruption of liquid crystalline lamella of olive oil while it was diluted with water.[22,26,27] It was also noted that SET was decreased with increase of X1, where versatile fatty acid composition of olive oil was typical. The same was portrayed in RS plot [Figure 3e] that SET was decreased with increase of olive oil and decreased at low levels of PEG 400. Effect of Y2 with respect to X2 and X3 at middle levels of X1 resulted with decreased Y2 with increased X3 as displayed in Figure 3f. To minimize SET, low levels of olive oil and span 80 and high levels of PEG 400 were required in optimization of stable SNEDDS of QP.

Variables form RS plot [Figure 3g] for *in vitro* dissolution (Y3) between X1 and X2 at intermediate levels of X3 were resulted with decreased T_{15}% on increase of span 80 (X2). This outcome was in agreement with SET as emulsification was delayed due to viscous crystalline gel formation on increased span 80. As hypothesized similarly in limpidity response, olive oil led interfacial fluidity decreased the drug release. On the other hand, fluidity led voids in amphiphile monomers facilitated emulsification in lesser SET.[28] As per RS analysis, increased trend in T_{15}% was observed between intermediary levels of X1 and at low levels of X1 and X3 [Figure 3h]. Similarly, Y3 between X2 and X3 at predetermined intermediate levels of X1 divulged with increased in X2 and vice versa, with an increase in X3 [Figure 3i]. To optimize the QP-loaded SNEDDS with maximized T_{15}%, high levels of olive oil (X1) and PEG 400 (X3), while intermediate levels of span 80 (X2) were considered.

Model adequacy plots of responses, as provided in Table 4, all regression coefficients of responses were in good agreement with r^2 and adjusted r^2 that indicated the best fit to the response for limpidity, SET and T_{15}%. Model equation of ANOVA with *F* values, probability >F (*P* value), and nonsignificant lack of fit of all the dependent variables exposed that the quadratic model was statistically significant. Further degree of precision and reliability of performed experiments were supplemented with squat value of coefficient of variation (%CV). Values of adequate precision and predicted residual error sum of squares of all responses employed in design were clearly explained that how the adapted model was fitted with each point of BBD.[29] As predicted and experimental responses lie nearer to and on straight line explained that derived model was able to envisage linear relationship between formulation variables and responses. Data were randomly dispersed within limits of ± 3 and thus confirmed that the selected statistical model was adequately fit for the experimental design of QP-loaded SNEDDS as shown in Figure 4a-f. Operability in actual preparation/process the optimal conditions were considered as X1 (olive oil) = 20 mg, X2 (span 80) = 55 mg, and X3 (PEG 400) = 34.5 mg with respective regression of 0.99, 0.99, and 0.70. Mean of triplicate BBD experimental

Table 4: Regression coefficients of actual values and model adequacies for responses

	Responses		
	Y1	Y2	Y3
X1	+1.69	−8.65	+0.30
P	0.0112*	0.0002*	0.0006*
X2	+4.37	+5.74	−2.63
P	0.0044*	0.0992	0.1747
X3	+4.48	−1.53	−1.48
P	0.0059*	0.0187*	0.1163
X1X2	+0.02	+0.03	−3.33E
P	0.3860	0.3141	0.8319
X1X3	+0.03	+0.07	+5.55E
P	0.3184	0.1671	0.8319
X2X3	−0.05	+0.01	+4.16E
P	0.0774	0.6402	0.8319
$X1^2$	−0.09	+0.10	−0.02
P	0.0249*	0.0368*	0.3781
$X2^2$	0.02	0.05	+0.01
P	0.1951	0.0514*	0.1680
$X3^2$	−0.03	−9.25E	+0.01
P	0.4954	0.8797	0.7603
Lack of fit (*F*)	6.46	0.60	7.67
Lack of fit (*P*)	0.139	0.6750	0.1176
Model (*P*)	0.0137	0.0040	0.0194
Model (*F*)	8.83	15.11	7.52
r^2	0.9408	0.9645	0.9312
Adjusted r^2	0.8342	0.9007	0.8075
% CV	3.59	4.92	2.58
Adequate precision	8.77	12.86	8.627
PRESS	734.5	708	372.5

*Represents significant effect on responses. CV: Coefficient of variation, Y1: Percentage limpidity, Y2: Self-emulsification time, Y3: *In vitro* drug release of QP at 15 min

values were resulted with 98% of transmittance, 66 s of SET, and 95.2% of T_{15}.

As shown in Table 3, the constraints applied for numerical optimization technique in BBD were to minimize SET, to maximize both percent limpidity and release of drug after 15 min. Regression equation for the preparation of QP SNEDDS were given in the coded form was found to be X1 = 0.99, X2 = 0.99, and X3 = 0.70. Operability in actual preparation/process the optimal conditions were considered as X1 (olive oil) = 20 mg, X2 (span 80) = 55 mg, and X3 (PEG 400) = 34.5 mg with respective regression of 0.99, 0.99, and 0.70. Mean of triplicate BBD experimental values were resulted in 98% of transmittance, 66 s of SET, and 95.2% of $T_{15.}$ Actual values of optimal factors for olive oil, span 80, and PEG 400 were found as 20, 55.07, and 34.78 mg, respectively, with experimental responses of limpidity, SET, and T_{15} as 94%, 69 s, and 93.4%. As a result, BBD was considered to be an accurate, robust, and significant tool in predicting the responses of QP-loaded liquid SNEDDS.

Mean droplet size of optimized nanodispersion was found to be 89.68 nm, electrokinetic potential was 27.2 mV and PDI was <1 and thus indicated the formation of stable nanodispersion of QP. Outcomes of study on electrokinetic parameters of interfaces of optimized SNEDDS were the direct results of thermodynamic stability of that isotropic system. Data on droplet size analysis and PDI indicated the uniform distribution of globules in self-nanodispersions of QP and were assisted by span 80 and PEG 400 with reduced interfacial interactions. As zeta potential was negative, QP-loaded SNEDDS follow intestinal lymphatic absorption, since intestinal cells carry characteristic negative charges due to luminal/mucosal fluidity.[30]

CONCLUSION

BBD was effectively employed to optimize QP-loaded SNEDDS composed of olive oil (X1), span 80 (X2), and PEG 400 (X3). These independent variables affected the limpidity, SET, and T_{15}% of SNEDDS through linear, quadratic, and interaction effects. Resulted data from BBD were utilized to design the optimized SNEDDS. These optimized SNEDDS were thermodynamically stable and chemically intact over a period. Overall, BBD was successful and most suitable in optimization of particulate emulsified systems such as QP-loaded SNEDDS. Further, *in vivo* studies are required to scale-up the process of SNEDDS.

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Conflicts of interest

There are no conflicts of interest.

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