IN VITRO STUDY FOR QF RELEASE BEHAVIOR OF OPTIMIZED FORMULATIONS OF NLC AND QF SUSPENSION

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ABSTRACT:

The optimal formulations contained 93.0.16% EE and 218.10.14 nm of PS. Findings showed that the proposed formulation with QF-loaded NLC was superior as a highly stabilized drug delivery system, offering greater bioavailability with the potential for treating psychological illnesses more effectively. The CCD consists of three factored designs with five levels: the centre point, plus and minus 1, plus and minus alpha (axial points), and plus and negative 1 (factorial points). Using Design Expert software Version 12, a mathematical link between variables was produced. According to the statistical analyses, three independent variables were the key elements influencing the PS and% EE of QF-loaded NLC. For PS and% EE, respectively, the best-fitting mathematical model was linear and quadratic. The goal of the current study is to determine whether nanostructured lipid carriers (NLCs) can increase the oral bioavailability of the second-generation antipsychotic medication quetiapine fumarate. NLC loaded with quetiapine fumarate (QF) was created by heat homogenization and ultrasonication. To optimise the NLC formulation, response surface methodology—central composite design (CCD)—was utilised to systematically assess the impact of the concentrations of capmul MCM EP, poloxamer 188, and egg lecithin on particle size (PS) and % entrapment efficiency (% EE).

KEYWORDS: *Central composite design; Nanostructured lipid carriers; Poloxamer 188; Quetiapine Fumarate; Response surface methodology.*

INTRODUCTION:

Colloidal drug carriers have been used to address the solubility issues of a drug. But these systems suffer from certain disadvantages like drug leakage, stability problems, high production cost and sometimes cytotoxicity. Similarly, lipid-containing delivery systems such as lipid drug conjugate (LDC), solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) can be effectively used to solve customary drug delivery systems issues such as low bioavailability and low drug loading. Since the last decades, a tremendous rise has been observed in psychological disorders, particularly bipolar disorder and Schizophrenia[1]. Quetiapine is one

of the extensively prescribed drugs as monotherapy in treating bipolar disorder and schizophrenia [2, 3., 4, 5] QF belongs to Biopharmaceutics Classification System class II [6, 7] drugs and exhibits 9% oral bioavailability. QF being weak acid get rapidly absorbs in the stomach but suffers from a high first-pass effect. QF possesses a half-life of 6 hours and requires frequent administration which in turn enhances the chance of dose skipping [8, 9]. Low oral bioavailability and high first-pass effect lead to a reduction in absorption which is a major obstacle in QF therapy. QF exhibits low solubility and low absorption at high pH. QF undergoes P-glycoprotein (P-gp) efflux leading to less concentration in the brain even after absorption.

However, LDC has a few drawbacks, including poor drug loading, unknown gelation tendency, rapid particle size expansion, and unpredictable gelation tendency [10, 11]. The SLN system was created to alleviate LDC drawbacks [8]. Small lipid-containing preparations with high surface areas are known as SLN and are thought to be biocompatible and biodegradable systems [12, 13, 14, 15].

The second-generation lipid carrier known as NLC (Lipid-based systems) is typically made up of both solid and liquid lipids combined in a system with a surfactant [16, 17]. Because of the mixing, substrates' melting points are lowered, and the mixture becomes solid at room temperature. In contrast to SLN, NLC exhibits a significant drug loading with a low drug expulsion rate [18, 19, 20]. NLC avoid P-gp efflux because of a different absorption mechanism (non-receptor mediated endocytosis). In light of these factors, the NLC system might be thought of as a successful substitute to get over problems with QF therapy.

METHODOLOGY

Materials:

QF was obtained from Pharma Industry in Hyderabad, India. For the production of NLC, Monegyl - T18 and Monegyl - D207 (Mohini organics Pvt. Ltd. Mumbai), Glyceryl monostearate, GMS (Micro Labs Limited, Bangalore), Capmul MCM EP (Intas pharmaceuticals Ltd., Ahmedabad), Poloxamer 188 (BASF India Limited, Navi Mumbai), Egg lecithin (Naproid life sciences Pvt. Ltd. From Mumbai) were used.

Methods:

Ratio optimization of solid to liquid lipids:

Solid lipid (Stearic acid, Monegyl- T18, Monegyl- D207, GMS) was screened by the method given in literature survey [21, 22]. Liquid lipid (oils) was selected depending on the solubility of the QF in liquid lipid [23, 24, 25, 26, 27].

The mixture comprising of both solid and liquid lipids was prepared homogenous. To optimize the ratio of lipids, a miscibility test between the selected lipids namely GMS and capmul MCM EP was performed. The selected solid lipids and oil were weighed in the different % ratios (60:40, 70:30, 63.636: 36.363) in glass vials. This blend was heated to a temperature 10°C above the M.P. of the solid lipid. Thereafter, the liquid blend vortexed and smeared on glass slide. Upon solidification, a dry filter paper was pressed on this lipid

blend and observed for sign of oil drops, if any. The mixture that does not show any oil drop on filter paper was considered as miscible and was selected for the development of QF-loaded NLCs [22].

Preparation of QF-loaded NLCs:

QF-loaded NLCs were fabricated by a slight modification of the hot homogenization method followed by the ultrasonication method. Preliminary batches were prepared, examined and optimized for various process parameters such as water volume, sonication time, amplitude, PS, Zeta potential (ZP), and Polydispersity index (PDI) of the NLC dispersion. This method resulted in the consistent production of smaller-size NLCs (<250 nm) with good PDI and good % EE. Egg lecithin was dissolved in a 3ml mixture of methanol and chloroforms (1:1) and vaporized for 15 min to completely evaporate the solvents to achieve a film of egg lecithin. The GMS and capmul MCM EP were melted (10ºC above the M.P. of the lipids used) and added to the above mixture. QF was added to attain a drug-lipid mixture. The hot surfactant solution (poloxamer 188) in water preheated to 10ºC above the lipid's M.P. (70ºC) was added to the clear lipid melt containing egg lecithin. The resulting mixture was exposed to high-shear homogenization at 2000 rpm for 10 min and heated to obtain a coarse emulsion. This coarse emulsion was then subjected to a probe sonicator (PCi *analytics* PKS -750FL, Mumbai) for 15 minutes at 50°C by maintaining output amplitude at 50% and a gap of 5 seconds to form NLC dispersion. Then hot NLCs obtained were cooled to room temperature to precipitate the lipid into the solid form to yield QF-loaded NLCs [28, 29, 30, 31]. This technique is simple to prepare NLCs without any sophisticated instruments and can also be used for large-scale production [32, 33].

Preliminary batches of NLC and optimization of process variables:

Based on the literature survey and trial batches, critical process variables were identified and subjected to optimization. QF suspension was formulated using 0.025gm of QF, 0.125gm of sodium carboxy methyl cellulose as a suspending agent and distilled water up to 25ml as a vehicle for further studies. Selected process variables used were temperature (70ºC), and magnetic stirrer rpm (2000 rpm) for 10 min. The sonicator process used variable amplitude at 50% and gap of 5 sec. for 15 min. The PS and% EE of NLC were significantly influenced by the variables capmul MCM EP concentration (X1), poloxamer 188 concentration (X2), and egg lecithin concentration (X3), according to preliminary batches. Thus, PS (nm) and% EE of the manufactured NLCs were examined systematically using response surface methodology (CCD) to account for the effects of these three independent variables. 15 runs were employed in this investigation since the CCD had three factors, five levels, plus and minus alpha (axial points), plus and minus 1 (factorial points), and the centre point. The optimal concentrations of various components were chosen based on the findings from preliminary batches (Table 1). Table 1 is a list of the formulation design's specifics.

Table 1: Independent variables, dependent variable and levels for design of NLCs

Variables	Levels				
1. Independent variables in gms	-1.68179	Low (-1)	Centre	High (+1)	+1.68179
Concentration of capmul MCM EP (X_1)	0.0829552	0.1	0.125	0.150	0.167045
Concentration of poloxamer 188 (X_2)	0.140343	0.2	0.2875	0.375	0.434657
Concentration egg lecithin (X_3)	0.11591	0.150	0.2	0.250	0.28409
2. Dependent variable	Constraint				
Y1: PS (nm)	Minimize				
Y2: EE (%)	Maximize				

For all batches the quantity of QF (0.025gm), GMS (0.175gm), water up to (25 ml) was kept constant. The formulations were prepared in triplicate[34]. Results of the study were validated through design expert software version 12 (State Ease, Inc., USA) for further analysis [35].

Optimization of QF-loaded NLC formulation:

The solution that has a 'Desirability Factor' close to 1 was selected as given by Design Expert software [31].

Freeze-Drying (FD) Study:

Several ratios of total lipids: cryoprotectant (Trehalose) at 1:2, 1:4, 1:6 and 1:8 were tried. The selected cryoprotectant was added after the homogenization process. The blend was then subjected to freeze-drying (Labconco, 25 Plus) at −40 °C for 24 hrs. The ratio that produced the smallest PS and PDI was selected[34, 35, 36].

Assessment and description of QF-loaded NLCs

Polydispersity index (PDI) and particle size:

The FD samples of QF-loaded NLCs were used after reconstitution with distilled water for analysis of PS and PDI. PS was calculated by using a photon correlation spectrometer (Malvern Zeta sizernano ZS 90) with a 4.0 mW internal laser. Dynamic light scattering was based on the particle diffusion associated with Brownian motion, which gives an idea about PS. PS was then estimated by the translational diffusion coefficient using the Stokes-Einstein equation by software[37]. PDI was determined to confirm the distribution of PS in formulation[38].

Zeta potential:

The FD samples were used after dilution with distilled water to determine ZP of the prepared formulation and was evaluated by using the Malvern Zetasizer Nano ZS 90. The electrophoretic mobility of particles was

transformed to the zeta potential [39, 40, 41]. Zeta potential was determined using the 'Dip cell' with the application of field strength 20 V/cm with 30 runs.

The efficiency of entrapment and loading of drug

The drug-loaded NLC was subjected to centrifuge at 45,000 rpm for 35 min. Then 1.0 ml of the supernatant was diluted with methanol. The samples were analyzed by using UV spectroscopy at 244 nm (UV 1650, Shimadzu, Japan) to calculate % EE[42]. For drug loading, QF from the freeze-dried powder was mixed with methanol. The QF quantity was analyzed by using UV spectroscopy at 244, against methanol as blank [41, 43].

Drug excipients compatibility studies, (FTIR) study:

FTIR was used to record the FTIR spectra of the final formulation and the QF (FTIR-4800, Shimadzu, Japan). In a nutshell, a solid sample (100 mg) and dry potassium bromide (100 mg) were crushed into a disc. For the liquid sample, a thin liquid membrane was created by dripping sample drops onto a NaCl or KBr aperture plate and sandwiching it under another aperture plate. After then, the sample's absorbance between 4000 and 400 cm-1 was measured. After that, the resultant spectrum was compared to the QF standard group frequencies. The resulting FTIR (4800, Shimadzu, Japan) spectrum was then compared with reference frequencies of QF [44, 45, 46, 47].

Morphological study (SEM):

The surface morphology of FD sample (batch F3) was studied using SEM (TESCAN MIRA3, Czech Republic). The sample was dusted on double-sided tape onto aluminium stub, coated with gold by using a cold sputter coater in an SEM chamber of thickness 400 Å. The graphs were recorded with a voltage of 15 kV electron beam [43].

Differential scanning calorimetry (DSC):

Thermal analysis data was recorded using a DSC (DSC 204 F1 Phoenix, NETZSCH). The freeze-dried sample (2 mg) was sealed in pin-holed standard 40 μl aluminumpan. The heating rate was10˚C/min from 30˚C to 400˚C and the nitrogen purged rate of flow was 10 ml/min. The data was also recorded for QF (drug), GMS (solid lipid) and physical mixture [48, 49].

X-ray diffraction (XRD):

XRD was used to assess the physical state of QF-loaded NLCs (Bruker AXS D8). In a low background sample holder, freeze-dried NLC was spread, and then secured in a goniometer onto the stage. B-B geometry was programmed into the gadget. XRD was measured with the voltage and current set at 35 mA and 40 mV, respectively. Also, the information was logged for the physical mixture [48], GMS (solid lipid), and QF (drug).

Drug release in vitro:

Using the dialysis bag diffusion method 50, the in vitro release of QF from the optimised formulation F3 and produced QF solution was monitored. The improved NLC formulation, corresponding to 5 mg of the medication, was added to a dialysis bag, sealed, and suspended for 2 hours in a USP (type II) apparatus with

900 ml of 0.1 N HCl (pH 1.2), followed by 24 hours at 50 rpm and 37°C 0.5°C in phosphate buffer (pH 6.8). In order to maintain sink conditions throughout the release period, 5 ml of the samples were taken out at intervals of 0, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, and 24 hours and filtered through a 0.45 m membrane filter. A UV-Visible spectrophotometer was used to examine the QF release after dilution at 207 nm while using the appropriate medium as a blank. For the suspension of QF, the same process was followed. Each measurement was performed three times 51. To determine the release kinetics of NLCs 52, data were fitted to various kinetic models. The greatest r2 value revealed the actual method of drug release [53, 54].

Stability studies:

The stability study of FD batch F3 of NLCs was carried out in accordance with ICH guidelines. Formulation F3 were stored at 25°C±2°C /60% RH, ambient temperature and 40°C±2°C /75% RH) for 6 months. Effects of temperature and RH on PS and % EE were studied at the end of 0, 3 and 6 months respectively [29, 31, 55].

Determination of pharmacokinetic parameters in rats Administration of optimized formulation of NLC and QF suspension in rats:

Present protocol was approval by Institutional Animal Ethical Committee (IAEC) of Pinnacle Biomedical Research Institute (PBRI), Bhopal (Reg. No. 1824/PO/ERe/S/15/CPCSEA) with reference no. PBRI/IAEC/PN-19025 dated 23/01/2019. In house breed wistar rats acquired from Pinnacle Biomedical Research Institute, Bhopal, (M.P.) were used for the present study. The male wistar albino rats (approx. 150-220g) were used for the present study under fasting conditions. Rats were divided in two groups (n-6), first group was treated with optimized formulation of NLC (equivalent to 25 mg of QF) via oral route whereas rats of second group were treated with pure drug suspension (equivalent to 25 mg of QF) orally.

Blood sample collection and processing:

Blood samples (250 µl) were serially taken out into heparinized tubes from retro-orbital plexus at intervals of 0.5, 1, 2, 4, 6, 8, 12 and 24 hours. The saline solution equivalent to blood samples withdrawn was injected to rats to compensate blood loss. Plasma was isolated by using centrifuge at 5000 rpm for 10 min at 4°C.The plasma samples were stored at -20ºC.

Extraction procedure for drug from blood plasma:

The QF in rat plasma samples were analyzed from the calibration curve. Drug in plasma was analyzed by HPLC (LC-2010CHT) equipped with C_{18} column, UV-Visible detector, an auto injector and a system controller. The mobile phase consists of a mixture with ratio 40:60 v/v of water and acetonitrile respectively at 25 ± 2°C. Mobile phase was passed through membrane filter (0.45 µm) followed by deaeration for 5 minutes using sonicator. The flow rate was adjusted to 1 ml/min. Sample injection volume was 20 µl and analyzed at 207 nm.

RESULTS:

Optimization of ratios of solid lipid to liquid lipid:

After solidification of selected solid lipid and liquid lipid in different % ratios (60:40, 70:30, 63.636: 36.363), they were applied to dry piece of filter paper and the sample which did not show any oil droplets on the surface of filter paper was considered miscible was selected for use in the development of trial batches of QF loaded NLCs.

Results of preliminary batches and optimization of process variables of NLCs:

Preliminary optimization of stirring time, rpm and temperature was decided by literature survey and by conduction of the different experiments [56]. NLC Trial 2 batch demonstrated good result with PS as 240.6nm, ZP of -25.3 mv, PDI of 0.492 good result.

Experimental design with results for NLC formulation

The experimental results were showed in Table 2.

Table 2: Experimental design for formulation of QF loaded NLC

Batch	Independent variables			Dependent variables			
				Observed value		Predicted value	
	X_1	X_2	X_3	Y_1	Y_2	Y_1	Y_2
F1	0.1	0.2	0.15	240.5±1.08	92.2±0.16	240.13	91.86
F2	0.15	0.2	0.15	242±0.81	83.5±1.22	241.94	83.84
F3	**0.1**	**0.375**	**0.15**	**218.1±0.14**	**93±0.16**	**220.46**	**92.59**
F4	0.15	0.375	0.15	220.8±0.16	84.2±0.29	222.26	84.16
F5	0.1	0.2	0.25	243.2±0.61	92.5±0.08	243.3	92.14
F6	0.15	0.2	0.25	245.1±0.08	83.5±0.4	245.1	83.51
F7	0.1	0.375	0.25	225±0.32	93.2±0.16	223.62	92.46
F8	0.15	0.375	0.25	225±0.81	83.5±0.08	225.43	83.43
F9	0.0829552	0.2875	0.2	231.3±0.08	95±0.81	231.26	95.91
F10	0.167045	0.2875	0.2	235±1.41	81.9±0.48	234.3	81.56
F11	0.125	0.140343	0.2	249.5±1.22	87±1.08	249.33	87.01
F12	0.125	0.434657	0.2	218.3±0.35	87±1.55	216.23	87.56
F13	0.125	0.2875	0.11591	232±0.73	88±0.81	230.12	88.08
F14	0.125	0.2875	0.28409	234.8±0.97	87.2±0.16	235.44	87.7
F15	0.125	0.2875	0.2	232.5±1.00	87.5±0.08	232.78	87.48

ean ± SD , *n*=3, X1: concentration of capmul MCM EP (gm); X2: concentration of poloxamer 188 (gm), X3: concentration of egg lecithin (gm), Y1: PS (nm), Y2:% EE (%)

Polynomial equation:

Polynomial equations for Y_1 and Y_2 were as follows:

$Y_1 = +254.26800 + 36.09216$

$X_1 -112.44751X_2 + 31.64568 X_3$ -------------------------------------(1)

$Y_2 = +115.34921 - 310.43642$

$X_1 + 17.39099X_2 - 3.41783X_3 - 45.71429$

$X_1X_2 - 120X_1X_3 - 22.85714X_2$

$X_3 + 708.09282X_1^2 - 9.15519 X_2^2 + 56.81505X_3^2$ -----------(2)

Response 1 (Y_1): Effect on PS

Results of all batches from ANOVA analysis, showed that independent factors were significantly affect the PS (p-value : <0.0001). The R^2 value (0.9862) found near to 1 showed linearity. The linear model F value of 381.13 showed that the model was significant. PS of all 15 batches were in the range of 218.1±0.14nm to 249.5±1.22nm (Table 3). Equation (1) shows factors X_1 (concentration of capmul MCM EP), X_3 (concentration of egg lecithin) have positive effect on PS of NLCs and X_2 (concentration of poloxamer 188) have a negative effect on PS of NLCs. This is also confirmed by equation of response Y_1.

The PS of NLCs increased with an increment in liquid lipid concentration, which was found similar to reports published by Hu et al., Tamjidi et al. and Dai et al [57, 58, 59]. This may be due to disruption of lipid wall followed by aggregation and ultimately particle size growth with increase in liquid lipid content [57]. Elevation in interfacial tension results in swelling of NLC and higher liquid lipid concentration causes increase in PS [58, 59]. Poloxamer in 1.5% concentration was sufficient to cover the surface of QF loaded NLC effectively during the formulation process due to reduction in interfacial tension between the water and oil phase which lead to separation of particle and surface area enlargement. But beyond the appropriate surfactant concentration, saturation occurs where the affinity between lipid and water phase gets decrease which would not permit the PS to decrease further [60].

Response 2 (Y_2): Effect on % entrapment efficiency:

Results from the ANOVA analysis indicates that all independent factors were greatly affect the % EE of NLCs (p-value: <0.0001). The R^2 value (0.9899) was found near to 1 and showed linearity. The quadratic model with F value of 108.73 showed the model was significant. The % EE of all batches was in the range of 81.9±0.48 to 95±0.81% (Table 3).

The equation (2) showed linear and quadratic interactions for response Y_2. Factors X_1 (concentration of capmul MCM EP) and X_3 (concentration egg lecithin) had negative impact on % EE of NLCs and X_2 (concentration of poloxamer 188) had a positive impact on % EE of NLCs.

The 3D plots showed the negative impact of X_1X_2, X_1X_3, X_2X_3 interactions on % EE of NLCs. The negative impact of X_1X_2 interaction (increase in concentration capmul MCM EP and concentration of poloxamer 188) or X_1X_3 interaction (increase in concentration capmulMCM EP and concentration of egg lecithin) or

X_2X_3 interaction (increase in concentration of poloxamer 188 and concentration of egg lecithin) would decrease the % EE of NLCs.

The drug QF is lipid soluble which gets entrapped in the lipid matrix and showed good % EE. Its solubility decreases during cooling of lipid melt. Further, capmul MCM EP in the lipid mixture increases drug dissolution and also help in preventing separation and crystallization of QF from lipid blend on cooling, which is in good accordance with the findings of the earlier studies of Lohan et al and Muller et al [28, 61].

The % EE of NLCs reduced greatly with an increment in liquid lipid concentration, which was found in agreement to earlier reports of Zhu et al., Ali et al., Soleimanian et al., Kudarha et al [62, 63, 64, 65]. This may be due to lipid precipitation during NLCs production. During cooling, re-crystallization of lipids results low drug entrapment. Thus, increase in the lipid concentration beyond certain limit leads to poor % EE.

Optimization of QF loaded NLC:

Optimization of QF loaded NLC was carried out to determine the appropriate concentration of independent variable. Results derived from design expert software 12, indicated that F3 has all charactistics to be considered as optimized batch (0.4% of capmul MCM EP, 1.5% poloxamer 188 and 0.6% egg lecithin).

Freeze-Drying Study:

Various total lipid: trehalose ratio (1:1, 1:2, 1:4, 1:6) were tried. But 1:4 ratio was used in the formulations for further investigation as this ratio showed superior result with PS of 220nm, ZP of -29.5mv and PDI of 0.380 than other ratios.

Evaluation and characterization of QF loaded NLCs

Particle size and polydispersity index of QF loaded NLCs:

The particle size and PDI of optimized batch F3 was found 218.1±0.14nm and 0.382 respectively. (Fig. 1)

Fig. 1: Particle size and polydispersity index of QF loaded NLCs

Zeta potential::

The ZP of F3 batch was found to be -29.5 mV, which imparts good stability of NLCs dispersion. (Fig. 2)

Fig. 2: Zeta potential of QF loaded NLCs

Percentage entrapment efficiency and drug loading:

All batches showed good % EE ranging from 81.9% to 95% (Table 3). The % EE for the batch F3 was found to be 93.00 ± 0.163 %. The DL was found to be 8.65 ± 0.067 %.

Fourier transforms infrared spectroscopy (FTIR) study:

As there is no change in the nature and position of the peak in the formulation, it can be concluded that the drug maintains its identification without any chemical interaction with excipients used.

Morphological study (SEM):

The micrograph of the optimized NLC (F3) demonstrated spherical droplets with average diameter of 50.47nm [66, 67].

Differential Scanning Calorimetry (DSC):

The absence of peak in the endotherm of optimized formulation was due to Kelvin effect (nanoparticles melt at a temperature lower than the M.P. of solid lipid) [68]. This finding suggests the conversion of the crystalline QF to its amorphous state. Thus indicate no incompatibility [53].

X-ray diffraction (XRD):

The XRD spectra of QF in Fig. 3 showed intense peaks at 2θ scale, indicating the high crystalline structure of QF (i.e., two sharp peaks at 2θ = 22.617°and 25.2341°). The peaks of QF were absent in the XRD pattern of physical mixture and QF loaded NLC formulation, confirmed that the QF molecule was converted to an amorphous complex with the lipid matrix.

Fig. 3: Overlaid XRD of pure drug, GMS, physical mixture and final formulation

***In vitro* drug release:**

The optimized formulations of NLC showed significant enhancement in QF release profile as compared with QF suspension (Fig. 4). Higuchi model ($r^2 = 0.9964$) was found to be best fitted model for optimized formulation of NLC F3 [69].

Fig. 4: *In vitro* QF release behavior of optimized formulations of NLC and QF suspension

Stability study:

From stability results it can concluded that the formulation had satisfactory stability over 6 months period [60]. (Table 3)

Table 3: Stability study of optimized formulation of NLC

Time (months)	0	3	6
Condition	25°C±2°C /60%±5 RH		
PS (nm)	218.1	220	220
% EE	93	93	93
Condition	Ambient temperature		
PS (nm)	218.1	218.1	218.1
% EE	93	93	93
Condition	40°C±2°C /75% ±5 RH		
PS (nm)	218.1	218.1	218.1
% EE	93	93	92

DISCUSSION:

The particle size of NLCs increased with an increment in liquid lipid cons., the same results as per earlier reports [57, 58, 59]. This may be due to disruption of the lipid wall then aggregation and ultimately particle size growth with an increase in liquid lipid content [57]. Interfacial tension increased and core of NLC swelled with greater liquid lipid concentration and thus observed an increase in PS [58, 59].

But beyond the appropriate surfactant conc., there exists saturation where the affinity between lipid and water phase gets explored entirely which would not permit the PS to decrease further [60].

The drug QF is lipid soluble, so it is mostly entrapped in the lipid matrix and showed a good % EE. The solubility decreases during the cooling of lipid melt. Further, capmul MCM EP in the lipid mixture increases drug dissolution and also helps in preventing separation and crystallization of QF from the lipid blend on cooling [61].

The % EE of NLCs reduced greatly with an increment in liquid lipid conc., which was the same that found in earlier reports [59, 60, 61, 62]. This may be due to lipid precipitation during NLC production. During cooling, re-crystallization of lipids results in a core with reduced drug content. So, an increase in the lipid conc. beyond certain limits leads to poor % EE. These results were in good agreement with earlier reports [70, 71, and 72].

QF loaded showed excellent stability specified by ZP, high % EE value, and drug loading capacity with sustained action. Thus it can be concluded that NLC is a smarter drug delivery system with unique advantages such as higher drug loading; higher entrapment of drug sustained drug release behaviour and eventually enhanced drug absorption as compared with other lipid-based drug delivery systems. The feasibility of large-scale production makes NLC a versatile delivery system. Thus, NLCs seem to be reasonable delivery systems for the oral administration of QF and may be used as an alternate strategy to achieve ameliorated release and prolonged action of QF. In future, QF-loaded NLCs may be used in clinical subjects for achieving better outcomes.

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