International Journal of Education and Science Research Review
E-ISSN 2348-6457
Volume-9, Issue-1 Jan-Feb-2022
P-ISSN 2349-1817
www.ijesrr.org
Email- editor@ijesrr.org

STUDY ON NANOSTRUCTURED LIPID CARRIER SYSTEM FOR EFFECTIVE DELIVERY OF POORLY WATER SOLUBLE DRUG QUETIAPINE FUMARATE

Vijay Kumar
Research Scholar
Dept. of Pharmacy
Sunrise University, Alwar
Rajasthan

Chatter Singh
Prof.
Dept. of Pharmacy
Sunrise University, Alwar
Rajasthan

ABSTRACT:

The aim of present study is to investigate the potential of nanostructured lipid carriers (NLCs) in improving the oral bioavailability of quetiapinefumarate, a second-generation antipsychotic drug. Quetiapine Fumarate (QF) loaded NLC were prepared by hot homogenization followed by an ultrasonication method. Response surface methodology - central composite design (CCD) was used to systemically examine the influence of concentration of capmul MCM EP, concentration of poloxamer 188 and concentration of egg lecithin on particle size (PS) and % entrapment efficiency (% EE) and to optimize the NLC formulation. The CCD consists of three factored design with five levels, plus and minus alpha (axial points), plus and minus 1 (factorial points) and the centre point. A mathematical relationship between variables was created by using Design Expert software Version 12. The statistical evaluations revealed that three independent variables were the important factors that affected the PS and % EE of QF loaded NLC. The best fitted mathematical model was linear and quadratic for PS and % EE respectively. The optimized formulations found with 218.1±0.14nm of PS and 93±0.16% of % EE. Results illustrated the superiority of developed QF loaded NLC formulation as a stable drug delivery system, providing better bioavailability with the possibility of better treatment for psychological disorders.

KEYWORDS: Central composite design; Nanostructured lipid carriers; Poloxamer 188; QuetiapineFumarate; Response surface methodology.

INTRODUCTION:

Since last decades a tremendous rise has been observed in the psychological disorders, particularly bipolar disorder and Schizophrenia[1]. Quetiapine is one of the extensively prescribed drugs as monotherapy in treatment bipolar disorder and schizophrenia [2, 3., 4, 5] QF belongs to Biopharmaceutics Classification

System class II [6, 7] drug and exhibits 9% oral bioavailability. QF being weak acid get rapidly absorbs in the stomach but suffers from high first-pass effect. QF possesses half-life of 6 hours and requires frequent administration which in turn enhances chance of dose skipping [8, 9].

Low oral bioavailability and high first pass effect lead to 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.

Colloidal drug carriers have been used to address 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.

However, LDC suffered with few demerits such as particle size growth, uncertain gelation tendency, sudden polymeric transitions and low drug loading [10, 11]. In order to address LDC disadvantages, SLN system was developed [8]. SLN can be characterized as small lipid containing preparations which possess large surface area and have been considered as biocompatible and biodegradable systems [12, 13, 14, 15].

NLC (Lipid based systems) - The second-generation lipid carrier is usually composed of solid lipids and liquid lipids together in a system with surfactant[16, 17]. This mixing causes depression in melting point of substrates and converts the mixture into solid form at body temperature. NLC shows a high drug loading with minimum drug expulsion [18, 19, 20] as compared to SLN. NLC bypass P-gp efflux due to other way for uptake (non receptor mediated endocytosis). In view of these facts, NLC system can be considered as effective alternate to overcome issues associated with QF therapy.

MATERIALS AND METHODS:

Materials:

QF was obtained as a gift sample from AurobindoPharma Limited, Hyderabad, India. For the production of NLC, Monegyl - T18 and Monegyl - D207 (Mohini organics Pvt. Ltd. Mumbai), Glycerylmonostearate, 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. Mumbai) were used. All the other reagents used were of analytical grade.

Methods:

Optimization of ratios of solid lipid to liquid lipid:

Solid lipid (Stearic acid, Monegyl- T18, Monegyl- D207, GMS) was screened by 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 were prepared homogenous. To optimize the ratio of lipids, 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 showed 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 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), Polydispersity index (PDI) of the NLC dispersion. This method resulted in consistent production of smaller size NLCs (<250 nm) with good PDI and good % EE.

Egg lecithin was dissolved in 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 was added to above mixture. QF was added to attain a drug-lipid mixture. The hot surfactant solution (poloxamer 188) in water preheated to 10^{o}C above the lipid's M.P. (70^{o}C) was added to the clear lipid melt containing egg lecithin. 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 probe sonicator (PCi *analytics* PKS -750FL, Mumbai) for 15 minutes at 50°C by maintaining output amplitude at 50% and gap of 5 second to form NLC dispersion. Then hot NLCs obtained was 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 prepared 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 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 vehicle for further studies. Selected process variable used were temperature (70^{o}C), magnetic stirrer rpm (2000 rpm) for 10 min. The sonicator process used variable were amplitude at 50% and gap of 5 sec. for 15 min. (Table 1)

Table 1: Preparation of trial NLC batches

Sr. No.	Components (gm)	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
	Ratio of Solid lipid % to liquid lipid %	63.636: 36.363	**63.636: 36.363**	60:40	60:40	70:30
1	QF	0.025	**0.025**	0.025	0.025	0.025
2	Monegyl T 18	0.175	-	0.0875	-	-
3	GMS	-	**0.175**	0.0875	0.165	0.1923
4	Capmul MCM EP	0.1	**0.1**	0.1	0.110	0.0827
5	Poloxamer 188	0.375	**0.375**	0.375	0.375	0.375
6	Egg lecithin	0.250	**0.250**	0.250	0.250	0.250
7	Distilled water (ml)	25	**25**	25	25	25

Experimental design for QF loaded NLC:

Preliminary batches indicated that the variables *viz.* concentration of capmul MCM EP (X_1), concentration of poloxamer 188 (X_2), and concentration of egg lecithin (X_3) were the important factors that affected the PS and % EE of NLC. Thus, response surface methodology- (CCD) was used to systemically examine the influence of these three independent variables on PS (nm) and % EE of the prepared NLCs. The CCD consisting of three factored design with five levels, plus and minus alpha (axial points), plus and minus 1 (factorial points) and the center point, hence 15 runs was used in this study. On the basis of the results obtained from preliminary batches, the optimize concentrations of different components was selected (Table 1). The details of the formulation design are listed in Table 2.

Table 2: Independent variables, dependent variable and levels for design of NLCs

Variables	Levels				
1. Independent variables in gms	-1.68179	Low (-1)	Center	High (+1)	+1.68179
Concentration of capmul MCM EP	0.0829552	0.1	0.125	0.150	0.167045

(X_1)					
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 '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 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].

Evaluation and characterization of QF loaded NLCs

Particle size and polydispersity index (PDI):

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 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 than estimated by the translational diffusion coefficient using the Stokes-Einstein equation by software[37]. PDI was determined to confirm distribution of PS in the formulation[38].

Zeta potential:

The FD samples were used after dilution with distilled water to determine ZP of prepared formulation 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 application of field strength 20 V/cm with 30 runs.

Entrapment efficiency and drug loading:

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 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:

The FTIR spectrum of QF and final formulation were recorded by FTIR (FTIR-4800, Shimadzu, Japan). Briefly, solid sample (1 mg) along with 100 mg dried potassium bromide was compressed into a disc. For liquid sample, sample drops were dripped onto NaCl or KBr aperture plate and sandwiched it under other aperture plate by forming thin liquid membrane. Then, sample was scanned for absorbance in between 4000 ~ 400 cm^{-1}. The obtained spectrum was then matched with standard group frequencies of QF.The obtained FTIR (4800, Shimadzu, Japan) spectrum was then compared with standard 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 aluminum stub, coated with gold by using cold sputter coater in SEM chamber of thickness 400 Å. The graphs were recorded with 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 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):

The physical state of QF loaded NLCs was evaluated by XRD (Bruker AXS D8).The freeze dried NLC was smeared on low background sample holder and fixed onto stage in goniometer. Device was set with B-B geometry. The voltage and current were set to 35 mA and 40 mV respectively and XRD was recorded. The data was also recorded for QF (drug), GMS (solid lipid) and physical mixture [48].

***In vitro* drug release:**

The *in vitro* release of QF from optimized formulation F3 and prepared QF suspension was recorded using the dialysis bag diffusion method [50]. The optimized NLC formulation equivalent to 5 mg of drug was transferred to a dialysis bag, sealed and suspended for 2 hrs in USP (type II) apparatus containing 900 ml 0.1 N HCl (pH 1.2), and then in phosphate buffer (pH 6.8) for 24 hrs with 50 rpm at 37°C ± 0.5°C. The 5 ml of the samples were taken out at intervals *viz.* 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 12 and 24 hrs filtered through 0.45 µm membrane filter and the same was replaced with fresh media to enables sink condition throughout release period. The QF release was analyzed after dilution at 207 nm using a UV–Visible spectrophotometer by taking the respective medium as a blank. Same procedure was repeated for QF

suspension. All measurements were run in triplicates [51]. Data was fitted to different kinetic models to obtain the release kinetics of NLCs [52]. The highest r^2 value showed the actual mode 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.

Estimation of various pharmacokinetics parameters:

Various pharmacokinetics parameters such as maximum concentration (C_{max} in ng/ml), time taken to reach C_{max} (T_{max} in hrs.), $AUC_{(0-24)}$ in nghr/ml, $AUMC_{(0-24)}$ in ng hr^2/ml, mean residence time (MRT in hrs), relative bioavailability (F in %) were determined using rat plasma samples and compared with drug (QF) suspension [21].

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 3.

Table 3: 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 ($\mathbf{r^2}$ =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 4)

Table 4: 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

Pharmacokinetic study:

Enhanced bioavailability of newly developed formulation of QF was validated using pharmacokinetic study in wistar albino rats**.** The drug in the plasma samples was analyzed by using HPLC method. Plasma concentration-time profile and various pharmacokinetic parameters *viz.* C_{max}, T_{max}, and $(AUC)_{0\text{-}24h,}$ $AUMC_{(0\text{-}24)}$, MRT and F were estimated using optimized formulation and QF suspension after an oral administration of dose equivalent to 25mg/kg has been presented in Fig. 5 and Table 5.

Table 5: Comparative pharmacokinetic parameters of optimized formulation of NLC and QF suspension in wistar rats

Parameters	**Optimized Formulation**	**QF suspension**
T_{max} (hr)*	2.00 ± 0.19	1.00 ± 0.36
C_{max} (ng/ml) *	2200.17 ± 200.36	364.83 ± 85.63
$AUC_{(0\text{-}24)}$ (nghr/ml)*	23966.41 ±1172.01	5110.83 ± 421.47
$AUMC_{(0\text{-}24)}$ (ng hr^2/ml)*	54249.89 ± 2119.12	11487.23 ± 967.24
MRT (hr)	2.26 ± 0.41	2.24 ± 0.35
Relative bioavailability (% F)	4.69	-

mean ± SD, *n*=3, *p<0.05 level of significant difference

Fig. 5: Comparative pharmacokinetic of optimized formulation of NLC and QF suspension in wistar rats

The C_{max} of optimized NLC (2200.17 ± 200.36ng/ml) was significantly higher than that of QF suspension (364.83 ± 85.63ng/ml). Peak plasma concentration and T_{max} of pure drug and formulation had significant difference. The mean observed plasma $(AUC)_{0\text{-}24h}$ of formulation was also significantly improved to 4.69-fold compared to pure drug suspension confirming improved bioavailability of drug from formulation. The optimized NLC formulation profile is superior to that of QF suspension. The oral bioavailability of QF from optimized batch F3 was higher than that of suspension.

Notably, at all time points, the NLC were remarkably higher than those administered with QF suspension defining performance superiority of NLC over QF suspensions. This increase in bioavailability of QF may be due to lymphatic transport from NLC formulation and avoiding first-pass metabolism of QF.

DISCUSSION:

The particle size of NLCs increased with an increment in liquid lipid conc., same results as per earlier reports [57, 58, 59]. This may be due to disruption of lipid wall then aggregation and ultimately particle size growth with increase in liquid lipid content [57]. Interfacial tension increased and core of NLC swelled with greater liquid lipid concentration and thus observed 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 permits the PS to decrease further [60].

The drug QF is lipid soluble, so it mostly entrapped in the lipid matrix and showed good % EE. The 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 [61].

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

QF loaded showed excellent stability specified by ZP, high % EE value, 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 behavior and eventually enhanced drug absorption as compared with other lipid-based drug delivery system. The feasibility of large-scale production makes NLC as versatile delivery system. Thus, NLCs seems to be reasonable delivery systems for oral administration of QF and may be used as 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.

CONFLICT OF INTEREST::

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

REFERENCES:

1. Merikangas KR, Akiskal HS, Angst J, et al. Lifetime and 12-month prevalence of bipolar spectrum disorder in the national comorbidity survey replication. Archives of General Psychiatry. 2007; 64(5):543-552.
2. Grunze H, Vieta E, Goodwin GM, et al. The World Federation of Societies of Biological Psychiatry (WFSBP) Guidelines for the Biological Treatment of Bipolar Disorders: Update 2010 on the treatment of acute bipolar depression. World Journal of Biological Psychiatry. 2010; 11(2):81-109.

3. Yatham LN, Kennedy SH, Parikh SV. Canadian network for mood and anxiety treatments (CANMAT) and international society for bipolar disorders (ISBD) collaborative update of CANMAT guidelines for the management of patients with bipolar disorder: update. Bipolar Disorders. 2013; 15(1):1-44.
4. Mehetre GD, Patki SS, Thenge RR, et al. QuetiapineFumarateBuccoadhesive Tablet- Formulation and In Vitro Evaluation. Research Journal of Pharmacy and Technology. 2020; 13(11):5095-5102.
5. Arulappa RX, Sundarapandian M, Venkataraman S, et al. Spectrophotometric Determination of QuetiapineFumarate in Bulk and Dosage Form. Research Journal of Pharmacy and Technology. 2009; 2(4):884-885.
6. Patel TM, Gohel MC. Development of Extended Release Pellets of QuetiapineFumarate by using HPMC and Eudragit RSPO. Research Journal of Pharmacy and Technology. 2014; 7(7):771-775.
7. Doppalapudi S, Suryadevara V, Yallam S et al. Formulation and Pharmacodynamic Evaluation of Quetiapine Solid Dispersions using Plasdone K-29/32 as Carrier. Research Journal of Pharmacy and Technology. 2020; 13(5):2359-2365.
8. Parvathi M, Prathyusha A, Kiran RS, et al. Preparation and evaluation of QuetiapineFumaratemicroemulsions: a novel delivery system. Asian Journal of Pharmaceutical and Clinical Research. 2014; 7(1):208-13.
9. Babu AK, Ramana MV. In vitro and in vivo evaluation of quetiapinefumarate controlled gastroretentive floating drug delivery system. International Journal of Drug Delivery. 2016; 8 (1): 12-22.
10. Olbrich C, Gessner A, Schroder W, et al. Lipid-drug conjugates nanoparticles of the hydrophilic drug diminazene - cytotoxicity testing and mouse serum adsorption. Journal of Controlled Release. 2004; 96(3): 425-35.
11. Das RJ, Baishya K, Pathak K. Recent advancement of lipid drug conjugates as nanoparticulate drug delivery system. International research journal of pharmacy. 2013; 4(1): 73-78.
12. Radtke M, Souto EB, Muller RH. Nanostructured lipid carriers: a novel generation of solid lipid drug carriers. Pharmaceutical Technology Europe. 2005; 17(4):45-50.
13. Cavalli R, Caputo O, Gasco MR. Solid lipospheres of doxorubicin and idarubicin. International Journal of Pharmaceutics.1993; 89:R9–R12.
14. Sarangi MK, Padhi S. Solid lipid nanoparticles- a review. Journal of Critical Reviews. 2016; 3(3): 5-12.
15. Baviskar A, Hiremath S, Akul M, et al. Effect of Lipids and Surfactants on Solid Lipid Nanoparticle Engineering. Research Journal of Pharmacy and Technology. 2011; 4(4):521-526.
16. Bornare AS, Saudagar RB. Nanostructured Lipid Carrier (NLC): A Modern Approach for Transdermal Drug Delivery. Research Journal of Pharmacy and Technology.2017; 10(8):2784-2792.

17. Sarma A, Das MK, Chakraborty T, et al. Nanostructured lipid carriers (NLCs)-based intranasal Drug Delivery System of Tenofovirdisoproxilfumerate (TDF) for brain targeting. Research Journal of Pharmacy and Technology.2020; 13(11):5411-5424.
18. Jaiswal A, Senthil V, Das TK. Design and Development of Valsartan Loaded Nanostructured Lipid Carrier for the Treatment of Diabetic wound Healing. Research Journal of Pharmacy and Technology.2019; 12(6):2922-2928.
19. Majumdar A, Dubey N, Dubey N. Cisplatin loaded Nano Lipid Carriers for the Treatment of Skin Cancer. Research Journal of Pharmacy and Technology.2020; 13(3):1483-1488.
20. Bakthavachalam A, Remya PN, Damodharan N. Review on Solid lipid nanoparticles. Research Journal of Pharmacy and Technology.2020; 13(9):4430-4434.
21. Shah B, Khunt D, Misra M, et al. Non-invasive intranasal delivery of quetiapinefumarate loaded microemulsion for brain targeting: Formulation, physicochemical and pharmacokinetic consideration. European Journal of Pharmaceutical Sciences.2016; 91:196-207.
22. Sansare V, Pathak G, Zambharakar B, et al. Box-behnken study design for optimization of clotrimazole loaded nanostructured lipid carriers. World Journal of Pharmaceutical Sciences. 2019; 7(2): 92-104.
23. Talele SG, Derle DV. Solubility and thermodynamic modeling of quetiapinefumarate in self nanoemulsifying drug delivery system (SNEDDS). International Journal of Applied Pharmaceutics. 2018; 10(4):127-132.
24. Shah B, Khunt D, Misra M, et al. Non-invasive intranasal delivery of quetiapinefumarate loaded microemulsion for brain targeting: Formulation, physicochemical and pharmacokinetic consideration. European Journal of Pharmaceutical Sciences.2016; 91:196-207.
25. Khunt D, Shah B, Misra M. Role of butter oil in brain targeted delivery of quetiapinefumaratemicroemulsion via intranasal route. Journal of Drug Delivery Science and Technology. 2017; doi: 10.1016/j.jddst.2017.05.004.
26. Mu H, Holm R, Mullertz A. Lipid-based formulations for oral administration of poorly water-soluble drugs. International Journal of Pharmaceutics. 2013; 453(1):215-24.
27. Sneh P, Koland M, Kumari SN. Nanoemulsion components screening of quetiapinefumarate: effect of surfactant and co surfactant. Asian Journal of Pharmaceutical and Clinical Research. 2015; 8(6): 136-140.
28. Lohan S, Sharma S, Murthy RR. Formulation and evaluation of solid lipid nanoparticles of quetiapinefumarate and quetiapinehemifumarate for brain delivery in rat model.Pharmaceutical Nanotechnology.2013; 1: 239-247.

29. Narala A, Veerabrahma K. Preparation, characterization and evaluation of quetiapinefumarate solid lipid nanoparticles to improve the oral bioavailability. Journal of Pharmaceutics. 2013; 2013:1-7. Article ID 265741

30. Yuan H, Wang LL, Du YZ, et al. Preparation and characteristics of nanostructured lipid carriers for control-releasing progesterone by melt-emulsification. Colloids and Surfaces B: Biointerfaces. 2007; 60: 174-179.