Original Article

Direct Brain Targeted Nanostructured Lipid Carriers for Sustained Release of Schizophrenic Drug: Formulation, Characterization and Pharmacokinetic Studies

Praveen Sivadasu[1], Devagowda Vishakante Gowda[1,*], Nithin Kundachira Subramani[2], Bhavya Malgur Vishweshwaraiah[1], Sachhidananda Shivanna[3], Siddaramaiah Hatna[3]

[1]Department of Pharmaceutics, JSS College of Pharmacy, JSSAHER, Mysuru, Karnataka, INDIA.
[2]Department of Chemistry, The National Institute of Engineering, Mysuru, Karnataka, INDIA.
[3]Department of Polymer science and Technology, Sri Jayachamarajendra College of Engineering, Mysuru, Karnataka, INDIA.

ABSTRACT

Background: Systemic drug delivery in schizophrenia is a major challenge, owing to the Blood-brain Barrier (BBB) and P-glycoprotein related effects. Consequently, herein an attempt is made to systemically deliver the most desirable schizophrenia drug, Quetiapine Fumarate (QF) via non-invasive intranasal route using Nanostructured Lipid Carrier (NLC) approach. **Materials and Methods:** The desired QF loaded NLCs were developed using central composite statistical design and the developed formulations were monitored for improving QF bioavailability and their brain targeting efficacies. **Results:** The optimized formulation displayed a 2-fold increase (compared to virgin QF) in *ex-vivo* nasal diffusion at the 6th hr, with no sign of structural damage (upon histopathological examinations). While, QF blood-brain ratio showed 10-fold increase for NLCs administered through nasal route (in comparison to intravenous route), thereby supporting prolonged retention of QF at the site of action. Similarly, the concentration of QF (in the brain) delivered via nasal route exhibited 4-fold increment at all-time points thereby supporting a potential nose to brain transport and effective bypassing of BBB. **Conclusion:** The results obtained infers that non-invasive intranasal route can be used as a potential alternative to conventional treatment options towards efficient management of schizophrenia.

Key words: Nanostructured lipid carriers, Quetiapine fumarate, Schizophrenia, Brain Targeting, Intranasal route.

Submission Date: 04-06-2019;
Revision Date: 04-09-2019;
Accepted Date: 18-11-2019

DOI: 10.5530/ijper.54.1.9

Correspondence:
Dr. Devagowda Vishakante Gowda,
Professor and Head,
Department of Pharmaceutics,
JSS College of Pharmacy, JSSAHER, Mysuru, Karnataka, INDIA.
Phone: +91 9663162455
E-mail: dvgowda@jssuni.edu.in

INTRODUCTION

Schizophrenia is a chronic re-occurring psychotic disorder, which originates at a young age and lasts for a lifetime.[1-3] The most common symptoms of schizophrenia include cognitive dysfunction, acute psychosis and related negative symptoms.[4,5] Additionally, whether schizophrenia represents a single or syndromal illness is yet to be ascertained and hence, defining its sub-groups is of considerate interest.[6,7] According to World Health Organization (WHO) statistics, more than 21 million people are affected by schizophrenia (worldwide) and the death rate of these affected population is (2-2.5) times higher than the general population.[8] Moreover, the effective method of its treatment demands a continuous presence of the drug at the target site (brain) for a prolonged duration of time. Nevertheless, systemic drug delivery to treat schizophrenia is a potential challenge, owing to BBB effects, which often obstructs the efficient transport of a rational drug (to the brain).[9-12]

Accordingly, the non-invasive route of drug administration as a potential alternative to conventional oral and parenteral routes has attracted considerable scientific interest.[13]

www.ijper.org

Besides, owing to its rich vascular structure, effective by-passing of first-pass metabolism and large surface area of absorption, nasal route displays increased efficiency in transporting the drug (to the brain) via olfactory and trigeminal region in upper nasal mucosa, thereby dodging the BBB.[14-16] All these positive attributes makes it logical to deliver the developed drug through the nasal route. Yet, toxicity related factors, the potential of the delivery system and its inherent ability to target specific delivery sites have often limited the wider scope of nasal drug delivery.[17]

Usage of colloidal carriers for drug delivery has gained a lot of attention towards the effective treatment of brain disorders as they target the active site very efficiently, (in comparison to other routes of administration).[18,19] Delivering these nanocarriers through nasal route also enables effective brain targeting and minimizing systemic exposure, when compared to oral route.[20] In recent years, NLC, which is considered as a modified version of solid lipid nanoparticle (SLN), where drug gets encapsulated in solid-lipid and liquid-lipid is increasingly preferred over SLN, as it overcomes major disadvantages of SLN, such as low drug loading and drug expulsion related issues.[21-23]

Further, QF is a second generation, atypical antipsychotic drug, with a half-life of 6hrs and requires repeated dosage (i.e. 25 to 300mg per day) with poor oral bioavailability. Additionally, the said drug also suffers from extensive first-pass metabolism and very low absorption. QF is a lipophilic drug, which displays pH-dependent solubility, thereby resulting in very low drug concentration in the brain, especially during oral administration. Accordingly, an alternative approach for administration is required to overcome these limitations.[24-29] Thus, herein we intend to establish the QF delivery efficacy (to the brain) via NLC based nasal route, which may aid to enhance the permeation and bioavailability and thereby act as an effective approach towards systemic drug delivery.

MATERIALS AND METHODS

Materials

Quetiapine fumarate was obtained as a gift sample from Divis Laboratories Pvt. Ltd., (Hyderabad, India). While, Gelucire 43/01 and 44/14, was gifted by Gattefosse (France). Further, Capmul MCM, Captex 200, Cremophor EL, Sterotex FL were also obtained as a gift sample from Abitec Group, (USA). However, Span 80, PEG 400 and Oleic acid were purchased from Merck (Mumbai, India). While, Tween 80, Tween 20 and polyvinyl alcohol (PVA) were purchased from Loba Chemie Pvt. Ltd., (Mumbai, India).

Experimental animals

The study was conducted after obtaining approval (No. 155/PO/Re/S/99/CPCSEA) from the institutional animal ethical committee of JSS College of Pharmacy, Mysuru. Wister albino rats weighing 180-200g were selected for bio-distribution studies, as per the guidelines set by National Institutes of Health, all the animals were humanely treated.

Methods

Solubility studies

The solubility of QF in various solid and liquid components was ascertained, so as to handpick the best suitable excipient towards the effective formulation of desired NLC's. In a typical procedure, an excess of drug (QF) was mixed in the respective excipients using a vortex mixer. The mixtures obtained were set aside for 72 h and followed by centrifugation at 5,000 rpm for 10 min. From obtained centrifuged solutions, 0.5 ml of supernatant was drawn out, diluted and analyzed for QF solubility using electronic spectral studies (UV1800, Shimadzu, Japan). While for solid lipids, the minimum amount of molten lipid required to solubilize the desired drug was quantified via visual observation, wherein the formation of a clear solution of melted lipid with QF was considered the end point.[30]

Compatibility studies

Differential Scanning Calorimetry (DSC) thermogram was obtained with Shimadzu DSC-60 calorimeter over a temperature range of 40 to 300ºC and at a constant heating rate of 20ºC/min.[31]

Construction of pseudo-ternary phase diagrams

Pseudo ternary phase diagrams which are essential in optimizing the S_{mix} ratio were constructed using aqueous titration technique. Wherein, the selected oil phase was heated and maintained at a temperature of 75°C, to which selected S_{mix} was added in varying ratios (so as to form homogeneous mixtures) as depicted in Table 1. The mixtures so obtained where then titrated against the desired aqueous phase and the changes observed were recorded visually. Finally, the pseudo-ternary phase diagrams were designed using TriPlot Software (Version 4.1.2).[32]

Formulation of NLCs containing quetiapine fumarate

The desired NLC formulations were prepared using hot homogenization, followed by rapid ultra-sonication, wherein selected quantity of desired drugs were dissolved in molten lipids and simultaneously, aqueous

Table 1: Excipient combinations.

Group	Oil phase (Oleic acid: Gelucire 44/14)	S_{mix} (Surfactant: Co-Surfactant)	Oil Phase: S_{mix}
1	1:1	1:0	1:9
			2:8
		1:1	3:7
			4:6
		2:1	5:5
2	1:2		6:4
		3:1	7:3
			8:2
			9:1

phase consisting of all other components were heated separately at a constant temperature. Post drug dissolution obtained blend was transferred to aqueous medium with vigorous stirring for 5 min at 10,000 rpm with a hot homogenization ((Polytron® PT1600E). Further, a probe sonicator was used to sonicate the obtained emulsion and desired NLC was stored in amber coloured glass vials at a temperature of 2-10ºC.[20]

Experimental design

In order to demonstrate the response surface model by attaining different combination of values Design expert software (Version 11, Stat-Ease Inc. and Minneapolis, U.S.A.) was employed. Central composite design (CCD) is used to form a second order quadratic equation without using complete three level factorial experiment. A 2 factor, 2 level central composite design was used to design the optimized procedure to formulate the desired NLC. Wherein the selected independent variables are oil phase and S_{mix}, while selected dependent variables were drug loading, entrapment efficiency and particle size. This leads to process of optimization with a small experimental design (13 runs).

Evaluation of QF-NLCs

Drug loading and encapsulation efficiency

The amount of QF loaded in relation to the lipid phase and its encapsulation efficiency was determined by centrifuging (REMI laboratory Instrument, India) a fixed volume of desired NLC for 20 min at 10000 rpm. The supernatant so obtained was diluted appropriately and the amount of QF loaded and encapsulation efficiency was quantified using UV spectrophotometric measurements at 248 nm (UV1800, Shimadzu, Japan),[33] using the following equations;

$$\text{Drug Loading (DL) (\%)} = \left(\frac{Wd - Ws}{Wn}\right) * 100 \quad (1)$$

$$\text{Entrapment efficiency (EE) \%} = \left(\frac{Wd - Ws}{Wd}\right) * 100 \quad (2)$$

Where W_d is Total weight of drug is taken, W_s is the weight of drug in the supernatant and W_n is the total weight of obtained NLC

Particle size and polydispersity index

Particle size and Polydispersity Index (PDI) of NLCs were measured using (Model Zetasizer Nano ZS, Malvern Ltd., UK).[34]

Surface morphology

The surface morphology of QF-NLC was recorded using a (Hitachi Noran System 7 scanning electron microscope (SEM), USA).[35]

In vitro drug release studies in optimized QF loaded NLC

The drug release studies for QF-NLC was accomplished using dialysis membrane (Hi-media, Mumbai, India) with a molecular weight limited to 10 kDa. In a typical experimental set-up, a NLC equivalent to 100mg of selected drug was measured and placed on dialysis membrane. The bag so formed, was placed in beaker containing a fixed quantity of simulated nasal fluid (SNF) (pH 6.4) i.e. 250 ml maintained at a temperature of 37°C. From which, samplings were carried out at predetermined intervals, during which 2 ml of aliquots were repeatedly withdrawn from receptor compartment and replenished with fresh buffer. Finally, the concentration of QF released was quantified spectrophotometrically at 248 nm (UV1800, Shimadzu, Japan).[36]

Ex-vivo permeation studies using sheep nasal mucosa

Sheep nasal mucosa was used to perform *ex-vivo* permeation studies for both pure drug and drug loaded NLCs. Sheep nasal mucosa was excised and collected from a slaughter house followed by washing with PBS solution and isopropyl alcohol, so as to remove excess of fat surrounding the tissue. In order to place the samples on donor and receptor component of diffusion cell, obtained nasal mucosa was sliced into small pieces with a thickness of 0.2mm. The mounted samples was finally stabilized using PBS treatment for 15 min at a temperature of 39±0.5°C. The resulting sample was then mounted on a rim of receptor compartment with freshly filled dissolution media and donor compartment was placed over it. Finally, the samples

were considerately drawn from the respective compartments and analyzed for the amount of QF permeated across the tissue using UV absorbance studies at 248 nm (UV1800, Shimadzu, Japan).[37]

Nasal ciliotoxicity studies

The nasal ciliotoxicity studies were performed with freshly isolated sheep mucosa, where each tissue sample was treated with Isopropyl Alcohol (IPA) (positive control), optimized NLCs (the formulation under study) and PBS (negative control). The tissue samples so processed were stored in 10% formalin solution for 2 hr, followed by H and E staining for histopathological imaging.[38]

In-vivo bio-distribution studies

For *in-vivo* bio-distribution studies, the selected animals were grouped into two, wherein, one group received intranasal formulations, while the other received a dose which is equivalent to 2.3 mg/kg body weight intravenously via injection through tail vein. Nevertheless, for intranasal administration, a 50 µl dose was administered by holding the rats from back in slanted position from formulation which is equivalent to 6 mg/ml.[39]

Pharmacokinetic and brain distribution study

The plasma and brain samples required for pharmacokinetic and brain distribution studies were collected by suitably anesthetizing the animals, before scarification. The typical plasma and brain sample collections involved drawing of 2ml of blood at different time intervals, by employing tail vein collection technique and the same was stored in Ethylene Diamine Tetraacetic Acid (EDTA) tubes under cold conditions. However for plasma separation, centrifugation was done for 15 min at 5000 rpm for the drawn blood samples. Further, obtained plasma was mixed with internal standard and 2 ml of acetonitrile was added to bring about protein precipitation. Post-precipitation, the plasma samples were further thoroughly mixed and centrifuged for a short period of time (5min at 4000rpm). To finish, supernatant obtained from the centrifuged solutions was collected and prior to analysis the obtained samples were dried at ambient temperatures and reconstituted with the selected mobile phase and filtered through membrane filter.

Post blood collection, animals were decapitated immediately and the skull was slit to excise the brain carefully. In order to get free from attached blood vessels the obtained brain tissue was quickly washed with saline water and marked with the help of a filter paper and stored in PBS. The stored tissues were then homogenized for 1 min at 10000 rpm followed by centrifugation and the obtained samples were analysed by using HPLC. Post analysis various pharmacokinetic parameters were calculated by using formulas which were cited elsewhere.[40,41]

RESULTS AND DISCUSSION

Screening of components and equilibrium solubility studies

The selection of right excipient towards the effective formulation of NLC is mostly dependent on the solubility of the desired drug (in actual excipients). Accordingly, the solubility of QF was determined in various vehicles (solid lipids, liquid lipids, surfactants and co-surfactants) and the results obtained are presented in Figure 1 and 2. Form the results it was evident that in liquid lipids Oleic acid, in surfactants Tween80 and Transcutol-p and in solid lipids Gelucire 44/14 had shown maximum solubility, which was considered for further studies.

Drug-excipient compatibility characterization

Differential scanning calorimetry (DSC) studies

The DSC thermograms of virgin QF and its physical mixture (QF, Oleic acid and Gelucire 44/14) are presented in Figure 3. The DSC thermogram of QF

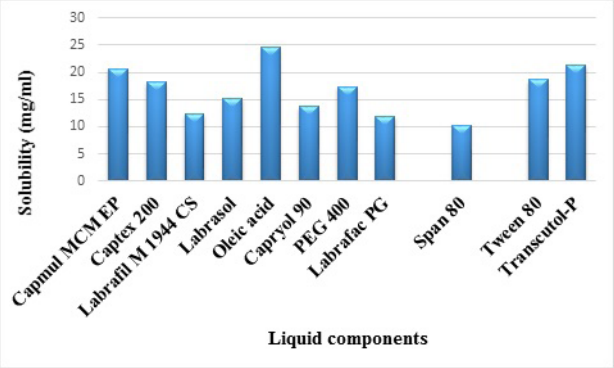


Figure 1: Solubility of QF in liquid components.

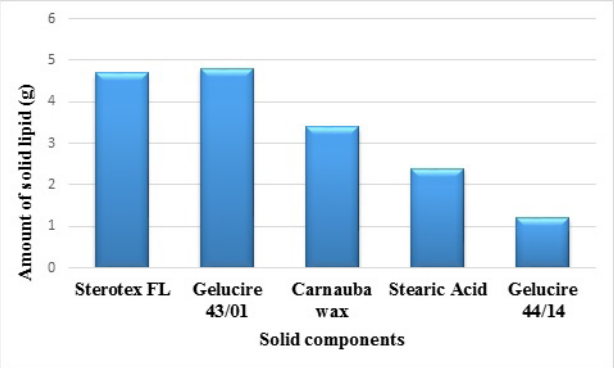


Figure 2: Solubility of QF in solid components.

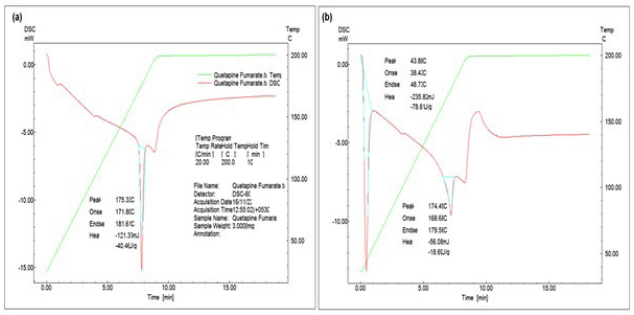


Figure 3: DSC thermogram of QF and physical mixture.

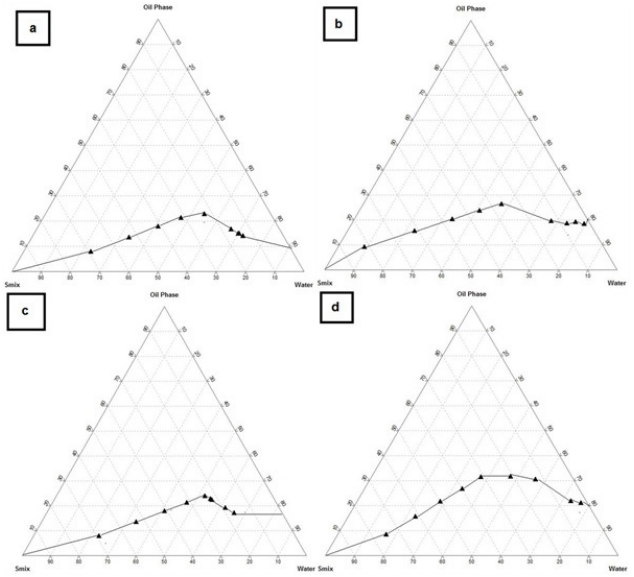


Figure 4: Pseudo-ternary Phase diagram for Group 1.

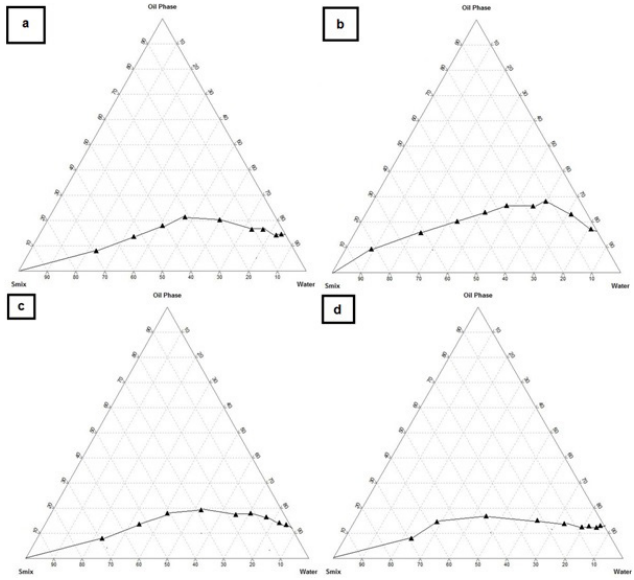


Figure 5: Pseudo-ternary Phase diagram for Group 2.

Table 2: Variables in central composite design of QF-NLC.

Independent factors		Design level	
Uncoded	**Coded**	**Uncoded**	**Coded**
Oil Phase (%)	X_1	5 7.5 10	-1 0 (Center point) 1
S_{mix} (%)	X_2	20 35 50	-1 0 (Center point) 1

displayed melting endotherm at 175.3º C, which is in agreement with the melting point of QF. While, the physical mixture displayed two peaks, first at 42.8ºC (corresponding to the oil phase), where the drug may be molecularly dispersed in corresponding lipids. While, the second peak at 174.5°C may be attributed to virgin QF, indicating the absence of undesirable drug-lipid interactions.

Pseudo-ternary phase diagrams

The (drug excipient) formulations which remained as nanoemulsions (when dispersed) were subjected to further studies. Based on the pseudo-ternary phase diagram Figure 4 and 5, nanoemulsions composed of Oleic acid and Gelucire 44/14 as the oil phase, Tween 80 and Transcutol-p as surfactant and co-surfactant were selected for further optimization. As can be seen from Figure 4 and 5, the nanoemulsion formation area increases with an increase in surfactant to co-surfactant ratio and was highest at 3:1 (Figure 4d). Consequently, the surfactant to co-surfactant ratio was maintained at 3:1 for further optimizations.

Formulation of QF-NLC

The QF-NLC consisting of Gelucire 44/14 + Oleic Acid (1:1 ratio), Tween 80 and Transcutol-p used as surfactants and co-surfactants in 3:1 ratio was formulated via homogenization (under hot) followed by rapid-ultra sonication. The as prepared, QF-NLC formulation existed as pale yellow color and milky dispersions.

Central composite design (Tables 2 and 3)[20] was employed towards formulating NLCs at 2 factors and 2 levels, with X_1 (Oil Phase) and X_2 (S_{mix}) as independent variables, while levels -1 and +1 were chosen as low and high, whereas 0 was chosen as the center point.

Response analysis for optimization of QF-NLC

Effect of dependent variables on entrapment efficiency and drug loading

The effect of all independent variables (under investigation) on drug loading and entrapment efficiency (as established by central composite design) is presented

Table 3: Observed responses in the central composite design of QF-NLC.

Formulation Code	Oil Phase	S_{mix}	Drug Loading (%)	Entrapment Efficiency (%)	Particle Size (nm)
QF1	7.5	35	11.85	49.85	152.9
QF2	10	20	10.58	52.48	220.6
QF3	7.5	35	11.85	49.85	152.9
QF4	5	20	4.94	35.84	98.7
QF5	7.5	13.7868	7.89	40.82	183.2
QF6	3.96447	35	20.36	77.49	102.6
QF7	7.5	35	11.85	49.85	152.9
QF8	11.0355	35	15.49	69.86	197.4
QF9	5	50	24.49	84.95	99.7
QF10	7.5	56.2132	14.86	60.98	137.89
QF11	10	50	18.64	81.67	169.7
QF12	7.5	35	11.85	49.85	152.9
QF13	7.5	35	11.85	49.85	152.9

in Table 3. As can be seen, both Oil phase and S_{mix} displayed a monotonous dependence on entrapment efficiency and drug loading (Figure 6 (a and b) and 7(a and b)). Accordingly, with an increase in the concentration of oil phase, there occurred a significant increment in both (entrapment efficiency and drug loading) responses, thereby reducing the escaping nature of the drug into the external phase. Moreover, for optimized formulation, entrapment efficiency was found to be 78.5% with a drug loading of 22.36%, while the rest of the drug remained free in the dispersion media (21.5% of the loaded 22.36%). Thus, 21.5% of loaded 22.36% drug (present in dispersion media) was considered as the loading dose. While, 78.5% of loaded 22.36% (amount of drug entrapped in NLC) was considered to be the maintenance dose (towards sustained delivery). Further the desired polynomial equations, showed a good fit of responses at different concentrations (Table 4), thereby supporting the significance of the said model.

Drug loading (R^1) = -103.22066 +23.18426 Oil phase+5.03266 Smix-0.994453Oil phase * Smix-1.17044 Oil phase^2-0.023299 Smix^2+0.047341 Oil phase^2 * Smix+0.002968 Oil phase * Smix^2

Entrapment efficiency (R^2) = -238.25937+56.96427 Oil phase+14.29878 Smix-2.87573 Oil phase * Smix-2.68312 Oil phase^2-0.076560 Smix^2+0.132772 Oil phase^2 * Smix+0.010734 Oil phase * Smix^2

Effect of dependent variables on particle size and PDI

From the above Table 3, it was evident that Oil phase have positive influence over particle size, while the effect is negative, with S_{mix} as shown in (Figure 6c and 7c). Further, results also suggested that an increase in concentrations of oil phase leads to formation of aggregates, which in turn increases the particle size from 98.7 to 220.6 (as shown in Table 3). With increase in the concentration of S_{mix} there is a rapid decrement in particle size. Further the desired polynomial equations, showed a good fit of responses at different concentrations (Table 4), thereby supporting the significance of the said model. The average particle size of optimized QF-NLC was found to be around 109.54 nm, while PDI was around 0.425 units. The results obtained infers that the selected excipients had led to effective development of desired NLC.

Particle Size (R^3) = +152.90+33.52 Oil phase -16.02 Smix-12.97 Oil phase * Smix-3.47 Oil phase^2+1.80 Smix^2+3.54 Oil phase^2 * Smix+14.46 Oil phase * Smix^2

Optimized formulation

The QF-NLC was developed by clearly defining the required limits of responses (Table 5). The combinations of variables, which resulted in NLCs achieving the required specifications were calculated using design expert software. The overlapping of the obtained results (over the predicted values) confirms the practicability and hence, validation of the model.

Surface Morphological behaviors of QF-NLC

The SEM photomicrograph of QF-NLC (Figure 8) supported that particles were spherical in shape and near homogeneous with relatively smoother surfaces.

Comparative account of drug release via *in vitro* studies

The *in vitro* drug release profiles of QF-NLC compared with virgin QF (Figure 9) supported that, QF-NLC displayed sustained drug release. Accordingly, the drug

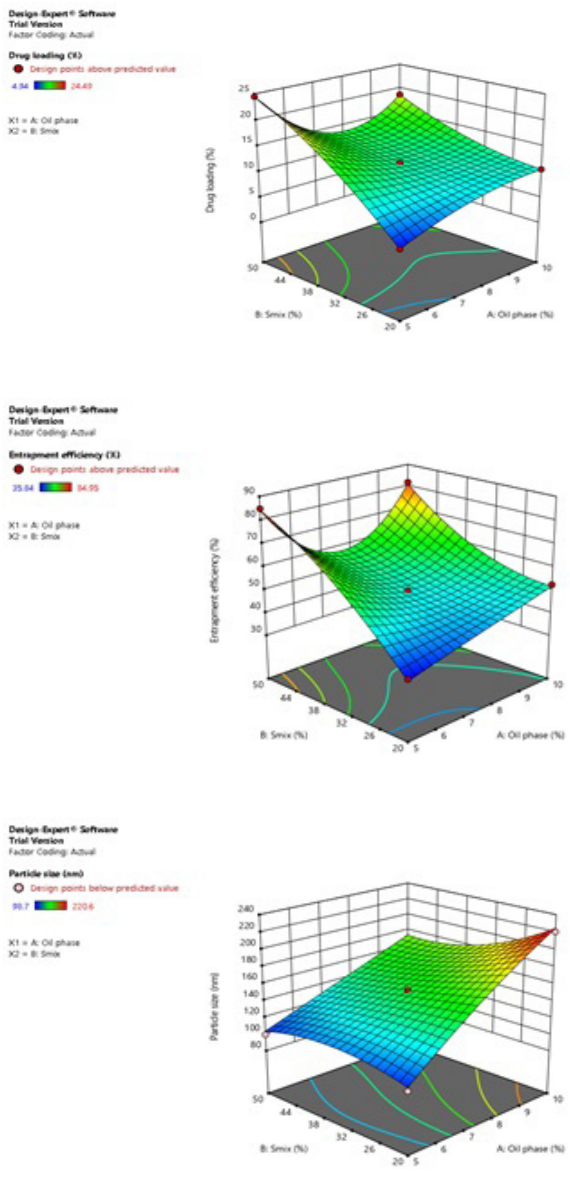


Figure 6: Three-dimensional response surface plot depicting the impact of oil Phase and S_{mix} on (a) drug loading (b) entrapment efficiency and (c) particle size of QF-NLCs.

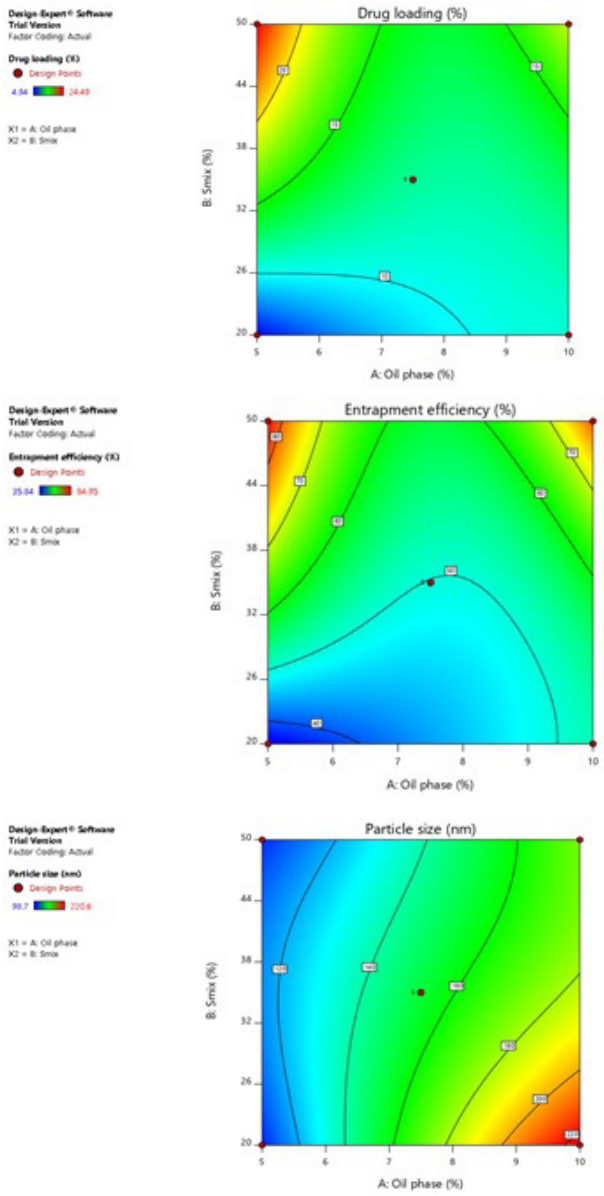


Figure 7: Contour plot showing the impact of oil Phase and S_{mix} on (a) drug loading (b) entrapment efficiency and (c) particle size of QF-NLC respectively.

release from virgin QF was rapid (95.34 ± 3.58% over 8 hr), in contrast to a prolonged release from QF-NLC (44.44 – 71.56% over 24 hr), thereby supporting sustained delivery of the desired drug and hence, the effectiveness of the developed formulation. Additionally, the QF release pattern from the optimized formulation was bi-phasic, associated with an initial short timed (2 hr) burst release, followed by a long term (24 hr) sustained release. The observed bi-phasic release may be owed to initial rapid QF release from the NLC surface, followed by later stage prolonged release from NLC core. Nevertheless, the kinetic analysis of *in vitro* QF release profile (from QF-NLC) conforming the Higuchi model supported that, the drug release profile was also a function of the solubility of drug in the desired excipient, lipid concentration and finally the particle size.[42]

Cumulative drug permeation measurements via *ex-vivo* diffusion studies

The *ex-vivo* diffusion studies of virgin QF and optimized QF-NLC with sheep nasal mucosa revealed that the cumulative drug permeated across the nasal mucosa was significantly higher for QF-NLC (in contrast to virgin QF). Accordingly, about 79.56±0.18% of QF

Table 4: Summary of ANOVA for responses of QF-NLC.						
Drug Loading (%)						
Source	**Sum of squares**	**df**	**Mean square**	**F value**	***P* value**	**Remarks**
Model	326.88	7	46.7	7.471E+05	< 0.0001	Significant
A-Oil Phase	11.86	1	11.86	1.897E+05	< 0.0001	
B-S_{mix}	24.29	1	24.29	3.886E+05	< 0.0001	
AB	33.01	1	33.01	5.281E+05	< 0.0001	
A^2	64.32	1	64.32	1.029E+06	< 0.0001	
B^2	0.38	1	0.3821	6114.13	< 0.0001	
A^2B	39.4	1	39.40	6.303E+05	< 0.0001	
AB^2	5.57	1	5.57	89170.54	< 0.0001	
Residual	0.0003	5	0.0001			
Lack of fit	0.0003	1	0.0003			
Pure error	0.0000	4	0.0000			
Cor Total	326.88	12				
Entrapment efficiency (%)						
Model	2960.55	7	422.94	504.63	< 0.0001	Significant
A-Oil Phase	29.11	1	29.11	34.73	0.0020	
B-S_{mix}	203.21	1	203.21	242.47	< 0.0001	
AB	99.20	1	99.20	118.36	0.0001	
A^2	1048.07	1	1048.07	1250.53	< 0.0001	
B^2	5.47	1	5.47	6.53	0.0509	
A^2B	309.87	1	309.87	369.73	< 0.0001	
AB^2	72.91	1	72.91	86.99	0.0002	
Residual	4.19	5	0.8381			
Lack of fit	4.19	1	4.19			
Pure error	0.0000	4	0.0000			
Cor Total	2964.74	12				
Particle size (nm)						
Model	16142.17	7	2306.02	87.92	< 0.0001	Significant
A-Oil Phase	4493.52	1	4493.52	171.33	< 0.0001	
B-S_{mix}	1026.50	1	1026.50	39.14	0.0015	
AB	673.40	1	673.40	25.68	0.0039	
A^2	83.97	1	83.97	3.20	0.1336	
B^2	22.49	1	22.49	0.8576	0.3969	
A^2B	25.13	1	25.13	0.9580	0.3726	
AB^2	418.08	1	418.08	15.94	0.0104	
Residual	131.14	5	26.23			
Lack of fit	131.14	1	131.14			
Pure error	0.0000	4	0.0000			
Cor Total	16273.31	12				

Table 5: Central composite design enabled QF-NLC (optimized formulation) obtained via independent variables and their effects on dependent responses.						
Value	**Oil Phase (%)**	**S_{mix} (%)**	**Drug loading (%)**	**Entrapment efficiency (%)**	**Particle size (nm)**	**Desirability**
Predicted	5.2	48	22.36	78.5	110	0.953
Actual	5.2	48	21.98	78.24	109	
% Error	0	0	0.38	0.26	1.0	

was permeated from the optimized NLC formulation, while the same was around 39.37±0.28% for the virgin QF (at the end of the same time interval (6 hr)). Consequently, the steady state flux achieved from virgin QF was found to be 1.56 μg/cm^2/h, whereas, for QF-NLC, it was recorded as 4.52 μg/cm^2/h. The observed higher flux may be attributed to the presence of permeation enhancing surfactants in QF-NLC. Moreover, the relatively higher lipophilicity of QF allows significantly better permeation through the nasal mucosa.[43]

Nasal ciliotoxicity studies

Nasal ciliotoxicity studies aimed to establish the toxicity of excipients employed towards the development of QF-NLCs revealed that the nasal mucosa samples treated with PBS (negative control) showed no nasociliary damage and the epithelial layers were intact. However, treatment with IPA (positive control) caused extensive damage to the nasal mucosa with subsequent loss of epithelial cells, loss of cilia and shrinkage of the mucosal layer. However, the nasal mucosa samples treated with optimized QF-NLC showed no adverse effects (Figure 10). Accordingly, the nasal ciliotoxicity studies established that all the excipients involved in the formulation of QF-NLC were safe enough for nasal administration.

Quantification of drug concentration via *in-vivo* bio-distribution studies

The brain and plasma concentrations after administration of QF-NLC via intranasal (IN) and intravenous (IV) routes and their corresponding pharmacokinetic parameters are shown in Table 6. While the mean plasma and brain concentration versus time profile of QF-NLC delivered through various routes are summarized in Figure 11. The distribution studies revealed that the concentration of QF in brain following IN administration was significantly higher at all time points. Nevertheless, when IV route was followed, the concentration of QF was highest in blood plasma (at all-time points) and lowest in the brain. The obtained results supported that the distribution of the drug in systemic circulation was lower with formulations administered intranasally then intravenously, thereby

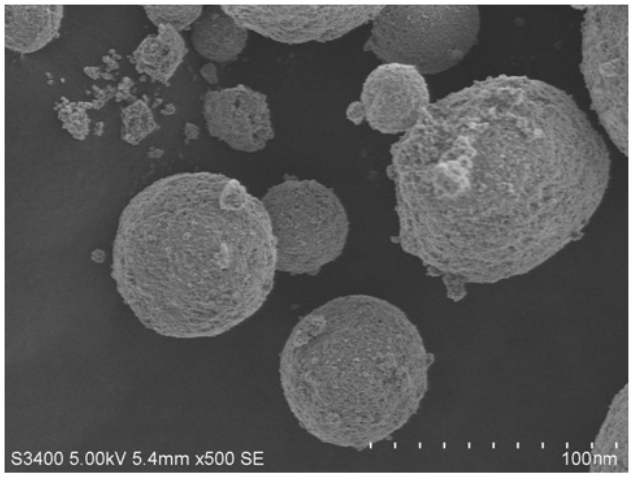


Figure 8: Surface morphology image of QF-NLC.

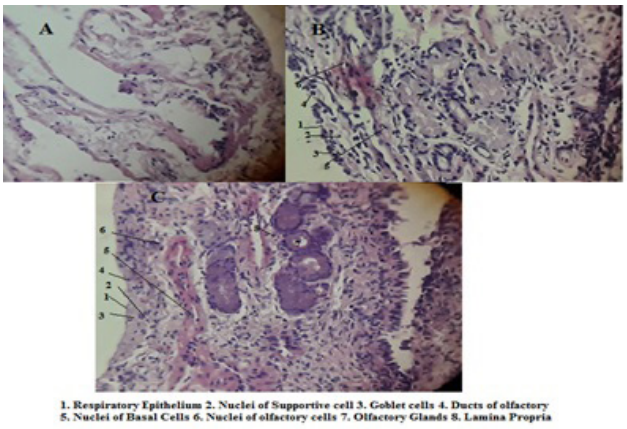


Figure 10: Histopathological sections of sheep nasal mucosa treated with (a) IPA (b) QF-NLC and (c) PBS for nasal ciliotoxicity studies.

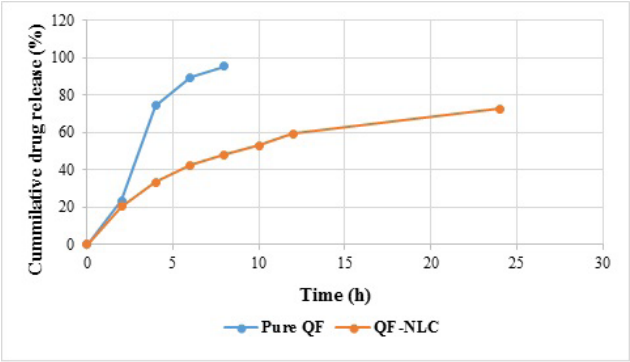


Figure 9: A comparative *in vitro* drug release profile of virgin QF and QF-NLC.

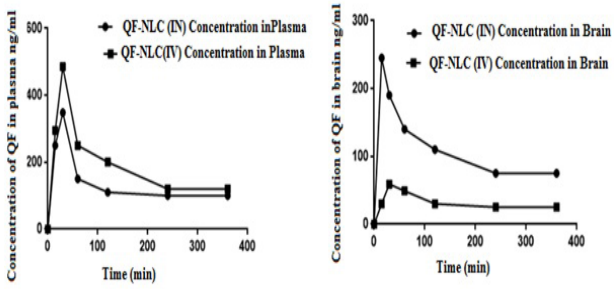


Figure 11: Mean concentration-time profile following IN and IV administration a) Plasma and b) Brain.

Table 6: Pharmacokinetic parameters following IN and IV administration.

Parameters	Tissue/organ	Formulation and route of administration	
		QF-NLC IN	QF-NLC IV
C_{max} (ng/ml) / (ng/gm)	Plasma	348±14.06	485±28.56
	Brain	245±26.97	59±24.24
T_{max} (min)	Plasma	30	30
	Brain	15	35
AUC_{0-360} (ng min ml^{-1})/ (ng min gm^{-1})	Plasma	45090±3235.24	65400±3267.71
	Brain	37650±2374.98	11183±726.22
$t_{1/2}$ (hr)	Plasma	1.85 ± 0.58	1.85 ± 0.38
	Brain	1.94 ± 0.56	3.22± 1.17
DTE (%)	Brain	485.76±10.25	-
DTP (%)	Brain	90.26±6.45	-
Nasal Bioavailability (%)	Plasma	60.71 ± 8.73	-
	Brain	356.87± 19.38	-

*Mean±SD, n = 3, C_{max}- Maximum concentration, T_{max} – Maximum time, AUC- Area under curve, DTE – drug targeting efficiency, DTP- direct transport percentage

indicating potential nose to brain transport. Further, the blood/brain ratio for a drug administered intranasally was significantly higher (0.15± 0.007), which supports increased and prolonged retention of the drug at the site of action (~ 3-5 times higher, than IV administration). Moreover, lower T_{max} (time taken to attain maximum concentration) value of 15 min in the brain, compared 30 min in plasma may be owed to direct nose to brain transport, following IN administration. Additionally, the DTE and DTP value for QF-NLC via nasal administration was found to be 455.76 ± 10.25 and 85.26 ± 6.45, supporting higher targeting efficiency. The lesser nasal bioavailability of QF in plasma may be attributed to the fact that, the nasally administered drug makes use of olfactory and trigeminal nerve for drug transport, thereby successfully by passing the BBB.[44]

CONCLUSION

The desired QF loaded NLCs were developed using central composite statistical design, which furnished the most favorable concentrations of the oil phase and S_{mix} to obtain optimal responses (minimum size and maximum drug loading and/or release). The current study demonstrated superior brain uptake of QF following non-invasive intranasal route, which may maximize the therapeutic index and reduce dosing frequency. Furthermore, the observed increase in QF permeation flux and higher DTE and DTP values for QF-NLC supports the favorable effect of NLC towards effective bypassing on BBB and modulation of tight junctions. The study also supports that the optimized QF loaded NLC (O-QF-NLC) can permeate through the nasal mucosa and into the receptor compartment (as such). To sum up, the current findings support that QF-NLC could be a promising approach towards direct brain targeting via the intranasal route.

ACKNOWLEDGEMENT

The authors express their gratitude to the JSS Academy of Higher Education and Research and JSS College of Pharmacy, Mysuru, for providing necessary support in due course of the work.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

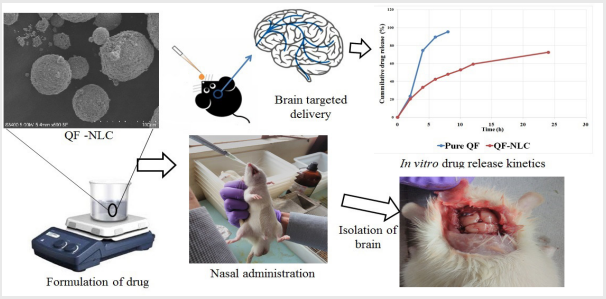
QF: Quetiapine fumarate; **NLC:** Nanostructured lipid carrier; **BBB:** Blood brain barrier; **IN:** Intranasal; **IV:** Intravenous; **C_{max}:** Maximum concentration; **T_{max}:** Maximum time; **AUC:** Area under curve; **DTE:** Drug targeting efficiency; **DTP:** Direct transport percentage; **PBS:** Phosphate buffer solution; **IPA:** Isopropyl alcohol; **SEM:** Scanning electron microscopy; **DSC:** Differential scanning calorimetry; **EDTA:** Ethylene diamine tetraacetic acid; **RPM:** Rotations per minute; **HPLC:** High Performance Liquid Chromatography.

REFERENCES

1. Kahn RS, Keefe RS. Schizophrenia is a cognitive illness: Time for a change in focus. JAMA Psychiatry. 2013;70(10):1107-12.
2. Murray V, McKee I, Miller PM, Young D, Muir WJ, Pelosi AJ, *et al*. Dimensions and classes of psychosis in a population cohort: A four-class, four-

dimension model of schizophrenia and affective psychoses. Psychol Med. 2005;35(4):499-510.
3. Upthegrove R, Marwaha S, Birchwood M. Depression and Schizophrenia: Cause, consequence or trans-diagnostic issue?. Schizophr Bull. 2016;22:240-44.
4. Craddock N, Owen MJ. The Kraepelinian dichotomy – going, going. But still not gone. Br J Psychiatry. 2010;196(2):92-5.
5. Messinger JW, Tremeau F, Antonius D, Mendelsohn E, Prudent V, Stanford AD, *et al*. Avolition and expressive deficits capture negative symptom phenomenology: Implications for DSM-5 and schizophrenia research. Clin Psychol Rev. 2011;31(1):161-8.
6. Koutsouleris N, Meisenzahl EM, Borgward S, Riecher-Rössler A, Frodl T, Kambeitz J, *et al*. Individualized differential diagnosis of schizophrenia and mood disorders using neuroanatomical biomarkers. Brain. 2015;138(7):2059-73.
7. Liemburg E, Castelein S, Stewart R, DerGaag MV, Aleman A, Knegtering H. Two subdomains of negative symptoms in psychotic disorders: Established and confirmed in two large cohorts. J Psychiatr Res. 2013;47(6):718-25.
8. World Health Organization (WHO). Schizophrenia. Fact sheet N 397. 2014.
9. Mittal D, Ali A, Md S, Baboota S, Sahni JK, Ali J. Insights into direct nose to brain delivery: Current status and future perspective. Drug Deliv. 2014;21(2):75-86.
10. Pires A, Fortuna AG, Falcao A. Intranasal drug delivery: How, why and what for. J Pharm Pharm Sci. 2009;12(3):288-311.
11. Jain R, Nabar S, Dandekar P, Hassan P, Aswal V, Talmon Y, *et al*. Formulation and evaluation of novel micellar nanocarrier for nasal delivery of sumatriptan. Nanomedicine. 2010;5(54):575-87.
12. Redzic Z. Molecular biology of the blood-brain and the blood cerebrospinal fluid barriers: Similarities and differences. Fluids Barriers CNS. 2011;8(1):1-23.
13. Song Y, Wang Y, Thakur R, Meidan VM, Michniak B. Mucosal drug delivery: Membranes, methodologies and applications. Crit Rev Ther Drug Carrier Syst. 2004;21(3):195-256.
14. Costantino HR, Illum L, Brandt G, Johnson PH, Quay SC. Intranasal delivery: Physicochemical and therapeutic aspects. Int J Pharm. 2007;337(1-2):1-24.
15. Johnson PH, Quay, SC. Advances in nasal drug delivery through tight junction technology. Expert Opin Drug Deliv. 2005;2(2):281-98.
16. Gastaldi L, Battaglia L, Peira E, Chirio D, Muntoni E, Solazzi I, *et al*. Solid lipid nanoparticles as vehicles of drugs to the brain: Current state of the art. Eur J Pharm Biopharm. 2014;87(3):433-44.
17. Battaglia L, Panciani PP, Muntoni E, Capucchio MT, Biasibetti E, DeBonis P, *et al*. Lipid nanoparticles for intranasal administration: Application to nose-to-brain delivery. Expert Opin Drug Deliv. 2018;15(4):369-78.
18. Saha RN, Vasanthakumar S, Bende G, Snehalatha M. Nanoparticulate drug delivery systems for cancer chemotherapy. Mol Mem Biol. 2010;27(7):215-31.
19. Tagami T, Ozeki T. Recent trends in clinical trials related to carrier-based drugs. J Pharm Sci. 2017;106(9):2219-26.
20. Alam MI, Beg S, Samad A, Baboota S, Kohli K, Ali J, *et al*. Strategy for effective brain drug delivery. Eur J Pharm Sci. 2010;40(5):385-403.
21. MuÈller RH, MaÈder K, Gohla S. Solid Lipid Nanoparticles (SLN) for controlled drug delivery–a review of the state of the art. Eur J Pharm Biopharm. 2000;50(1):161-77.
22. Wissing SA, Müller R. Solid lipid nanoparticles as carrier for sunscreens: *In vitro* release and *in vivo* skin penetration. J Control Release. 2002;81(3):225-33.
23. Eskandari S, Varshosaz J, Minaiyan M, Tabbakhian M. Brain delivery of valproic acid via intranasal administration of nanostructured lipid carriers: *In vivo* pharmacodynamic studies using rat electroshock model. Int J Nanomedicine. 2011;6:363-71.
24. Miyamoto S, Duncan GE, Marx CE, Lieberman JA. Treatments for schizophrenia: A critical review of pharmacology and mechanisms of action of antipsychotic drugs. Mol. Psychiatr. 2005;10(1):79-104.
25. Soeiro-de-Souza MG, Dias VV, Missio G, Balanzá-Martinez V, Valiengo L, Carvalho AF, *et al*. Role of quetiapine beyond its clinical efficacy in bipolar disorder: From neuroprotection to the treatment of psychiatric disorders (Review). Exp Ther Med. 2015;9(3):643-52.
26. Ignacio ZM, Reus GZ, Abelaira HM, Maciel AL, DeMoura AB, Matos D, *et al*. Quetiapine fumarate treatment reverses depressive-like behaviour and reduces DNA methyltransferase activity induced by maternal deprivation. Behav Brain Res. 2017;320:225-32.
27. Osborne V, Davies M, Layton D, Shakir SAW. Utilisation ofextended release quetiapine fumarate (Seroquel XLTM): Results from an observational cohort study in England. Eur Psychiatry. 2016;33:61-7.
28. Krishnaraj K, Chandrasekar MJ, Nanjan MJ, Muralidharan S, Manikandan D. Development of sustained release antipsychotic tablets using novel polysaccharide isolated from Delonixregia seeds and its pharmacokinetic studies. Saudi Pharm J. 2012;20(3):239-48.
29. Patil HV, Nithin KS, Sachhidananda S, Chandrashekara KT, Kumar S, Siddaramaiah H. Mycofabrication of bioactive silver nanoparticle: Photo catalysed synthesis and characterization to attest its augmented bio-efficacy. Arab J Chem. 2016.
30. How CW, Abdullah R, Abbasalipourkabir R. Physicochemical properties of nanostructured lipid carriers as colloidal carrier system stabilized with polysorbate 20 and polysorbate 80. Afr J Biotechnol. 2011;10(9):1684-9.
31. Montenegro L, Campisi A, Sarpietro MG, Carbone C, Acquaviva R, Raciti G, *et al*. *In vitro* evaluation of idebenone-loaded solid lipid nanoparticles for drug delivery to the brain. Drug Dev Ind Pharm. 2011;37(6):737-46.
32. Abdelwahab SI, Sheikh BY, Taha MM, How C, Abdullah R, Yagoub U, *et al*. Thymoquinone-loaded nanostructured lipid carriers: Preparation, gastroprotection, *in vitro* toxicity and pharmacokinetic properties after extravascular administration. Int J Nanomedicine. 2013;8:2163-72.
33. Shah B, Khunt D, Bhatt H, Misra M, Padh H. Intranasal delivery of venlafaxine loaded nanostructured lipid carrier: Risk assessment and QbD based optimization. J Drug Deliv Sci Technol. 2016;33:37-50.
34. Malik DS, Kaur G. Nanostructured gel for topical delivery of azelaic acid: Designing, characterization and *in-vitro* evaluation. J Drug Deliv Sci Technol. 2018;47:123-36.
35. Pardeshi CV, Belgamwar VS. Direct nose to brain drug delivery via integrated nerve pathways bypassing the blood–brain barrier: An excellent platform for brain targeting. Expert Opin Drug Deliv. 2013;10(7):957-72.
36. Madane RG, Mahajan HS. Curcumin-loaded Nanostructured Lipid Carriers (NLCs) for nasal administration: Design, characterization and *in vivo* study. Drug Deliv. 2016;23(4):1326-34.
37. Illum L. Nasal drug delivery-possibilities, problems and solutions. J Control Release. 2003; 87(1-3):187-98.
38. Sood S, Jain K, Gowthamarajan K. Optimization of curcumin nanoemulsion for intranasal delivery using design of experiment and its toxicity assessment. Colloids Surf B Biointerfaces. 2014;113:330-7.
39. Shah B, Khunt D, Misra M, Padh H. Non-invasive intranasal delivery of quetiapine fumarate loaded microemulsion for brain targeting: Formulation, physicochemical and pharmacokinetic consideration. Eur J Pharm Sci. 2016;91:196-207.
40. Zhang Q, Jiang X, Jiang W, Lu W, Su L, Shi Z. Preparation of nimodipine-loaded microemulsion for intranasal delivery and evaluation on the targeting efficiency to the brain. Int J Pharm. 2004;275(1-2):85-96.
41. Gabal YM, Kamel AO, Sammour OA, Elshafeey AH. Effect of surface charge on the brain delivery of nanostructured lipid carrier's *in situ* gels via the nasal route. Int J Pharm. 2014; 473(1-2):42-57.
42. Müller RH, Runge S, Ravelli V, Mehnert W, Thünemann AF, Souto EB. Oral bioavailability of cyclosporine: Solid Lipidnanoparticles (SLN) versus drug nanocrystals. Int J Pharm. 2006;317(1):82-9.
43. Khan A, Imam SS, Aqil M, Ahad A, Sultana Y, Ali A, *et al*. Brain targeting of temozolomide via the intranasal route using lipid-based nanoparticles: Brain pharmacokinetic and scintigraphic analyses. Mol Pharm. 2016;13(11):3773-82.
44. Dhuria SV, Hanson LR, Frey II WH. Intranasal delivery to the central nervous system: Mechanisms and experimental considerations. J Pharm Sci. 2010;99(4):1654-73.

PICTORIAL ABSTRACT



About Authors

Mr Praveen Sivadasu, is working as a Research scholar at JSS College of Pharmacy, JSSAHER, Mysuru. His research area is Nasal drug delivery and nanotechnology.

Dr. Devagowda Vishakante Gowda, is working as Professor and Head for Department of Pharmaceutics at JSS College of Pharmacy, JSS Academy for Higher Education and Research, Mysuru. His research area is Tablets, oral vaccines, nano particles, nasal delivery.

Dr. Nithin Kundachira Subramani, is working as Asst. Professor in Chemistry Department at The National Institute of Engineering, Mysuru. His research interest is polymer composites, nano composites and material science.

Ms. Bhavya Malgur Vishweshwaraiah, is working as a Research scholar at JSS College of Pharmacy, JSSAHER, Mysuru. Her research interest is nasal drug delivery, pulmonary drug delivery and nanotechnology.

Mr. Sachhidananda Shivanna, is working as a research scholar at Sri Jayachamarajendra College of Engineering, Mysuru. His research interest is polymer composites, nano composites and material science.

Dr. Siddaramaiah Hatna, is working as Professor and Head for Department of Polymer Science and Technology at Sri Jayachamarajendra College of Engineering, Mysuru. His research interest is polymer composites, nano composites and material science.

SUMMARY

- In this present study an attempt was made to systemically deliver the most desirable schizophrenia drug, quetiapine fumarate (QF) via non-invasive intranasal route using nanostructured lipid carrier (NLC) approach.
- Results obtained from solubility studies suggested that Oleic acid and Gelucire 44/14 are selected as solid lipid and liquid lipid to formulate QF-NLC. From DSC thermograms it was observed that QF was compatible with the selected lipids. Based on ternary phase diagrams obtained 3:1 S_{mix} ratio (Oleic acid and Gelucire 44/14 as the oil phase, Tween 80 and Transcutol-p as surfactant and co-surfactant) was selected for further optimization of QF-NLC.
- Based on the results obtained from the pre-formulation studies further optimization to formulate desired NLCs was done by using central composite design by employing homogenization followed by ultra-sonication technique. Formulated drug loaded NLCs had shown enhanced entrapment efficiency and drug loading with decreased particle size.
- SEM photo-micrograph of QF and QF-NLCs had revealed that particles were spherical in shape and near homogeneous with relatively smoother surfaces. Formulated QF- NLCs had shown a sustained release when compared with pure drug solutions and also achieved higher flux when compared with pure drug with no structural damage to nasal mucosa. *In vivo* bio distribution studies suggested that concentration of QF in brain following IN administration was significantly higher at all-time points. Thereby, indicating potential nose to brain transport when compared with intravenous route.

Cite this article: Sivadasu P, Gowda DV, Nithin KS, Bhavya MV, Sachhidananda S, Siddaramaiah H. Direct Brain Targeted Nanostructured Lipid Carriers for Sustained Release of Schizophrenic Drug: Formulation, Characterization and Pharmacokinetic Studies. Indian J of Pharmaceutical Education and Research. 2020;54(1):73-84.