Original Article

# Development and Characterization of Paliperidone Loaded Nanostructured Lipid Carrier

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## ABSTRACT

**Background:** Paliperidone is indicated for the treatment of schizophrenia. It has an absolute oral bioavailability of about 28% as it is poorly soluble in water and also undergoes hepatic first-pass metabolism. **Objectives:** The purpose of the present study is to improve the solubility, *in vitro* bioavailability of paliperidone by formulating nanostructured lipid carrier (NLC). **Materials and Methods:** High shear homogenization followed by the ultra-sonication technique was used for the preparation of NLCs loaded with Paliperidone, and were prepared by varying weight of solid and liquid lipid in different ratios. Lyophilization of the selected NLC was carried out to further improve its stability. **Results:** Glyceryl monostearate, Linoleic acid, and Tween 80 to Tween 20 (2:1) were selected as solid lipid, liquid lipid, and surfactant correspondingly for the development of NLC. NLC formulation F5 was selected as the best formulation as it exhibited lowest particle size, PDI with stable zeta potential and drug release for 12 hr. Selected NLC F5 was further lyophilized to improve the stability using different cryoprotectants *vis-à-vis* sucrose, sorbitol and aerosol. Lyophilized NLC showed a slight increase in particle size. DSC and P-XRD study revealed molecular dispersion of paliperidone in lipid matrix. **Conclusion:** Hence the approach of formulating lyophilized NLC for paliperidone has the potential of improving *in-vitro* bioavailability.

**Keywords:** High shear homogenization, Ultrasonication, Lyophilization, Bioavailability, Stability.

## INTRODUCTION

Even though the recent scenario of the Pharmaceutical field seems to be highly perceptive owing to the development of new technologies and well-advanced instruments for the profit of civilization, it still fails to circumvent the troubles allied with BCS class II drugs such as poor aqueous solubility, and low permeability. That's why there is exigency to focus on other carriers as a substitute for the conventional dosage form to evade such drugs' interrelated difficulties.[1] Right now, lipid nanoparticles have a wide range of applicability in the therapeutic field as an alternative drug carrier. Lipid nanoparticles are lipid-oriented drug delivery systems and act as a carrier for the delivery of the loaded drug to accomplish curiosity such as enhancement of solubility as well as bioavailability of pharmaceuticals that have low solubility in water.[2]

Amongst lipid nanoparticles, NLCs are trendier because of their convinced benefits in contrast to other lipid nanoparticles as well as conventional lipid-oriented dosage forms.[3] NLCs (2nd generation of SLNs) are proficient to prevail over the limits such as low entrapment efficiency, seepage of drug from formulations during storage, etc., associated with SLNs.[4] NLCs are made up of a specially blended binary mixture of long-chain solid lipid with short-chain liquid lipid are regarded to be in the "generally regarded as safe" category and are biodegradable as well as biocompatible in nature. For fabrication of NLCs, the proportion of solid lipid and liquid lipid usually vary between70:30 to 99.9:0.1 correspondingly.

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The melting point of the resultant lipid matrix shows a lower melting point as compared to the original solid lipid. Nevertheless, the matrix remains solid at body temperature.[5] SLNs are made up of solid lipid and surfactants (devoid of liquid lipid), while NLCs consist of solid lipid, liquid lipid, and surfactants. Thus we can speak that the only distinction between components of SLNs and NLCs is liquid lipid.[6] NLC acts as a hybrid carrier for drug delivery and has an average particle size of about 10-500 nm.[7] The drug entrapment efficiency of NLC is high due to its highly disordered lipid matrix having many imperfections.[8] Because of nanometric particle size and unique composition of lipid matrix, NLC protects drugs and biologicals from degradation by the pH and various enzymes present in the GIT, enhance the drug solubilization in the intestinal milieu by the formation of micelles and alter the drug transport pathway from the portal system to the lymphatic system, thereby reducing the first-pass metabolism and altering the enterocyte-based drug transport and deposition.[9-11] Paliperidone (PD) is a primary active metabolite product of Risperidone.[12] It is a benzisoxazole derivative. Chemically, it is (RS)-3-{2-[4-(6-fluoro-1,2-benzisoxazol-3-yl) piperidin-1-yl] ethyl}-9-hydroxy-2-methyl-6,7,8,9 tetrahydropyrido [1,2-a] pyrimidin-4-one. It has been accepted for schizophrenia treatment by FDA since 2006. Even if the mechanism of action of paliperidone is not completely well-known, principally it acts as an antagonist of dopamine D (2) and serotonin 5HT (2A) receptors.[13] It is marketed as a racemic mixture.[14] under the brand name 'Invega' by Janssen pharmaceuticals. The primary objective of the current research is to improve the *in-vitro* bioavailability of paliperidone by formulating NLC.

## MATERIALS AND METHODS

### Materials

Pure PD drug was lifted by Lupin pharmaceutical Pvt. Ltd. (Pune, India). Stearic acid (SA) was purchased from Niram chemicals (Mumbai, India). Glyceryl monostearate (GMS) was obtained from Labdhi chemicals, (Ahmedabad, India). Compritol 888 ATO, Precirol ATO 5, Labrasol, Lauroglycol FCC, Labrafac, Labrafil 1944 CS were procured from Gattefosse India Pvt. Ltd. (Mumbai, India). Cremophor RH 40, Soya lecithin, Linoleic acid (LA), Tween 20, Tween 80 were purchased from Himedia Laboratory Pvt. Ltd. (Mumbai, India). Glycerol, PEG 300, PEG 400, PEG 600 were obtained from Merck life science Pvt. Ltd. (Mumbai, India). Capmul MCM, Captex 355, Captex 200 P were procured from Abitec Corporation (Columbus, USA). Poloxamer 188 and Polaxomer 407 were obtained from Transchem Corporation (Mumbai, India).

### Methods

#### *Screening of lipid components*

Choice of appropriate lipid (solid lipid and liquid lipid) as well as surfactant(s) for NLC formulation is the most critical step for designing NLC.[15] Because of lack of specific regulatory guidelines for lipid components selection, drug solubility in different lipid components has been considered as the most appropriate method till now.[16]

### Screening of Solid Lipids

Due to the solid nature of lipids equilibrium solubility technique is not a viable process for solid lipid selection.[17] Thus, a different strategy for selecting solid lipid for NLC formation was used.[18] The solubility of PD in four different solid lipids (SA, GMS, Compritol 888 ATO, Precirol ATO 5) was determined by adding PD to the molten state of lipids in increment order of 1 mg at each time till its saturation.[19] The lipids were heated above 5-10°C than their corresponding melting point. Ultimately, the total amount of drugs that could be dissolved in solid lipids was determined. The experiment was triplicated.

### Screening of liquid lipids and surfactants

To identify the best liquid lipid (oil) and surfactant system for NLC development, an equilibrium solubility technique was followed.[17] Excess quantity of the drug was added to 1 ml of each oil and surfactant component contained in a centrifuge tube (2 ml). The mixture was then vortexed and kept in the rotary shaker for 72 hr to equilibrate at 25°C. The centrifugation was carried out at 3000 rpm for 15 min at equilibrium followed by collection of supernatant by passing through a membrane filter of 0.45 µm diameter.[20] Finally, a double beam UV visible spectrophotometer at 271 nm was used for the analysis of PD.

### Selection of Binary Lipid Phase

The degree of miscibility between solid and liquid lipids having the best solubilizing tendency for PD was assessed by mixing them in 90:10, 80:20, 70:30 and 60:40 ratios. By magnetic stirrer (Remi Motors, Mumbai), lipid mixture was agitated at 1200 rpm for an hour at 80°C. After 1 hr, the lipid mixture was cooled and poured into a filter paper. Then the solid mixture was inspected visually for the occurrence of any oil droplets on it. A binary mixture with a melting point above 40°C and no evidence of oil droplets on the filter paper was chosen for NLC development.[21]

## Percentage transmittance study of surfactants

The percentage transmittance of surfactants were carried out by dissolving 100 mg solid and liquid lipid mixture in 3 ml of methylene chloride and followed by drop wise addition with a syringe to 10 mL of 5% of surfactant solution while continuously stirring with a magnetic stirrer operated at a speed of 1200 rpm for 30 min at room temperature. After that, for complete removal of methylene chloride, the above dispersion was kept in a rota evaporator operated at a speed of 50 rpm at 40°C for 30 min. The product was then collected, and 1ml of the collected sample was diluted ten times with milli-Q water. The diluted sample was then scanned at 540 nm with a double beam UV-Visible spectrophotometer,[22] to determine the percentage transmittance value of the surfactant or their combinations.

## Preparation of NLCs

High shear homogenization followed by ultrasonication technique,[23-26] was used for PD-loaded NLC development. In this technique, two phases (lipid and aqueous surfactant solution phase) were prepared independently. At first, to evade recrystallization of lipid during NLC development mixture of solid and liquid lipid was overheated 5-10°C more compared to the melting point of solid lipid, followed by incorporation of PD into that mixture. At the same time, an aqueous surfactant solution of Tween 80 to Tween 20 in 2:1 ratio was prepared at the same temperature as that of the lipid phase and stirred uninterruptedly for 15 min at 2400 rpm. Later, the drug contained lipid phase was added to the hydrophilic surfactant solution and homogenized for 30 min at 12000 rpm by high shear homogenizer (Ika, Germany), which yields primary emulsion (micro-emulsion). The primary emulsion thus formed was ultrasonicated by using a probe sonicator (Orchid scientific and Innovative India Pvt. Ltd., Mumbai) at a voltage of 220v with 50HZ frequency for 2 min. In order to get the dispersion phase, the dispersion was then cooled to room temperature, resulting in the formation of NLCs.

## Freeze drying of optimized NLC formulation

The selected liquid NLC was mixed with sucrose, sorbitol, and aerosil in concentrations of 3% w/v, 5% w/v, 7% w /v.[27] The sample was subjected to deep freezing near -20°C temperature for a period of 24 hr. The frozen NLC dispersion was subjected to lyophilization at a temperature of -50°C and 0.002 mbar vacuum for two days using Alpha 1 Martin Christ Lyophilizer, Germany, to get the lyophilized product.

## *In vitro* evaluation of Paliperidone loaded NLC and lyophilized formulation

### *Entrapment efficiency (EE)*

EE of NLC indicates the fraction of drug that has been entrapped in the core of the lipid matrix. Percentage entrapment of drug in NLC formulations was determined by high-speed cooling centrifugation at 6000 rpm for 20 min. at 4°C. The free drug present in the supernatant was quantified using a double beam UV-Visible Spectrophotometer,[26] at 271 nm and the percentage entrapment efficiency was calculated by following Eq$^{n}$ (1).[28]

$$EE(\%\ w/v) = \frac{(\text{Drug loaded in NLC}) - (\text{free drug})}{\text{Drug loaded in NLC}} \times 100 \quad \text{Eq}^{n}\ (1)$$

## Particle size and Zeta Potential

Particle size (PS), Poly-dispersibility index (PDI), and Zeta potential (ZP) of the liquid NLC formulation and lyophilized NLC product were determined by Malvern Zetasizer (Nano ZS; Malvern Instruments, Malvern, UK) at room temperature. Measurement of particle size was based on the photon correlation spectroscopic (PCS) technique,[29] while ZP was based on the electrophoretic light scattering technique.[30] Samples were diluted by double distilled water (100 times), filtered through millipore filter paper, and were analyzed.

## Drug Release Kinetics

*In vitro* drug release kinetics of PD-loaded NLC and the lyophilized formulation containing 9 mg equivalent of the PD were performed by dialysis bag technique,[31] using 0.1N HCl for 2 hr, and then with phosphate buffer (pH 6.8) for 10 hr as dissolution media. The formulation was kept inside the dialysis bag with proper sealing at both ends. Thereafter, it was dipped in a 100 ml beaker containing dissolution media maintained at a temperature of 37±0.5°C with continuous stirring at 50 rpm. Samples were collected as per the planned time by following sink conditions. Collected samples were analyzed by double beam UV-Visible spectrophotometer after suitable dilution. The time required for 95% drug release (T95%).[32] was calculated from this experiment.

## Differential scanning calorimeter (DSC)

DSC thermogram of pure PD drug, GMS, physical mixture (PD+ GMS, PD+ GMS+ LA), and lyophilized NLC formulation was recorded using DSC-60 (Shimadzu, Japan). For this experiment, 2 mg of sample was kept in an aluminum pan, then sealed. The sealed pan was heated under nitrogen flow (20 mL/min) at a

scanning rate of 10°C/min from 25 to 250°C by keeping an empty aluminum pan as reference.[33]

### FT-IR Study

FT-IR study of drug PD, GMS, physical mixture (PD+ GMS, PD+ GMS+ LA), and lyophilized NLC were conducted using IR Affinity-1 (Shimadzu, Japan). Over the range of 4000-400 $cm^{-1}$, samples were analysed.[32]

### Scanning Electron Microscopy (SEM)

By using SEM (Hitachi, Tokyo, Japan), the surface texture of pure PD drug and lyophilized NLC formulation was recorded. Initially, the samples adhered to the metallic stub (carbon-coated) afterward Platinum coating. Then under high vacuum conditions, surface analysis of the pre-coated samples was carried out.[34]

### Thermo Gravimetric analysis (TGA)

TGA studies were performed to observe the loss in weight of the sample with the steady change of temperature.[35] By taking 9 mg of pure PD drug and lyophilized NLC formulation (equivalent to 9 mg of pure PD drug), thermal analysis experiments were conducted with the help of the Perkin Elmer Pyris 1 TGA instrument. Under continuous nitrogen flow, samples were heated in-between temperature ranges of 50°-700°C with a usual heating rate of 10°C/min.

### Powder X-ray diffraction (PXRD)

Ultima IV X-ray Diffractometer (Rigaku, Tokyo, Japan) with Cu as a source of radiation was used to investigate the physical states of pure PD drug and its freeze-dried NLC formulation. The diffraction angle (2θ) range was in between 5-80°C with a scanning speed of 5°/min. A voltage of 40 kV and a current of 30 mA were used to measure the XRD pattern.

### Micromeritic properties of lyophilized NLC

#### (a) Carr's Index

Carr's index is a measure of powder's compressibility. Lower the value of Carr's index better is the flow property of the powder and vice-versa. It was calculated by using the Eq$^{n}$ (2).[36]

$$\text{Carr's index} = \frac{\text{Tapped density} - \text{bulk density}}{\text{Tapped density}} \times 100 \qquad \text{Eq}^{n}\ (2)$$

#### (b) Hausner's ratio

Interparticulate friction can be quantified by Hausner's ratio. It was calculated by using the Eq$^{n}$ (3).[37]

$$\text{Hausner's ratio} = \frac{\text{Tapped density}}{\text{Bulk density}} \qquad \text{Eq}^{n}\ (3)$$

### *Stability Study of optimized NLC and lyophilized NLC*

A six month accelerated stability study was conducted for both the optimized NLC and lyophilized NLC according to ICH guideline in order to inspect any significant change that would result in various evaluation parameters during storage and transport. The entire study was conducted at 40°C and 75% RH.

## RESULTS AND DISCUSSION

### Screening of Solid Lipids

The solubility study of PD was performed in the following lipids i.e., SA, GMS, Compritol, and Precirol (Figure 1) by equilibrium solubility technique. PD exhibited the highest solubility in GMS (8 mg), and hence it was selected as solid lipid. The solubility of the drug in lipid plays a vital role in drug loading capacity and entrapment efficiency. Higher solubility of PD in GMS can be attributed to the chemical nature of the GMS.

### Screening of liquid lipids and surfactants

The solubility of PD in a series of liquid lipids (Figure 1) revealed the highest solubility in LA (13.42 mg/ml). However, PD also showed solubility of more than 10 mg/ml in Labrasol and PEG-300. As LA showed the best solubilizing property for PD, it was selected as liquid lipid. Solubility study of PD in different surfactants (Figure 1) showed maximum solubility in Tween 80 followed by Tween 20.

### Selection of binary phase

The selected solid lipid (GMS) and liquid lipid (LA) were assorted at altered ratios to decide the best composition of binary mixture for the NLC formulation. All the four binary mixtures of solid and liquid lipid showed the absence of oil droplets on the filter paper (Table 1).

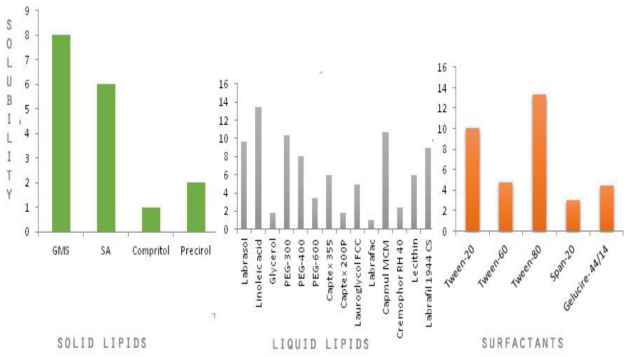


**Figure 1: Solubility of PD in different solid lipids, liquid lipids and surfactants.**

## Percentage transmittance study of surfactants

Surfactant combination shows better emulsification property and also affects particle size of the NLC formulation. Percentage transmittance study of Tween 80 and Tween 20 alone and in combination (1:1, 2:1, 3:1 respectively) were performed, and the results have been shown in (Table 2). This study confirmed that transmittance percentage was highest when the surfactant mixture was taken in a 2:1 ratio.

## Preparation of NLCs

PD-encapsulated NLCs were formulated by high shear homogenization and Ultrasonication technique. A total of 12 formulations (Table 3) were prepared by varying the proportion of solid lipid and liquid lipid. The formulations were manufactured at three different quantities of total lipid, i.e., 500, 750, and 1000 mg, with a fixed quantity of drug, i.e., 9 mg. To improve the stability of NLC, formulation $F_5$ was lyophilized using different cryoprotectants such as sucrose, sorbitol, and aerosil at three different concentration levels (i.e., 3, 5, 7%).

## Characterization of NLC

### *Entrapment efficiency*

The EE of the formulations $F_1$ to $F_4$ was less than 60%, whereas it was relatively higher for $F_5$ to $F_8$ and still higher for $F_9$ to $F_{12}$ (Table 4). This can be attributed to an increase in the total amount of lipid available for dissolving/dispersing the drug. It was observed that increased concentration of solid lipid (GMS) results in higher EE. This can be attributed to the chemical nature of GMS. The entrapment efficiency for the lyophilized formulation was nearly the same as that $F_5$ formulation (Table 5). Hence, it can be said that lyophilization did not have any destruction of the structure during the dehydration process.

**Table 1: Selection of binary lipid phase.**

| Sl. No. | Lipid combination | Solid lipid: Liquid lipid (GMS:LA) | Visualization of oil droplet |
|---|---|---|---|
| 1 | GMS with Linoleic acid | 90:10 | No |
| 2 | GMS with Linoleic acid | 80:20 | No |
| 3 | GMS with Linoleic acid | 70:30 | No |
| 4 | GMS with Linoleic acid | 60:40 | No |

**Table 2: Percentage transmittance study of surfactant (s).**

| Surfactant | % TRANSMITTANCE |
|---|---|
| Tween-20 | 55.30 ±1.3 |
| Tween-80 | 68.35±2.1 |
| **Surfactant combination** | **% TRANSMITTANCE** |
| Tween 80 : Tween 20 (1:1) | 56.42±1.7 |
| Tween 80 : Tween 20 (2:1) | 79.63±1.1 |
| Tween 80 : Tween 20 (3:1) | 70.2 ± 2.4 |

**Table 3: Formulation composition of NLC.**

| Formulations | Total amount of lipid (mg) | Amount of drug (mg) | Amount of Stearic acid(mg) | Amount of Linoleic acid (mg) | Tween-80: Tween-20 (0.5%v/v) 2 : 1 |
|---|---|---|---|---|---|
| F1 | 500 (6:4) | 50 | 300 | 200 | 10 mL |
| F2 | 500 (7:3) | 50 | 350 | 150 | 10 mL |
| F3 | 500 (8:2) | 50 | 400 | 100 | 10 mL |
| F4 | 500 (9:1) | 50 | 450 | 50 | 10 mL |
| F5 | 750 (6:4) | 50 | 450 | 300 | 10 mL |
| F6 | 750 (7:3) | 50 | 525 | 225 | 10 mL |
| F7 | 750 (8:2) | 50 | 600 | 150 | 10 mL |
| F8 | 750 (9:1) | 50 | 675 | 75 | 10 mL |
| F9 | 1000 (6:4) | 50 | 600 | 400 | 10 mL |
| F10 | 1000 (7:3) | 50 | 700 | 300 | 10 mL |
| F11 | 1000 (8:2) | 50 | 800 | 200 | 10 mL |
| F12 | 1000 (9:1) | 50 | 900 | 100 | 10 mL |

**Table 4: Characterization of NLCs.**

| Formulations | Entrapment efficiency (%) | Particle size (nm) | PDI | Zeta potential | Time required for 95% of drug release, T95% (h) |
|---|---|---|---|---|---|
| $F_1$ | 57.7 ±1.8 | 296.2 ± 1.7 | 0.321 | - 25.5 | 6.5 ± 0.12 |
| $F_2$ | 58.8 ± 2.3 | 332.5 ± 2.6 | 0.234 | - 21.7 | 7.2 ± 0.22 |
| $F_3$ | 53.4 ±1.5 | 367.2 ± 1.4 | 0.345 | -22.5 | 8.7 ± 0.35 |
| $F_4$ | 59.6 ±2.6 | 438.5 ± 1.8 | 0.312 | -21.6 | 9.6 ± 0.52 |
| $F_5$ | 69.3 ±1.9 | 173.4 ± 0.7 | 0.123 | - 26.7 | 11.7 ± 0.72 |
| $F_6$ | 65.5 ±2.2 | 183.2 ± 3.1 | 0.342 | -23.5 | 12.8 ± 0.25 |
| $F_7$ | 76.1 ±2.5 | 189.4 ± 1.6 | 0.412 | -24.6 | 13.8 ± 0.75 |
| $F_8$ | 78.7 ±2.1 | 194.5 ± 1.3 | 0.253 | -23.5 | 14.5 ± 0.39 |
| $F_9$ | 72.6 ±2.4 | 445.5± 1.9 | 0.325 | -22.7 | 15.6 ± 0.51 |
| F10 | 78.3 ±1.4 | 476.8 ± 2.8 | 0.254 | -24.5 | 16.2 ± 0.85 |
| F11 | 79.8 ±1.1 | 489.3 ± 3.3 | 0.315 | - 32.2 | 17.7 ± 0.24 |
| F12 | 77.9 ±2.7 | 560.2 ± 3.7 | 0.424 | -24.6 | 18.5 ± 1.23 |

Mean± SD, $n = 3$.

**Table 5: Evaluation of lyophilized product of optimized formulation.**

| Sl. No | Evaluation parameter | $F_5$ | Lyophilized NLC |
|---|---|---|---|
| 1 | Carr's index | * | 15 ± 0.6 |
| 2 | Hausner's ratio | * | 1.06 ± 0.2 |
| 4 | Particle size (nm) | 173.4 ± 0.7 | 186.5 ± 1.1 |
| 5 | Zeta potential (mV) | - 26.7 | -28.4 |
| 6 | PDI | 0.123 | 0.294 |
| 7 | Entrapment efficiency (%) | 69.3 ± 1.9 | 67 ± 1.6 |
| 8 | % drug Diffusion<br>At 2 h<br>8 h<br>12 h | <br>26.32 ± 2.3<br>77.87 ± 1.5<br>98.35 ± 1.8 | <br>24.43 ± 0.9<br>74.85 ± 2.3<br>98.15 ± 1.2 |

Mean ± SD, *n* = 3, *Could not be determined as it was a liquid formulation.

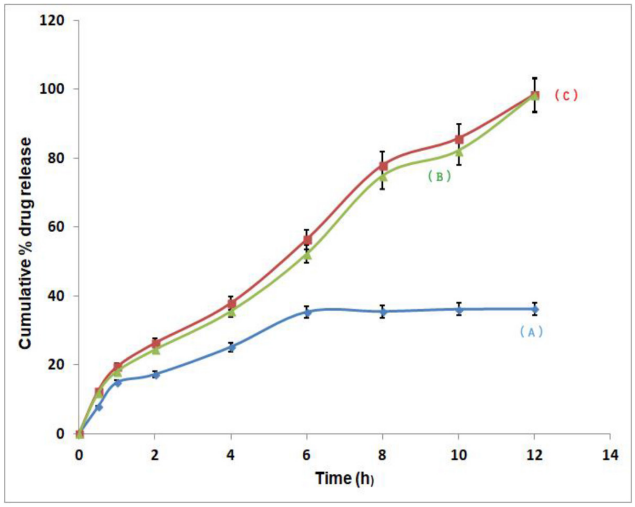


**Figure 2: Cumulative % drug release study of pure drug, optimized NLC and lyophilised NLC.**

## Particle size, Zeta potential, and PDI

PS of the prepared NLC formulations is presented as mean hydrodynamic diameter. The NLCs showed particle size in the range of 173 to 560 nm (Table 4). With the increase in the proportion of lipid, an increment in size of the particle was noticed. The mixture of surfactants in the ratio of 2:1 was found to be optimum for stabilizing the NLC by creating a steric barrier on the NC surface and also helpful to prevent agglomeration. Probe Sonication is also responsible for reducing the particle size due to the Sonication energy. The particle size of the lyophilized $F_5$ formulation almost increased by two times compared to its liquid NLC formulation (Table 5). This may be due to the particle aggregation during the freeze-drying process.[38]

PDI indicates the homogeneity of the prepared NLC formulations. It plays a pro-vital role in defining the stability of NLC. A lower PDI value indicates the particles are homogeneous with narrow size distribution. The PDI value of all liquid NLC formulations along with lyophilized formulation was within the range of 0.123 to 0.424 (Table 4 and 5), suggesting homogeneous and narrow size distribution, which can be attributed to the optimum selection of surfactant and Sonication time.

ZP of all the NLCs and in the lyophilized formulation was within the range of -21.7 to -32.2 mv (Table 4 and 5). Higher ZP for all formulations guarantees the physical stability of NLCs.

## Drug Release Studies

The *in-vitro* drug release studies (Table 4) for NLCs suggest that formulations $F_6$ to $F_{12}$ exhibited very slow drug release with T95% more than 12 hr, whereas $F_1$ to $F_4$ showed nearly 95% of drug within 10 hr only. Only

**Table 6: Comparison of correlation coefficient of optimized formulation and pure drug.**

| Formulation | Correlation coefficient | | | Slope |
|---|---|---|---|---|
| | Zero order | First order | Higuchi | Korsmeyer and Peppa's Release exponent |
| $F_5$ | 0.943 | 0.921 | 0.977 | 0.806 |
| Lyophilized NLC | 0.991 | 0.939 | 0.987 | 0.828 |

$F_5$ formulation exhibit nearly 12 hr of T95%. Figure 2 represents cumulative % drug release profile of pure drug, optimized NLC and lyophilised NLC.

$F_5$ formulation was selected as the best formulation as its particle size was lowest (173.4 nm) with low PDI (0.123) and relatively stable ZP (- 26.7 mv). Both lyophilized NLC formulation optimized NLC formulation (Table 5) showed a similar diffusion profile.

Table 6 illustrates a comparison of correlation coefficient values for the dissolution profiles plugged into various release models between optimized NLC i.e. $F_5$ and lyophilized NLC of F5 by different drug release model. The drug release kinetics is fallowing diffusion as correlation coefficient is highest for higuchi model. The value of Korsmeyer and Peppa's release exponent suggest a non-fickian transport mechanism of drug release for both optimized NLC and lyophilized NLC.

## Differential scanning calorimeter

DSC thermogram of pure PD drug, GMS, physical mixture (PD+ GMS, PD+ GMS+ LA), and optimized lyophilized NLC formulation are presented in (Figure 3).

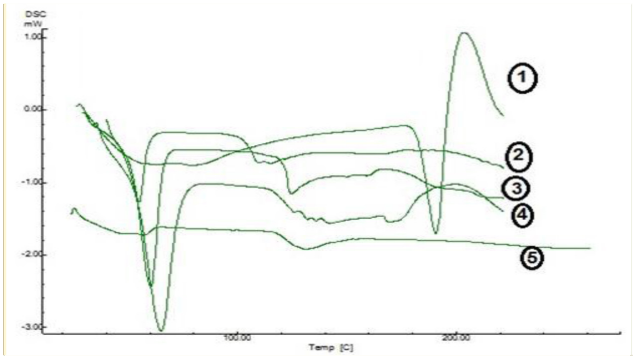

**Figure 3: Overlay DSC thermogram of 1-paliperidone pure drug, 2-Physical mixture of paliperidone and GMS, 3-GMS, 4-Physical mixture of paliperidone, GMS and LA, 5-Lyophylized NLC.**

The DSC thermogram of Paliperidone exhibited a sharp endothermic peak at 190.7°C which is close to the reported melting point of pure paliparidone (179.8°C) with a narrow range of onset and endset temperatures of 182.7°C and 197.5°C, respectively. This indicates the crystalline nature of PD. The DSC thermogram of GMS revealed a sharp endothermic peak at 60.3°C with the onset and endset temperature of 52.2°C and 65.5°C, respectively. DSC thermogram of the physical mixture (PD+ GMS, PD+ LA) and freeze-dried optimized NLC formulation did not exhibit any peak in the melting point range of PD. The absence of a peak for the drug can be attributed to the molecular level of dispersion of the drug losing its crystalline nature.

## FT-IR Study

FT-IR study of drug PD shows absorption spectra at 1050 $cm^{-1}$ for –OH, 1750 $cm^{-1}$ for–CO (ketone), 1338 $cm^{-1}$ for $–CH_3$, 1336 $cm^{-1}$ for $–CH_2$, 3070 $cm^{-1}$ for Aromatic hydrogen (Ar-H), 1230 $cm^{-1}$ for –CF, and 3365 $cm^{-1}$ for –NH bonds. FT-IR study of GMS shows absorption spectra at 1050 $cm^{-1}$for –OH, 1750 $cm^{-1}$ for —CO (ketone), and 1300 $cm^{-1}$for –C-O bonds. The physical mixtures different carriers and lyophilized NLC formulation shows similar absorption spectra. It showed the compatibility between PD with different carriers. The overlaid FT-IR spectrum is shown in (Figure 4).

## Scanning Electron Microscopy

Surface morphology and shape of drug PD and lyophilized NLC formulation were revealed from the SEM study. SEM of pure PD drug Figure 5 (A) showed its crystallinity nature, whereas SEM of lyophilized NLC formulation Figure 5 (B) revealed its porous and smooth surface.

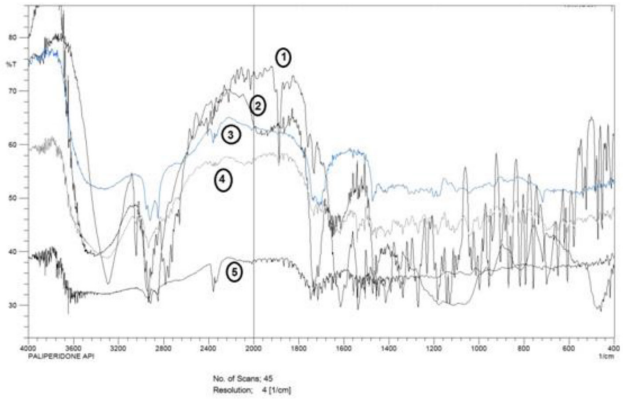

**Figure 4: Overlay FTIR spectra of 1-paliperidone pure drug, 2-physical mixture of paliperidone and GMS, 3-GMS, 4-Physical mixture of paliperidone, GMS and LA, 5-lyophylized NLC.**

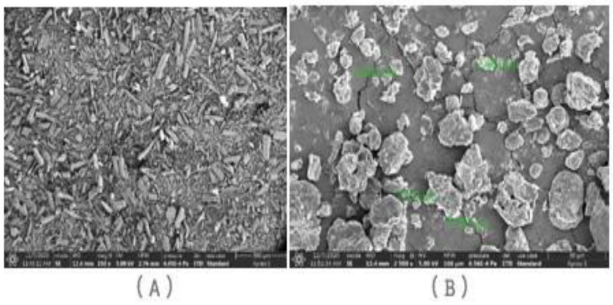


**Figure 5: SEM of (A) pure PD drug and (B) lyophilized product.**

## Thermogravimetric Analysis

Figure 6 (a) shows constant weight within 380°C with an overall weight loss of 56.52%. Whereas lyophilized NLC Figure 6 (b) showed a weight loss of 79.68%. The initial weight loss can be due to loss of moisture, and further weight loss was due to the decomposition.

## Powder-XRD

P-XRD of drug PD Figure 7 (a) exhibited sharp peaks at diffraction angle (2θ) of 10.39, 13.85, 14.60, 20.74, 22.18, 24.71, 24.80, 25.16, and 28.04 degrees. These indicate the crystalline nature of PD. In the case of lyophilized NLC Figure 7 (b), only two distinct peaks at 20.12 and 23.87 degrees, whereas all other peaks were absent. This can be attributed to partial amorphization or molecular dispersion of PD in solid lipid.

## Evaluation of Micromeritic Properties

Carr's index and Hausner's ratio of the lyophilized formulation were determined as per the standard procedure (Carr's index 12-16 and Haussner's ratio less than 1.25 are considered as good flow) In the case of sucrose and sorbitol as a cryoprotectant, the obtained

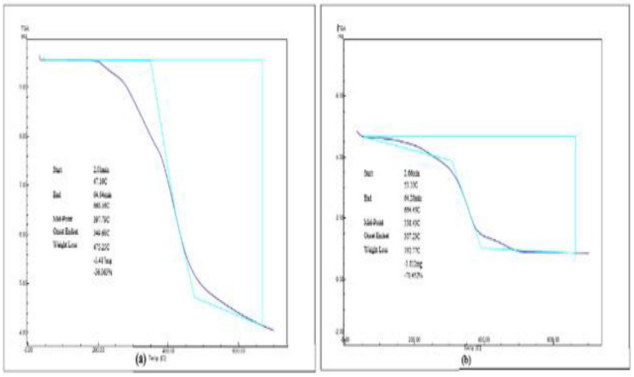

Figure 6: TGA of (a) pure PD drug and (b) lyophilized product.

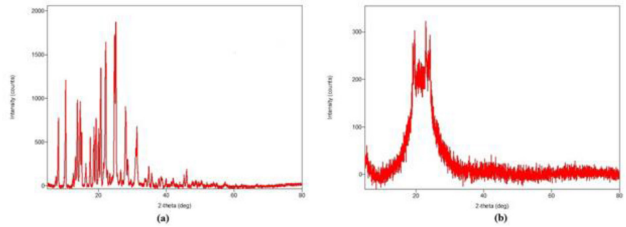

Figure 7: P-XRD of (a) pure PD drug and (b) lyophilized product.

**Table 7: Freeze-dried product formulation table.**

| Sl. No | Matrix former | Concentration (% w/v) | Observation |
|---|---|---|---|
| 1 | Sucrose | 3 | Sticky powder |
| 2 | Sucrose | 5 | Sticky powder |
| 3 | Sucrose | 7 | Free-flowing powder |
| 4 | Sorbitol | 3 | Sticky powder |
| 5 | Sorbitol | 5 | Sticky powder |
| 6 | Sorbitol | 7 | Free-flowing powder |
| 7 | Aerosil | 3 | Free-flowing powder |
| 8 | Aerosil | 5 | Free-flowing powder |
| 9 | Aerosil | 7 | Free-flowing powder |

lyophilized NLC was sticky and did not exhibit good flowability with 3 and 5% w/v of cryoprotectant whereas, with 7% w/v of cryoprotectant, the lyophilized NLC exhibited desirable flowability (Table 7). At all the three concentration levels of aerosil i.e., 3, 5, and 7% w/v. Keeping in view to use minimum quantity of cryoprotectant, we have selected 3% w/v of cryoprotectant for further study.

### Stability Study of optimized NLC and lyophilized NLC

The sample of optimized NLC and lyophilized NLC were tested at 0, 3 and 6 months of stability study period for various evaluation parameters. Table 8 shows the various study parameters value at different time interval. The result infers that during the study period there is significant change occurs in the value of particle size and zeta potential in case of optimized NLC. While in case of lyophilized NLC there is no significant change occur for the same parameter. Hence on the stability point of view lyophilized NLC is superior in comparison to optimized NLC.

**Table 8: Accelerated stability study of optimized formulation.**

| Time (month) | $F_5$ | | | Lyophilized NLC | | |
|---|---|---|---|---|---|---|
| | Particle Size (nm) | Zeta Potential (mV) | Entrapment efficiency (%) | Particle Size (nm) | Zeta Potential (mV) | Entrapment efficiency (%) |
| 0 | 173.4 | - 26.7 | 69.3 | 186.5 | -28.4 | 67 |
| 3 | 226.7 | - 20.1 | 68.5 | 188.6 | -27.2 | 66.8 |
| 6 | 289.5 | - 18.5 | 68.9 | 187.4 | -28.8 | 68.5 |

## CONCLUSION

Currently, NLC plays a provital role in the therapeutic field owing to its numerous paybacks. PD-encapsulated NLCs were formulated by high shear homogenization and Ultrasonication method by taking solid lipid, liquid lipid, and surfactants in different proportions. The lipid matrix formed by blending solid and liquid lipid was solely responsible for the higher entrapment efficiency of the NLC formulations. A diffusion study revealed a biphasic pattern of drug release in a controlled manner. Optimized NLC formulation and its lyophilized product were relatively stable, as confirmed from the ZP value. Obtained results were encouraging to formulate PD - loaded NLC. However, further *in vivo* studies are necessary to get an idea of the safety and efficacy profile of PD – loaded NLC.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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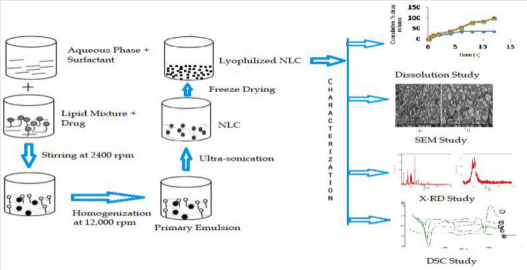
## PICTORIAL ABSTRACT




## SUMMARY

Nano structured lipid carriers (NLC) were prepared for paliperidone to improve its oral bioavailability. High shear homogenization followed by the ultra-sonication technique was used for the preparation of NLCs. Glyceryl monostearate, Linoleic acid, and Tween 80 to Tween 20 (2:1) were selected as solid lipid, liquid lipid, and surfactant correspondingly for the development of NLC. NLC formulation F5 was selected as the best formulation as it exhibited lowest particle size, PDI with stable zeta potential and drug release for 12 h. Selected NLC F5 was further lyophilized to improve the stability using different cryoprotectants *vis-à-vis* sucrose, sorbitol and aerosol. Lyophilized NLC showed a slight increase in particle size. DSC and P-XRD study revealed molecular dispersion of paliperidone in lipid matrix. Hence the approach of formulating lyophilized NLC for paliperidone has the potential of improving In-vitro bioavailability.

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