RESEARCH PAPER

Formulation of Nateglinide-Loaded Solid Lipid Nanoparticles: An iv-vitro and Pharmacokinetic with Pharmacodynamic Evaluation

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ABSTRACT

Background: Nateglinide, a postprandial glucose lowering medication in type 2 diabetes, exhibits low oral bioavailability (50-60) and extremely short half -life (1.5-1.9h), requiring patients to take thrice per day. Our goal was to establish a once-daily solid lipid nanoparticle (SLN) formulation that would enhance drug delivery and maintain glucose reduction. **Methods:** A face-centered central composite design was used to prepare nateglinide-loaded SLNs with Geleol as lipid, Poloxamer 188 as a surfactant and Phospholipoid 90G as stabilizer. The optimized batch (NSLN19) was defined in terms of particle size, zeta potential, entrapment efficiency, drug loading, in-vitro release and stability. The pharmacokinetics and pharmacodynamics of in-vivo were tested on alloxan-induced diabetic rats. **Results:** NSLN19 showed a particle size of 255.3 ± 2.0 nm, entrapment efficiency of 94.6 ± 1.2%, and drug loading of 92.2 ± 1.3%. In-vitro release was sustained (58.4 ± 1.8% at 24 h). The SLN formulation increased the half-life of nateglinide (1.9 ± 0.3h) to 3.8 ± 0.5h, T_{max} to 3.2 ± 0.4 h, and the blood glucose level was not above 200 mg/dl after more than 18 h, compared with pure nateglinide. The formulation was kept at 25°C ± 2°C / 60% ± 5% RH & 4°C ± 2°C for stability and was found to be stable for six months. **Conclusion:** The solid lipid nanoparticles with nateglinide have the potential to provide a better once-a-day oral option with improved bioavailability, prolonged glycemic regulation, and excellent stability that can better patient compliance.

Keywords: Controlled release; Diabetes mellitus; Entrapment efficiency; Nateglinide; Pharmacokinetics; Solid lipid nanoparticles; Stability

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INTRODUCTION

Nateglinide was selected as it suits the requirements of postprandial glucose in type 2 diabetes since it is rapid, and it exits the body soon, therefore, reducing the chances of late-night hypoglycemia. However, there are two big flaws with the drug. First, it is insoluble in water, and of an oral dose only about half to sixty per cent. of the dose is absorbed by the blood. Second, it has a half-life of about 1.5-1.9 hours, and therefore patients must take three times a day, immediately before each main meal. Even at that time, a significant portion of the dose is lost as a result of incomplete absorption and first pass metabolism [1-2]. This was a real opportunity we saw. Since nateglinide is mainly absorbed in the upper small intestine, a lipid-based nanoparticle has the potential to retain it longer and increase its absorption. That prompted us to solid lipid nanoparticles (SLNs) small, fat-based nanoparticles that shield the drug against enzymes, assist the drug penetrate the intestinal wall, and release it gradually. They also are safe and well tolerated [3].

The primary objective of our research was to achieve a high drug loading and entrapment of nateglinide loaded SLNs. We also desired to determine the effects of the levels of lipid, surfactant, and stabilizer on the end product. We prepared twenty batches of different entrapment and content of the drug and measured them using a face centered central composite design. The optimal formula provided us with a daily dose of nateglinide one that had the potential to enhance patient compliance and offer a more precise control of blood sugar levels. This paper takes us through our preparation, optimization and our important results.

MATERIALS AND METHODS

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Nateglinide was acquired under the Yarrowchem, Mumbai. The lipid (core matrix) was Geleol which was purchased at Gattefosse India Pvt. Ltd., Mumbai. The surfactant used was Poloxamer 188, which was purchased in BASF India Limited. Phospholipid 90G was generously donated to Lipoid, Germany, as a gift sample and used as a critical stabilizing agent. The other chemicals and reagents that were used in the study were all of an analytical grade and were not refined.

Standard Calibration Curve:

An 1mg/mL stock solution of nateglinide was made by dissolving the 10mg of the drug in 10mL of methanol. The stock solution was diluted (with the mobile phase) to produce working standard solutions with concentrations of 5-50 ug/mL (5, 10, 15, 20, 30, 40, 50 ug/mL). HPLC was run on a C18 column (250 x 4.6 x 5 mm) at ambient temperature and run at 1.0 ml/min, injection volume 20 µL and UV detection at 210 nm. The triplicate injections of each standard solution were made, and the average peak area was taken. The mean peak area verses the concentration formed the calibration curve[4].

Drug Excipients Interaction Study:

In the case of FTIR, pure nateglinide, Geleol, Poloxamer 188 and phospholipoid 90G were milled with KBr and pressed into discs. Spectra were measured between 4000 and 400cm minus to identify any shifting or widening of any peak that could signify any interactions. In the case of DSC, 3-5 mg of samples were contained in aluminium pans and heated between 25°C and 220°C in the presence of nitrogen. The analysis of thermograms was based on the changes in melting behaviour in the absence of new or relocated endotherms confirmed compatibility between drug and excipients [5].

Method of Preparation:

To create nateglinide loaded solid lipid nanoparticles, the first step is to heat Geleol (the lipid) at 120mg of the drug up to approximately 70°C. The temperature 5-10°C was kept above the melting point of the lipid until the molten mixture is clear. Individually, distilled water was brought up to the same temperature and Poloxamer 188 (surfactant) and Phospholipoid 90G (stabilizer) was dissolved in it. The resulting hot aqueous phase was added to the lipid drug blend and mixed instantly at 20, 000 rpm in five minutes to form a coarse emulsion. The mixture was then probe sonicated at 40 percent amplitude and sonicated over a period of 10 minutes (pulse cycle of four seconds on and two seconds off) in an ice water bath. Nano emulsion was quickly cooled to 4°C with stirring to solidify the nanoparticles and then lyophilized and stored in amber vials at 4°C.[6-7].

Table 1: Variables used to Prepare Solid Lipid Nanoparticles

Variable	Low (-1)	Medium (0)	High (+1)
Primary Lipid (%)	1.0 % w/v	2.0 % w/v	3.0 % w/v
Surfactant (%)	0.5 % w/v	1.0 % w/v	1.5 % w/v
Stabilizer (%)	0.1 % w/v	0.2 % w/v	0.3 % w/v

Table 2: Formulation Chart with Dependent and Independent Factors

Batch	Primary Lipid (%)	Surfactant (%)	Stabilizer (%)	Particle Size (nm)	Entrapment Efficiency (%)
NSLN1	-1	-1	-1	312.5 ± 4.2	68.2 ± 2.1
NSLN2	0	0	0	254.8 ± 1.5	84.6 ± 1.1
NSLN3	1	0	0	268.4 ± 2.1	87.3 ± 1.3
NSLN4	0	0	0	259.2 ± 1.3	85.1 ± 0.9
NSLN5	0	0	0	255.5 ± 1.6	84.9 ± 1.0
NSLN6	0	-1	0	278.6 ± 2.8	78.5 ± 1.5
NSLN7	0	0	0	254.9 ± 1.4	85.3 ± 0.8
NSLN8	0	1	0	235.2 ± 2.0	89.4 ± 1.2
NSLN9	-1	0	0	243.7 ± 2.3	80.2 ± 1.6
NSLN10	0	0	1	240.5 ± 2.2	88.6 ± 1.3
NSLN11	0	0	0	275.0 ± 1.4	84.2 ± 1.0
NSLN12	1	-1	-1	328.9 ± 5.1	72.5 ± 2.0
NSLN13	0	0	-1	268.3 ± 2.5	81.3 ± 1.4
NSLN14	-1	-1	1	285.4 ± 3.0	76.8 ± 1.8
NSLN15	0	0	0	285.1 ± 1.2	84.5 ± 1.1

NSLN16	-1	1	-1	225.6 ± 2.4	83.4 ± 1.5
NSLN17	1	1	-1	275.3 ± 3.1	90.2 ± 1.4
NSLN18	1	-1	1	305.7 ± 4.0	82.7 ± 1.7
NSLN19	1	1	1	255.3 ± 2.0	94.6 ± 1.2
NSLN20	-1	1	1	218.5 ± 2.1	86.9 ± 1.5

Evaluation Parameters:

Particle Size and Polydispersity Index (PDI):

To get the optimal scattering intensity, 1:10 to 1:100 of the SLN dispersion was diluted with distilled water, and 1 mL of the diluted sample was pipetted into a clean polystyrene cuvette. The cuvette was put in a DLS apparatus (e.g. Malvern Zetasizer) and equilibrated at 25 0 C and measurements were taken at a backscatter angle of 173°. All samples were run three times and the size of the particles was measured as the Z average diameter (nm), and the size distribution breadth was measured as the PDI [8].

Zeta Potential (ZP):

To prevent multiple scattering, the SLN dispersion was diluted 1:10-1:50 with 1mM NaCl solution (pH = 7.0), and the diluted sample was filled into a disposable folded capillary cell, which was free of air bubbles. The cell was placed in the DLS instrument with the capability of zeta potential and an electric field of ±150 mV was applied [9]. Electrophoretic mobility was determined at 25°C and repeated 3-5 times and the average zeta potential (mV) value taken as a measure of particle surface charge and physical stability.

Entrapment Efficiency (EE) and Drug Loading (DL):

In the case of EE, the dispersion of SLN was centrifuged (15000-20000 rpm, for 30-60 min, at 4°C) and the supernatant of free nateglinide was collected, diluted in the mobile phase and the mixture was subjected to HPLC (C18 column, 210 nm, acetonitrile:0.025 EE% was determined as: (total drug - free drug)/total drug x 100. In the case of DL%, the pellet was washed with distilled water twice, followed by lyophilization and weighing of the pellet, then the drug was dissolved in a known mass of lyophilized nanoparticles using methanol, sonicated and filtered and analyzed through HPLC and finally, DL% was calculated as (mass of drug in nanoparticles / mass of lyophilized nanoparticles) x 100 [10-11].

In-Vitro Drug Release Test:

The dialysis membrane (MWCO 1214 kDa) was moistened with distilled water 12 hours prior to introducing 5 mL of the optimized SLN dispersion (synonymous with 120 mg nateglinide) in the sealed bag. The bag was incubated under sink conditions in 900 mL of phosphate buffered saline (pH 6.8) with 0.5% w/v SLS to ensure sink conditions and stirred with a USP dissolution apparatus II (paddle) at 37°C + 0.5°C. Kinetic studies were carried out by taking 5 mL aliquots at fixed time intervals (0, 0.5, 1, 2, 4, 6, 8, 12, 24 hours) and replacing with fresh pre warmed medium, then filtered with 0.45 µm syringe filters and analyzed by HPLC to find the cumulative percentage of drug released [12].

Surface Morphology (Scanning Electron Microscopy):

The dispersion of SLN was diluted 1:10 with distilled water and one drop was put on a clean aluminum stub and dried at room temperature and in a mild vacuum. A sputter coating (1020 nm) of the dried sample with gold/palladium was done in the presence of argon to ensure no charging occurred [13]. The stub was then attached to the SEM chamber and images recorded at increasing voltages of 5-15 kV with images of the stub at different magnifications to view the shape and smoothness of the surface of the particles and their aggregation.

X-Ray Diffraction (XRD):

Optimized SLN formulation was lyophilized into powder (100 200 mg) onto a quartz or zero background silicon sample holder. An X ray diffractometer (at 40 kV, 40 mA) with Cu K 0 radiation (1.5406 A) was used to record XRD patterns, scanning 3° to 50° (2θ) at a rate of 0.02° per second. Pure nateglinide, physical mixture of excipients, blank SLNs and drug loaded SLNs were examined by the same procedure and the diffractograms were compared to the disappearance or the lesser intensity of the typical drug peaks, which is a sign of amorphization or dispersal of the molecules [14-15].

In-vivo Study:

Induction of diabetes: Rats were induced with diabetes by starving them during 36 hours where they were free to utilize water. Alloxan monohydrate (20mg/mL in ice-cold saline) was recently prepared and 150mg/kg of body weight of each rat was single intraperitoneally injected [16].

We promptly prevented hypoglycemic shock by giving 5 percent glucose drinking water and subsequently by giving 2 ml of 50 percent dextrose orally after every two hour in the first 12 hours. The blood glucose of the fasting was followed on day 3, and on day 7, and, in both cases, it was more than 200 mg/dL, which proved the success of the induction. We also prescribed 1 unit of long-acting insulin daily, five days to avoid ketoacidosis.[17-18].

Treatment Protocol:

Adult Wistar rats (200-400g) were maintained at normal conditions. Alloxan (150mg/kg) was injected intraperitoneally after 36 hrs to induce diabetes, and glucose was administered to avoid hypoglycemia. The stable hyperglycemia (>200 mg/dL) was established, and the animals were separated into three groups (n=6 each). Group I was administered with the oral saline (control), Group II with nateglinide (120mg/kg) and Group III with nateglinide loaded solid lipid nanoparticle (120mg/kg). Samples of blood were taken at various time intervals until 24 hours at the lateral tail vein. Pharmacokinetics of plasma was determined by HPLC analysis, and blood glucose decrease was evaluated by the GOD POD method [19-20]. The procedures were all in accordance with CPCSEA and IAEC guidelines (Approval No.:1504//PO/RE/S/11/CPCSEA/17).

Stability Studies:

Three batches of the optimized nateglinide loaded SLNs were prepared in three independent batches filled in sterile amber glass vials (10 mL each) which were sealed under ambient air conditions and kept in a temperature/humidity-controlled stability chamber at two temperatures: 25C + 2C / 60C + 5C RH (room temperature) and 4ºC + 2ºC. Samples were sampled at 0 (initial), 1, 3 and 6 months and assessed using visual appearance (color, aggregation, sedimentation), particle size, PDI, zeta potential, entrapment efficiency and in vitro drug release profile as outlined above [21-23]. One way ANOVA, with Tukey post hoc test ($p < 0.05$ was discussed as significant) was used to statistically compare 6-month data with initial data.

RESULTS AND DISCUSSION

Standard Calibration Curve:

The calibration curve indicates that the relationships between the concentration of nateglinide (5-50 ug/mL) and the mean of the peak areas are excellent ($R^2 = 0.9999$). The equation is $y = 358.23x + 284.8$ which implies high sensitivity and low intercept. This confirms the HPLC technique of precise nateglinide quantification in SLN samples.

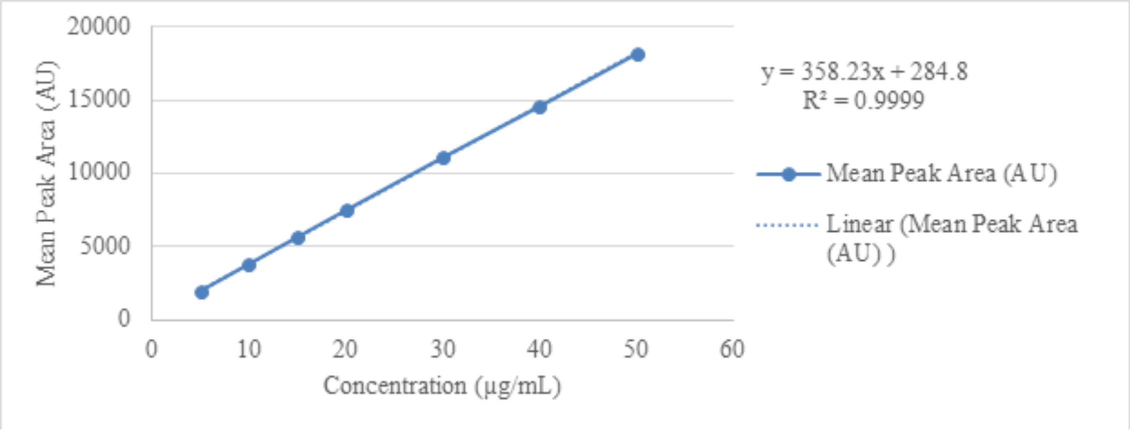


Figure 1: Standard Calibration Curve of Nateglinide

Drug Excipients Interaction Study:

The optimized formulation NSLN19 retained the entire core structure of Nateglinide. The FTIR revealed important peaks, such as NH (~3300 -1), amide C=O (~1650 $^{-1}$), and CN (~1280 $^{-1}$), did not change, and only small changes indicated beneficial hydrogen bonding with excipients. DSC confirmed that the melting point of the drug at 138.20ºC was precisely the same, and it was not chemically incompatible with any other chemical. There was a new small peak at 175.06ºC, which was probably due to melting excipients or a eutectic mixture. A combination of these findings in Figure 2 demonstrate that the drug and excipients are physically stable, chemically compatible, and has a high chance of enhancing drug absorption without destroying the drug.

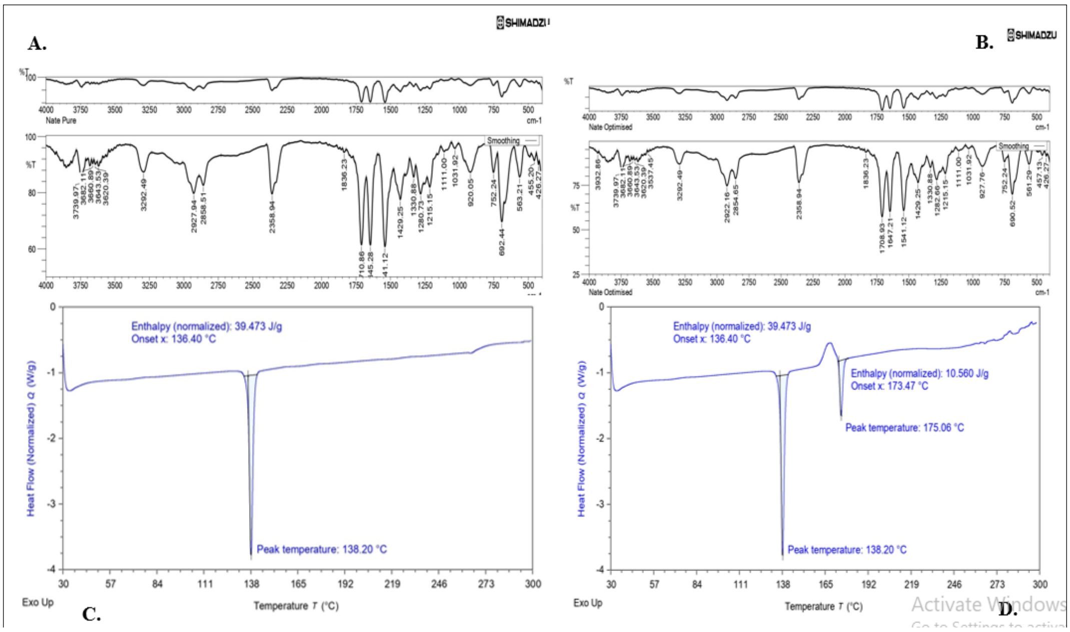


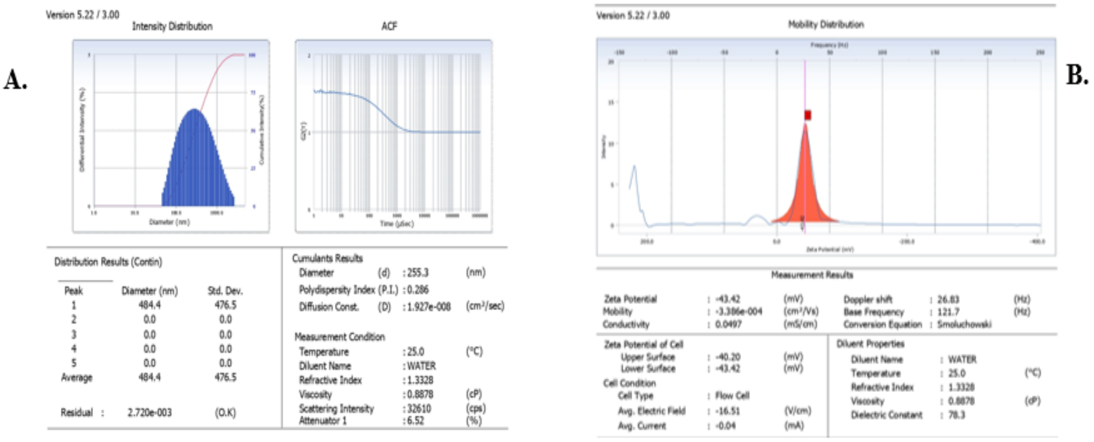
Figure 2: Drug Excipients Study, A) FTIR of Pure Drug, B) FTIR of Optimized Formulation, C) DSC of Pure Drug& D) DSC of Optimized Formulation

Particle Size:

Particle size ranged from 218.5 ± 2.1 nm (NSLN20) to 328.9 ± 5.1 nm (NSLN12). The biggest particles occurred with low surfactant and stabilizer because of the coalescence of droplets. Either increased size led to a significant reduction. The tiniest particles were mixed with high surfactant and stabilizer yet low lipid, but with reduced drug loading. Optimized NSLN19 provided 255.3 ± 2.0nm with PDI of 0.286 ± 0.011, to balance high entrapment and uniformity and stability. Figure 3 shows the results.

Zeta Potential:

Zeta potential ranged from −15.7 ± 2.1 mV (NSLN12) to –46.2 ± 1.5 mV (NSLN8). The values were all negative as a result of Phospholipid 90G. The smallest negative values indicated the aggregation risk, and the possibility was more negative with the increase of surfactant or stabilizer. Optimized NSLN19 provided -43.42 ±1.2 mV, which is far above the stability range of ±30 mV. This intense repulsion of the ions aided in avoiding agglomeration and provided oral delivery stability through colloidal stability. Figure 3 can be regarded as the results of the study. Polydispersity Index PDI ranged from 0.274 ± 0.012 (NSLN20) to 0.521 ± 0.035 (NSLN12). Batches with inadequate surfactant or stabilizer provided distributions greater than 0.48. The joint increase in the two reduced the PDI significantly. The best NSLN19 was 0.286 ± 0.011 which is near the lowest value. Reproducibility was also confirmed with six batches of center points with PDI of about 0.29-0.30 and SDs of minor variations. Higher concentrations of surfactant and stabilizer were necessary to achieve uniform particle size due to improved interfacial stabilization. Figure 3A illustrates particle size and PDI, while Figure 3B presents the zeta potential of the optimized formulation.



Distribution Results (Contin)

Peak	Diameter (nm)	Std. Dev.
1	484.4	476.5
2	0.0	0.0
3	0.0	0.0
4	0.0	0.0
5	0.0	0.0
Average	484.4	476.5

Residual : 2.720e-003 (O.K)

Cumulants Results
Diameter (d) : 255.3 (nm)
Polydispersity Index (P.I.) : 0.286
Diffusion Const. (D) : 1.927e-008 (cm²/sec)

Measurement Condition
Temperature : 25.0 (°C)
Diluent Name : WATER
Refractive Index : 1.3328
Viscosity : 0.8878 (cP)
Scattering Intensity : 32610 (cps)
Attenuator 1 : 6.52 (%)

Measurement Results
Zeta Potential : -43.42 (mV)
Mobility : -3.386e-004 (cm²/Vs)
Conductivity : 0.0497 (mS/cm)
Doppler shift : 26.83 (Hz)
Base Frequency : 121.7 (Hz)
Conversion Equation : Smoluchowski

Zeta Potential of Cell
Upper Surface : -40.20 (mV)
Lower Surface : -43.42 (mV)
Cell Condition
Cell Type : Flow Cell
Avg. Electric Field : -16.51 (V/cm)
Avg. Current : -0.04 (mA)

Diluent Properties
Diluent Name : WATER
Temperature : 25.0 (°C)
Refractive Index : 1.3328
Viscosity : 0.8878 (cP)
Dielectric Constant : 78.3

Figure 3: A) Particle Size and PDI & B) Zeta Potential of Optimized Formulation

Entrapment Efficiency:

EE varied from 68.2 ± 2.1% (NSLN1) to 94.6 ± 1.2% (NSLN19). Less than 73% of the drug was trapped with low surfactant and stabilizer. EE was enhanced to approximately 89 by increasing either of them alone. The highest combination of the three elements was the best outcome. NSLN19 reached 94.6%, which was 4.4% higher than NSLN17. There was consistent EE of about 84-85% around six center-point batches (SD 1.1 or less). NSLN19 was the best to retain the drug.

Drug Loading:

Drug loading ranged from 64.3 ± 2.3% (NSLN1) to 92.2 ± 1.3% (NSLN19). Values had a similar trend as EE with a 2-3 percent lower range because of mechanical losses in the homogenization and sonication. In the case of NSLN19, 92.2% loading corresponded to almost 111 mg of nateglinide being absorbed. Batches that contained high surfactant and low stabilizer produced 81-88% loading. There were six center batches with averages of 82.5%. The greatest loading was obtained with high amounts of all three excipients.

Table 3: Evaluation Parameters of Prepared SLN of Nateglinide

Batch	PDI	Zeta Potential (mV)	Drug Loading (%)
NSLN1	0.485 ± 0.031	–18.3 ± 1.9	64.3 ± 2.3
NSLN2	0.296 ± 0.015	–38.7 ± 1.2	82.5 ± 1.3
NSLN3	0.312 ± 0.018	–35.2 ± 1.4	85.1 ± 1.5
NSLN4	0.291 ± 0.014	–39.1 ± 1.1	82.9 ± 1.2
NSLN5	0.294 ± 0.016	–38.5 ± 1.3	82.7 ± 1.4
NSLN6	0.398 ± 0.022	–25.4 ± 1.6	76.2 ± 1.7
NSLN7	0.293 ± 0.014	–38.9 ± 1.2	83.1 ± 1.1
NSLN8	0.267 ± 0.013	–46.2 ± 1.5	87.6 ± 1.4
NSLN9	0.324 ± 0.019	–30.7 ± 1.4	78.0 ± 1.8
NSLN10	0.258 ± 0.012	–44.8 ± 1.3	86.5 ± 1.5
NSLN11	0.352 ± 0.021	–32.4 ± 1.7	82.0 ± 1.3
NSLN12	0.521 ± 0.035	–15.7 ± 2.1	69.8 ± 2.2
NSLN13	0.382 ± 0.024	–27.9 ± 1.5	79.2 ± 1.6
NSLN14	0.412 ± 0.027	–22.6 ± 1.8	74.5 ± 2.0
NSLN15	0.428 ± 0.029	–26.8 ± 1.9	82.3 ± 1.4
NSLN16	0.334 ± 0.020	–41.5 ± 1.6	81.1 ± 1.7
NSLN17	0.305 ± 0.017	–37.4 ± 1.4	88.3 ± 1.6
NSLN18	0.445 ± 0.030	–20.1 ± 2.0	80.5 ± 1.8
NSLN19	0.286 ± 0.011	–43.42 ± 1.2	92.2 ± 1.3
NSLN20	0.274 ± 0.012	–42.8 ± 1.3	84.6 ± 1.7

In-Vitro Drug Release Study:

The release profiles of all twenty batches of low surfactant and low stabilizer were collected and released 89.3% and 91.2% of drug in 24h, respectively - the drug must have settled near the particle surface. Increasing the concentration of

Surfactant alone (NSLN8, 65.7 ± 2.3) or stabilizer alone (NSLN10, 67.2 ± 2.4) slowed release significantly which is evident in Figure 4. The slowest release came from NSLN19 (58.4 ± 1.8% at 24 h), matching its highest entrapment (94.6 ± 1.2%) and most negative zeta potential (–43.42 ± 1.2 mV). Kinetics revealed that all batches had abnormal transport (value of n was between 0.48 and 0.65). The highest n values (0.61-0.65) belonged to NSLN1, NSLN12, NSLN14, and NSLN18. NSLN19 is the least diffusion dominated with an intact matrix of release (0.48) and highest Korsmeyer Peppas R^2 (0.988). Generally, NSLN19 was the most sustained, predictable release, which is optimal in once daily dosing.

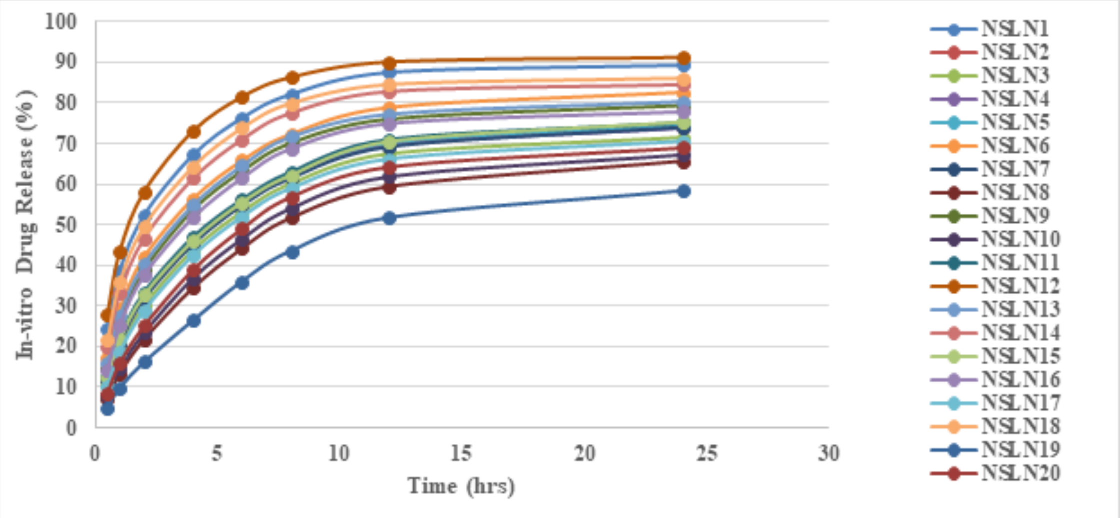


Figure 4: In-vitro Drug Release Profile of the Prepared SLN of Nateglinide

Surface Morphology (SEM):

The SEM image of NSLN19 revealed predominantly smooth-surfaced, uniform-shaped, spherical particles. It was possible to observe some small aggregates, which were likely to be the result of drying the sample. The sizes were less than 1.0 um, which is in line with the measured size of 255.3 ± 2.0nm. No cracks and deformations were observed and it was demonstrated that the solid lipid matrix was not destroyed like shown in Figure 5. The good physical stability, entrapment efficiency and sustained release properties that emerged in our experiments was facilitated by the non-aggregated, spherical morphology.

X-Ray Diffraction (XRD):

Pure nateglinide showed sharp and intense XRD peaks of 10.2, 15.3, 18.9, 20.1 and 24.5 indicating that it is crystalline in nature. The NSLN19 peaks were broad low intensity humps and new sharp peaks of the lipid matrix appeared at 20.9 degrees and 21.4 degrees partly crystallizing and dispersing the drug molecules as in Figure 5. These results were similar to that of DSC (endotherm present) and FTIR (no degradation) which indicated the high entrapment (94.6 ± 1.2%) and release sustained drug maintenance of the optimized formulation.

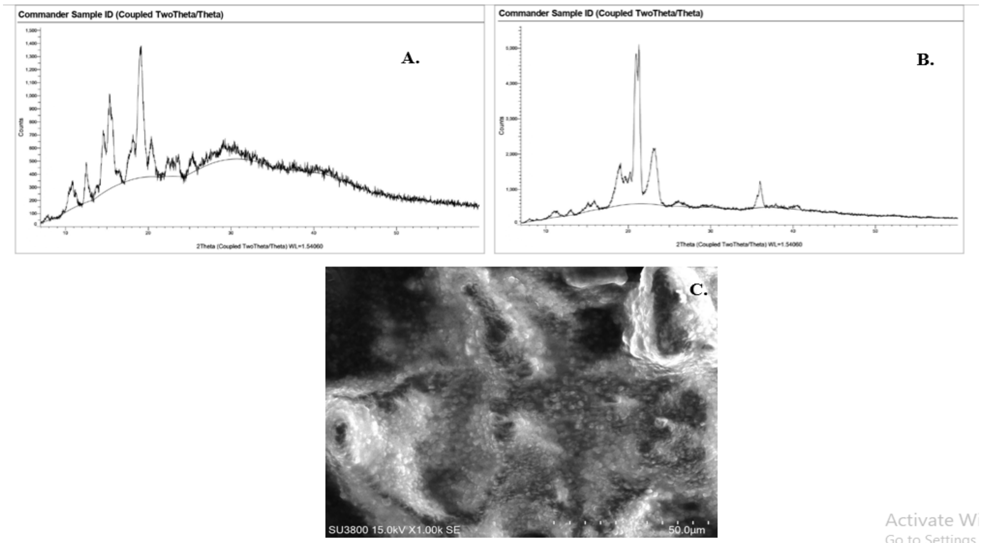


Figure 5: A) XRD of Pure Drug, B) XRD of Optimized Formulation & C) SEM of Optimized Formulation

In-vivo Study:

The SLN formulation increased the oral bioavailability of nateglinide by 1.2× not only marginally but significantly i.e. 65% to 78%. More to the point it changed the peak time to 3.2 ± 0.4h instead of 1.0 ± 0.2h, reduced the peak concentration, and increased the half-life to 3.8 ± 0.5h as opposed to 1.9 ± 0.3h. These changes transformed the glucose lowering profile. Pure drug resulted in a sharp reduction, which vanished in 08 hrs and the SLN maintained the blood glucose below 200/dL, over 18 hours. The sustained effect was due to prolonged release of drugs, rather than higher total absorption. The SLN was able to develop nateglinide into once day formulation, enhancing the compliance without the risk of hypoglycemia.

Table 4: Pharmacokinetic Parameters of optimized Formulation (Mean ± SD, n=6)

Parameter	What it indicates	Pure Nateglinide (120 mg/kg)	Nateglinide SLN (120 mg/kg)	Significance
C_{max} (μg/mL)	Peak drug concentration in blood	10.2 ± 1.1	8.4 ± 0.9*	Lower peak suggests reduced risk of side effects
T_{max} (h)	Time to reach peak concentration	1.0 ± 0.2	3.2 ± 0.4*	Delayed T_{max} confirms sustained release from SLN matrix
AUC_{0-24} (μg·h/mL)	Total drug exposure over 24 hours	45.8 ± 4.2	54.9 ± 4.8*	Increased AUC indicates better overall absorption
$AUC_{0-\infty}$ (μg·h/mL)	Total lifetime drug exposure	48.3 ± 4.5	58.2 ± 5.0*	It confirms sustained absorption beyond 24 h
t½ (h)	Half-life (time for drug level to drop by 50%)	1.9 ± 0.3	3.8 ± 0.5*	Prolonged half-life means drug stays longer in body
MRT (h)	Mean residence time of drug in body	3.7 ± 0.4	7.2 ± 0.9*	Longer MRT confirms prolonged drug presence at target site

Absolute Bioavailability (%)	Fraction of oral dose reaching systemic circulation	65.2 ± 4.5	78.3 ± 5.2	SLN improved absorption by 1.2× relative to pure drug

Table 5: Pharmacodynamic Parameters of optimized Formulation (Blood Glucose Levels in mg/dL, mean ± SD, n=6)

Time (h)	Control (Saline)	Pure Nateglinide (120 mg/kg)	Nateglinide SLN (120 mg/kg)
0	285 ± 18	291 ± 15	288 ± 16
0.5	282 ± 20	210 ± 14*	222 ± 13*
1	279 ± 19	178 ± 12*	195 ± 11*
2	286 ± 21	162 ± 10*	172 ± 9*
4	291 ± 22	185 ± 11*	148 ± 8*†
6	288 ± 20	212 ± 13*	135 ± 7*†
8	290 ± 19	228 ± 14	128 ± 6*†
12	287 ± 21	252 ± 15	148 ± 8*†
18	289 ± 20	268 ± 16	175 ± 10*†
24	285 ± 18	277 ± 17	212 ± 11*†

*Significantly different from control ($p < 0.05$).

†Significantly different from pure drug at same time point ($p < 0.05$).

Stability Studies:

The NSLN19 formulation remained highly stable at 4°C for six months, with minimal changes: particle size (255.3±2.0 to 257.4±2.4 nm), PDI (0.286±0.011 to 0.292±0.013), zeta potential (-43.4±1.2 to -42.5±1.3 mV), entrapment efficiency (94.6±1.2% to 93.5±1.5%), and 24h drug release (58.4±1.8% to 59.8±2.1%). Moderate but satisfactory shifts were observed at 25°C temperature: the particle size reached 263.5±3.1 nm, EE decreased to 91.8±9.1 nm, and release grew to 62.1± 2.4. Refrigeration is ideal but room temperature storage is within practical use of six months.

Table 6: Visual Appearance and Stability of NSLN19 at Various Storage Conditions

Time point	Storage condition	Aggregation	Sedimentation	Colour change
0 month	4°C / 25°C	None	None	None (milky white)
1 month	4°C	None	None	None
	25°C	None	Slight trace	None
2 months	4°C	None	None	None
	25°C	None	Mild, easily re-dispersible	None
3 months	4°C	None	None	None
	25°C	None	Moderate, re-dispersible	None
6 months	4°C	None	None	None
	25°C	None	Moderate, re-dispersible	Slight off-white

25°C ± 2°C / 60% ± 5% RH

Time point	Particle Size (nm)	PDI	Zeta Potential (mV)	Entrapment Efficiency (%)	Drug Release after 24h (%)
0 month	255.3 ± 2.0	0.286 ± 0.011	–43.4 ± 1.2	94.6 ± 1.2	58.4 ± 1.8
1 month	256.9 ± 2.3	0.291 ± 0.013	–42.9 ± 1.3	94.1 ± 1.4	59.0 ± 2.0
3 months	259.7 ± 2.7	0.299 ± 0.015	–42.1 ± 1.4	93.2 ± 1.6	60.3 ± 2.2

6 months	263.5 ± 3.1	0.308 ± 0.018	–41.0 ± 1.5	91.8 ± 1.9	62.1 ± 2.4
4°C ± 2°C					
Time point	**Particle Size (nm)**	**PDI**	**Zeta Potential (mV)**	**Entrapment Efficiency (%)**	**Drug Release after 24h (%)**
0 month	255.3 ± 2.0	0.286 ± 0.011	–43.4 ± 1.2	94.6 ± 1.2	58.4 ± 1.8
1 month	255.8 ± 2.1	0.287 ± 0.011	–43.2 ± 1.2	94.4 ± 1.3	58.7 ± 1.9
3 months	256.5 ± 2.2	0.289 ± 0.012	–42.9 ± 1.2	94.0 ± 1.4	59.1 ± 2.0
6 months	257.4 ± 2.4	0.292 ± 0.013	–42.5 ± 1.3	93.5 ± 1.5	59.8 ± 2.1

Optimization Study:

Particle Size (Y_1):

Lipid enlarges the size of the particle whereas surfactant and stabilizer decreases the particle size. The X_1X_2 interaction is negative indicating that surfactant opposes the growth which is lipid-driven. Presence of positive quadratic terms implies an optimal range to move any factor excessively far out of the middle and the particles will be drawn back again to influence the choice of optimized batch. Figure 6 can be regarded as the results.

Y_1 (Size) $= 262.4 + 18.6\,X_1 - 22.3\,X_2 - 15.7\,X_3 - 8.1\,X_1X_2 + 4.3\,X_1X_3 - 5.6\,X_2X_3 + 11.2\,X_1^2 + 9.8\,X_2^2 + 7.5\,X_3^2$

Entrapment EfficiencyY_2:

Lipid, surfactant, and stabilizer are all stronger in enhancing drug entrapment, with lipid being the strongest. The positive interactions (e.g., lipid surfactant) enhance EE beyond individual contributions explaining NSLN19 with 94.6% value. The use of negative quadratic terms caution against diminishing returns at extreme levels, thus guaranteeing balanced formulation design. Figure 6 can be considered the results of the results.

Y_2 (EE) $= 84.7 + 6.2\,X_1 + 5.8\,X_2 + 4.9\,X_3 + 2.1\,X_1X_2 + 1.7\,X_1X_3 + 2.5\,X_2X_3 - 3.4\,X_1^2 - 2.9\,X_2^2 - 2.1\,X_3^2$

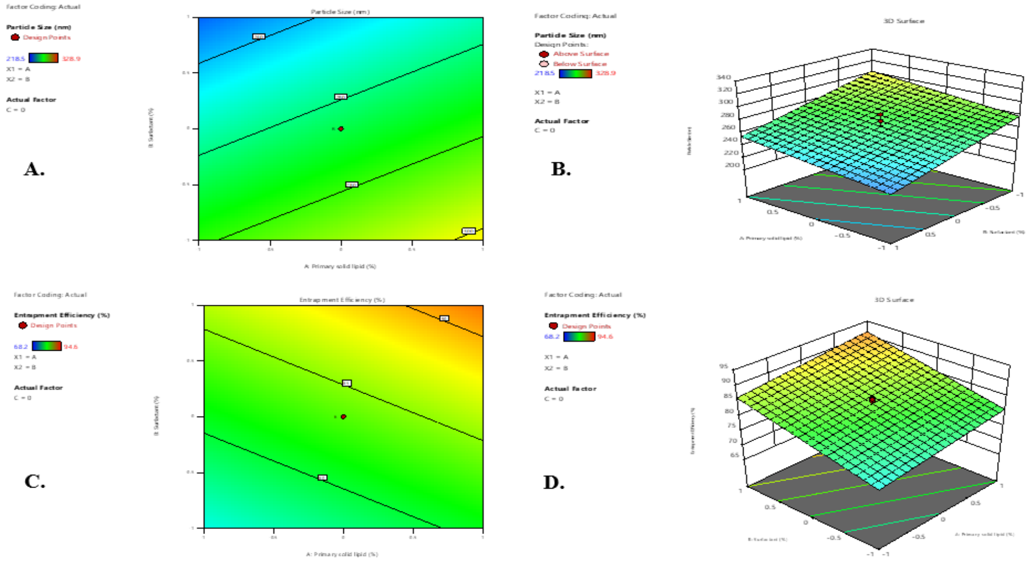


Figure 6: A) Contour Graph of Particle Size, B) 3D Graph of Particle Size, C) Contour Graph of entrapment Efficiency & D) 3D Graph of Entrapment Efficiency

CONCLUSION

In this study, we have been able to reform nateglinide as a once daily solid lipid nanoparticle formulation that is reduced to a short acting, thrice daily drug. The optimized NSLN19 balanced particle size (255.3 ± 2.0 nm) with exceptionally high entrapment (94.6 ± 1.2%) and drug loading

(92.2 ± 1.3%). The sustained release profile only 58.4 ± 1.8% released in 24 h compared to only 8 h of pure nateglinide. Improved pharmacokinetics included doubled half life (3.8 ± 0.3 h vs. 1.9 ± 0.2 h)) and shifted peak time (3.2 ± 0.2 h vs. 1.0 ± 0.1 h), which reduced the risks of sharp glucose spikes and late hypoglycemia. Through refrigeration and little modification, the formulation was able to maintain the six months of stability. Combined with the above findings, these results indicate that SLNs is a feasible, scalable solution to address the clinical limitations of nateglinide, enhancing patient adherence and postprandial glucose regulation.

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Ethics Approval: All the animal experiments were performed in compliance with the CPCSEA guidelines, Government of India. The protocol of the study was approved by the Institutional Animal Ethics Committee (IAEC) (Approval No.: 1504//PO/RE/S/11/CPCSEA/17). All was done to ensure that animals were not suffering.

Patient Consent: Not Required.

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