RESEARCH ARTICLE

Design, Development and Characterization of Nisoldipine Solid Lipid Nanoparticles: Statistical and Characterization Perspectives

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Received: 06th January, 2024; Revised: 03rd March, 2024; Accepted: 11th July, 2024; Available Online: 25th September, 2024

ABSTRACT

The objective of this study was to develop and characterize solid lipid nanoparticles (SLNs) loaded with Nisoldipine (NIS), aiming to enhance the drug's bioavailability and provide a sustained release profile. SLNs were prepared using a solvent evaporation method and evaluated for several critical parameters. The superfluity test assessed the homogeneity of the SLN suspension, while particle size, drug entrapment efficiency, shapes, and surface morphology were analyzed using SEM/DLS/TEM. Researchers studied the process of how drugs are released and performed *in-vitro* drug release tests to establish the substance's release profile. HPLC and FT-IR were used to verify the drug's chemical stability and encapsulation effectiveness. Stability studies assessed the formulation's stability over time throughout different storage settings.

The SLNs exhibited a high degree of homogeneity in the superfluity test, with no phase separation. The drug entrapment effectiveness was over 80%, and the typical particle size was 150-250 nm. Microscopy and transmission electron microscopy revealed that the SLNs had a flat, spherical top. NIS exhibited a regulated sequence of kinematics and prolonged release during dissolution investigations. FTIR and HPLC confirmed the stability and integrity of the drug within the SLNs. Stability studies indicated that the formulation remained stable over time.

NIS-loaded SLNs show significant potential as an effective drug delivery system, offering enhanced bioavailability, sustained release, and excellent stability.

Keywords: Solid lipid nanoparticles, Nisoldipine, Drug delivery, Sustained release, Nanoparticles, Bioavailability.

International Journal of Drug Delivery Technology (2024); DOI: 10.25258/ijddt.14.3.53

How to cite this article: Chauhan NK, Malik A. Design, Development and Characterization of Nisoldipine Solid Lipid Nanoparticles: Statistical and Characterization Perspectives. International Journal of Drug Delivery Technology. 2024;14(3):1623-1634.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Worldwide, cardiovascular illnesses continue to rank as the top cause of death and disability, with hypertension identified as a primary risk factor. Hypertension, often termed a silent killer, contributes significantly to the development of conditions such as stroke, heart failure, and myocardial infarction. Managing hypertension effectively is critical to reducing the associated health risks, and this necessitates the use of potent antihypertensive drugs.[1]

An effective method of reducing blood pressure is vasodilation, which nisodipine does by blocking the entry of calcium ions into cardiac cells and vascular smooth muscle. This family of calcium channel blockers is known as the dihydropyridine class. Despite its clinical efficacy, nisoldipine's therapeutic potential is compromised by several pharmacokinetic challenges. Due to its minimal bioavailability by the mouth of around 5-10% and its significant first-pass hepatic metabolism, its limited absorption in the gastrointestinal system is caused by its inability to dissolve in water.[2] These limitations often necessitate higher doses or more frequent administration to achieve the desired therapeutic effect, which in turn increases the risk of adverse effects such as headaches, flushing, and peripheral edema.[3] In light of these obstacles, NDD strategies have to be developed immediately to improve nisoldipine's therapeutic efficacy by increasing its bioavailability, stability, and solubility.[4]

In recent years, nanotechnology has emerged as a transformative approach in the field of drug delivery, offering solutions to many of the challenges posed by conventional dosage forms. Many researchers are interested in SLNs as a nanocarrier system because of the hope that they might enhance the delivery of nisoldipine and other poorly soluble medicines.[5] SLNs are solid lipid fragments, submicron in dimensions that stay solid at ambient and physiological temperatures. The benefits of both conventional liposomes and polymeric nanoparticles are combined in these tiny particles, which also manage to sidestep some of the drawbacks, including fragility and possible toxicity, of both.[6]

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SLNs offer several key benefits that make them attractive for drug delivery applications. To start with, the medication's stability is improved since the encapsulated drug is shielded from environmental variables, including sunlight, air to circulate, and dampness by the solid lipid matrix.[7] Second, the lipid core of SLNs can incorporate lipophilic drugs like nisoldipine, potentially improving their solubility and bioavailability.[8] Third, regulated or prolonged drug release is an engineering goal for SLNs; this feature may assist in maintaining therapeutic levels of medication over lengthy periods, which in turn reduces treatment recurrence and improves adherence among patients.[9] Additionally, SLNs are typically biocompatible and biodegradable, minimizing the risk of adverse reactions.[10] The development of SLNs involves several critical steps, including the selection of appropriate lipids and surfactants, optimization of the formulation process, and thorough characterization of the final product. The choice of lipid is particularly important, as it influences the drug loading capacity, release profile, and overall stability of the SLNs.[11] Commonly used lipids include triglycerides, fatty acids, and waxes, which are selected based on their melting points and compatibility with the drug.[12] Surfactants and co-surfactants are used to stabilize the nanoparticles and prevent aggregation, which is essential for maintaining the desired particle size distribution.[13]

It is possible to create SLNs using a number of different processes, involving as phase inversion, solvent evaporation, microemulsion techniques, and high-pressure homogenization.[14] When designing SLNs, there is important to consider the target qualities, including drug release characteristics, polydispersity index, and particle size, since each approach offers its own set of pros and cons.[15] For example, high-pressure homogenization is a widely used technique that can produce SLNs with small and uniform particle sizes, which is critical for enhancing drug absorption and bioavailability.[16] Comprehensive characterization of the SLNs is necessary after their preparation to guarantee they fulfill the required criteria for clinical application. Characterization involves assessing the particle size and size distribution, which affect the stability and bio-distribution of the nanoparticles.[17] Smaller particle sizes are generally preferred as they can enhance the drug's absorption and bioavailability.[18] The zeta potential, which indicates the surface charge of the nanoparticles, is another critical parameter, as it influences the stability of the SLNs in suspension.[19] A high zeta potential, either positive or negative, can help prevent particle aggregation by repelling similarly charged particles, thereby maintaining a stable formulation.[20]

Encapsulation efficiency is another key factor in the characterization of SLNs, as it determines the amount of drug that is successfully incorporated into the nanoparticles.[21] High encapsulation efficiency is desirable as it maximizes the therapeutic potential of the formulation while minimizing waste.[22] The *in-vitro* drug release profile of SLNs is also crucial, as it provides insights into the release kinetics and mechanism, helping to predict how the drug will behave *in-vivo*.[23] A controlled or sustained release profile is often desired to maintain therapeutic drug levels for longer durations, which can improve treatment efficacy and patient adherence.[24] Stability studies are an essential part of the development process, as they determine the shelf life and robustness of the SLNs under various storage conditions.[25] Factors such as temperature, humidity, and pH can influence the stability of the nanoparticles, and it is important to assess how these conditions affect the physical and chemical properties of the SLNs overtime.[26] Ensuring the stability of the formulation is crucial for its success in clinical applications, as it affects both the efficacy and safety of the drug.[27]

Given the potential benefits of SLNs in enhancing the delivery of nisoldipine, this study aims to develop and characterize nisoldipine-loaded SLNs with the goal of improving the drug's bioavailability and providing a controlled release formulation. The study involved the formulation of SLNs using different lipid compositions and surfactant concentrations, followed by a comprehensive evaluation of their physicochemical properties, drug loading capacity, *in-vitro* release profile, and stability. The successful development of nisoldipine-loaded SLNs could pave the way for more effective management of hypertension, offering a new approach to optimizing the delivery and therapeutic efficacy of antihypertensive drugs.

MATERIALS AND METHODS

Chemicals

Nisoldipine was acquired via gift simple from Emcure Pharmaceutical Ltd., Pune, Maharashtra. Himedia Laboratories Pvt. Ltd., Mumbai, Maharashtra, provided Egg lecithin, Poloxamer 188, Sodium glycocholate, Soy lecithin, Palmitic acid, and ethanol (99%). Glyceryl Monostearate, Tween 80, and Stearic acid were obtained from Loba Chemie Pvt. Ltd., Mumbai, Maharashtra. Merck Life Science Pvt. Ltd., Bengaluru, Karnataka supplied Tricaprin and Trilaurin. The chemicals used during the experiment were of analytical grade exclusively. A distilled water apparatus (Borosil®, India) was used for the experiment.

Instrumentations

Sophisticated instruments such as Digital balance (Denver Digital Balance, MA, USA), Ultracentrifuge (Remi R4C Laboratory Centrifuge, Remi Motors Pvt. Ltd., Mumbai, India), UV-Vis Spectrophotometer (Shimadzu UV-1800 Spectrophotometer, Kyoto, Japan), FTIR Spectrophotometer (Shimadzu 8400S FTIR Spectrophotometer, Kyoto, Japan), Magnetic Stirrer with Hot Plate (Remi Motors, Mumbai, India), Zetasizer (Malvern Instrument Ltd., Mumbai, India), and Transmission Electron Microscope (ZEISS EVO 18 Special Edition, LA, USA) were employed for this study.

Formulation Development

Considerations for formulation development

A variety of biodegradable materials, including metals, lipids, natural or synthetic polymers, and others, may be used to create NPs, which can then be used as a drug delivery mechanism.

The medication is either contained inside the core of these nano-particulate drug delivery devices or absorbed/conjugated into their outer surface. The physicochemical characterization of lipids and the modifications that those lipids experience during preparation constitutes several of the main issues connected with developing SLN preparation. Prior to and during lyophilization, SLN must undergo comprehensive physical-chemical characterization, and it is crucial to use the right quality of lipids in its synthesis.

Preparation of SLNs by microemulsion process

To prepare nisoldipine SLNs employing solely the microemulsion approach, Glycerol Monostearate, Stearic Acid, and Palmitic Acid were first dissolved in ethanol and heated until fully melted, forming the solid lipid phase. This lipid phase was then combined with Tricaprin and Trilaurin to create a uniform oil phase. In a separate step, the aqueous phase was prepared by dissolving soya lecithin, egg lecithin, tween 20, Poloxamer 188, and sodium glycocholate in deionized water (Table 1). The oil phase was gradually introduced into the aqueous phase with continuous stirring to create a stable microemulsion. Throughout this process, the temperature was carefully controlled to maintain stability, and high shear mixing was employed if needed to ensure uniformity. Once the microemulsion was established, it was cooled to allow the lipid phase to solidify, resulting in the formation of SLNs. The nanoparticles were then isolated via centrifugation and washed with deionized water to remove any remaining surfactants and co-surfactants. Finally, the SLNs dispersion was freeze-dried for 24 hrs at a temperature of −50ºC and pressure below 15 Pascal using a freeze dryer.[28]

Characterization of SLNs

Particle size

The Horiba scientific nanopartica device was used for determining the particle size utilizing the dynamic light scattering method. For the particle size examination, the samples were diluted using distilled water preceding assessment and then examined at an angle set of 165 at 25ºC. These specimens were mixed with distilled water in order to evaluate the zeta potential.[29]

Table 1: The nisoldipine SLN

Ingredients	*F1*	*F2*	*F3*	*F4*
Nisoldipine (mg)	10	10	10	10
Glycerol monostearate (mg)	10	15	20	25
Soya lecithin (mg)	0.25	0.5	0.75	1
Tricaprin (mg)	1	1	-	-
Trilaurin (mg)	-	-	1	1
Egg lecithin (mg)	1	0.75	0.5	0.25
Stearic acid (mg)	2	2	-	-
Palmitic acid (mg)	-	-	2	2
Tween 20 (mL)	0.5	0.5	0.5	0.5
Poloxamer 188 (mg)	1.5	1.5	1.5	1.5
Sodium glycocholate (mg)	1	1	1	1
Ethanol (mL)	5	5	5	5
Distilled water	q.s.	q.s.	q.s.	q.s.

Drug entrapment efficiency

Taking readings of the free Nisoldipine concentration in the dispersion medium allowed us to calculate the nisoldipine entrapment efficiency. Using 0.45 µm membrane filters, 10 mg of freeze-dried SLNs of nisoldipine were dissolved in aliquot amounts of phosphate buffer with a pH of 7.4. The filtered solutions were measured at 237 nm for absorbance using a UV-visible spectrophotometer.[30] Here is the formula that was used to compute the percentage entrapment efficiency (%EE):

$$\%EE = (\text{Mass of the drug in submicron particles}) / (\text{Mass of drug used in formulation}) \times 100$$

Shape and surface morphology

Optimal freeze-dried Nisoldipine-loaded SLNs were analyzed using transmission electron microscopy (TEM) to examine their shape and surface morphology. A small sample of the SLN suspension was first prepared by diluting it with deionized water to achieve an appropriate concentration. A drop of this diluted suspension was then placed onto a carbon-coated copper grid and allowed to adhere for a few minutes. The excess liquid was carefully blotted away using filter paper, and the grid was then stained with a contrasting agent, such as uranyl acetate or phosphotungstic acid, to enhance the visibility of the nanoparticles. After staining, the grid was air-dried to remove any residual liquid. The prepared grid was examined under a TEM microscope, where the morphology, size, and shape of the drug-loaded SLNs were analyzed by capturing high-resolution images. This allowed for a detailed assessment of the nanoparticles' structure and uniformity.[31]

In-vitro drug release studies

Using a dialysis bag and a pH 7.4 phosphate buffer with 0.5% v/v Tween 80, researchers conducted *in-vitro* drug release investigations. Next, a 100 mL beaker containing a pH 7.4 phosphate buffer solution was used to hold the 5 mg of nisoldipine medication dispersion, which was poured into the dialysis membrane bag and secured at both ends. Using a magnetic stirrer, the speed was maintained at 100 rpm and the temperature at 37ºC ± 1ºC. To keep the sink conditions constant, samples were removed at regular intervals and replaced with a new buffer solution of the same volume at the same time. A UV-visible spectrophotometer was used to examine the samples at a wavelength of 237 nm. The quantity of Nisoldipine medication release was subsequently used to compute the cumulative percentage release.[32]

Drug release kinetics

For the optimal composition, the drug release kinetics were calculated using kinetic equations such as zero-order (accumulative % release vs. time) and first-order (log % drug remaining vs. time). The models developed by Higuchi and

Korsmeyer-Peppas illustrate the relationship between drug release and time in two different ways: cumulative percentage vs square root of time, and log drug release versus log time. The linear curve that was created from the regression analysis of plots was used to compute the values of r^2. Numerical values for n were calculated using the Korsmeyer-Peppas model.[33]

FTIR spectroscopy

The samples of both free Nisoldipine and Nisoldipine-loaded SLNs were first prepared. Each sample was finely ground with potassium bromide (KBr) to form a homogenous powder mixture. This mixture was then compressed into a thin pellet using a pellet press. The FTIR spectra of these pellets were recorded over the range of 4000 to 400 cm^{-1} using an FT-IR spectrometer. The resulting spectra were analyzed to identify any shifts or changes in the characteristic absorption peaks, which would indicate interactions or changes in the chemical environment of nisoldipine when encapsulated in SLNs. By comparing the spectra of free nisoldipine and the Nisoldipine-loaded SLNs, differences in functional group vibrations and peak positions were assessed to evaluate the impact of the SLN formulation on the drug's molecular structure.[34]

HPLC study

A sample of the Nisoldipine SLN formulation was first processed to separate the free drug from the encapsulated portion. This was achieved by centrifuging the SLN suspension to separate the nanoparticles from the supernatant containing the free nisoldipine. To eliminate any particles before HPLC analysis, the supernatant was filtered using a 0.45 µm syringe filter. An example of a reverse-phase C_{18} column used in the chromatographic system is 250 mm × 4.6 mm with a particle size of 5 µm. Methanol, water, and 0.1% phosphoric acid (or another suitable mobile phase as determined by optimization) were usually mixed in a 70:30 (v/v) ratio to form the mobile phase. The flow rate was adjusted to 1.0 mL/min, and the detector was calibrated to 273 nm, the wavelength at which nisoldipine had the highest absorption. We put a little portion of the filtered supernatant into the HPLC machine and studied the chromatogram that came out of it. By comparing the sample's retention duration and peak area to those of a standard calibration curve that was constructed with known concentrations of nisoldipine, the quantity of free nisoldipine was measured. This method allowed for accurate quantification of the free drug present in the SLN formulation.[35-41]

Stability studies

The best SLN formulation underwent stability assessment by being stored at 30 ± 2ºC / 65 ± 5% RH for 90 days. Modifications in particle size and %entrapment efficiency were monitored frequently.[42-48]

Statistical analysis

A one-way analysis of variance (ANOVA) and Dunnett's paired- comparison test were used for statistical analysis of the collected data. A statistically noteworthy result was defined as a *p-value* below 0.01.

RESULTS AND DISCUSSION

Formulation Development

The microemulsion process was used to manufacture nisoldipine SLN. Due to the importance of microemulsion composition in SLN synthesis, the research sought to establish a stable system by screening a variety of lipids and surfactants. The lipid phase for nanoparticles was chosen as nisoldipine because of its excellent solubility in the GMS/tricaprin combination. We used the HLB technique to choose the surfactants. A combination of a hydrophilic and a lipophilic surfactant was used in place of a single surfactant. The hydrophilic surfactant used was Tween 20. Egg lecithin and span 20, two lipophilic surfactants, were combined with tween 20 in the first trials. Combinations of egg lecithin and tween 20 to make solid lipid nanoparticles were not physically stable and had poor drug entrapment efficiency, in contrast to formulations that included tween 20, which were stable and had good entrapment efficiency. A transparent oil-in-water microemulsion was produced by adjusting the tween 20 concentration to the desired HLB value of the lipid phase. Additionally, the impact of other co-surfactants on nisoldipine SLN was investigated. These included stearic acid, palmitic acid, tween 20, and poloxamer 188. In contrast to SLN dispersions with ethanol and stearic acid, which did not exhibit stability for more than 48 hours, SLN dispersions with palmitic acid exhibited good entrapment efficiency and did not exhibit drug precipitation in formulation even after 30 days of storage. Different surfactant: co-surfactant ratios, such as 1:2, 1:1, and 2:1, were optimized for the formulations of solid lipid nanoparticles. The results showed that surfactant: co-surfactant ratios of 2:1 and 1:2 reduced drug entrapment. It was discovered that nanodispersions with higher concentrations of co-surfactant were unstable and exhibited precipitation when stored. We used a surfactant: co-surfactant ratio of 1:1 since it was the optimal concentration for Nisodipine entrapment.

Design Expert® based optimization by Box-Behnken design

Table 2 shows the results of the Design Expert® software analysis of the influence of unrelated variables on reaction, efficiency of entrapment (%). Table 3 shows the effects on entrapment efficiency (Y1) of Smix: Lipid ratio (X1), loading of drugs quantity in lipid phase (X2), and GMS content in lipid stage (X3). After running the numbers through a test for regression, we arrived to polynomial equation-1:

$$\text{Entrapment Efficiency}(Y) = 88.65 - 3.12X_1 + 1.78X_2 + 0.67X_3 - 1.85X_1X_2 + 2.02X_1X_3 + 0.56X_2X_3 + 1.92X_1^2 + 2.89X_2^2 - 8.45X_3^2$$

Through the use of analysis of variance (ANOVA), the statistical validity of the equation was confirmed. Using a student t-test, we determined if the regression coefficient was statistically significant. Research on the effect of external variables on SLN effectiveness in entrapment was carried out by creating responsive surface and contour plots, as shown in Figure 1. Scientific studies have shown that the drug loading

Table 2: Preliminary optimization of variables of Nisoldipin

Ingredients	*F1*	*F2*	*F3*	*F4*
Nisoldipine (mg)	10	10	10	10
Glycerol Monostearate (mg)	10	15	20	25
Soya lecithin (mg)	0.25	0.5	0.75	1
Tricaprin (mg)	1	1	-	-
Trilaurin (mg)	-	-	1	1
Egg lecithin (mg)	1	0.75	0.5	0.25
Stearic acid (mg)	2	2	-	-
Palmitic acid (mg)	-	-	2	2
Tween 20 (mL)	0.5	0.5	0.5	0.5
Poloxamer 188 (mg)	1.5	1.5	1.5	1.5
Sodium glycocholate (mg)	1	1	1	1
Ethanol (mL)	5	5	5	5
S: Co-S ratio	1:1	2:1	1:2	3:1
Sonication time (min)	20	20	20	20
Entrapment efficiency (%)*	81.3±0.8	78.9±0.9	67.8±0.5	84.6±0.2

*Mean ± SD (n = 3); Ratio of lipid: water phase was 1:3

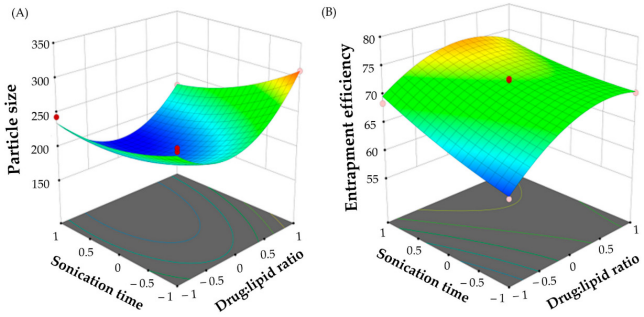

Figure 1: 3D surface plot (A) Size of Particles, and (B) Efficiency of Entrapment

quantity in the phase of lipids and the Smix: Lipid ratio impact the effectiveness of entrapment of NPs. When the Smix: Lipid ratio was smaller, increasing the drug concentration resulted in a little improvement in entrapment efficiency; however, when the ratio was larger, drug concentration led to a reduction in entrapment. One possible explanation is that when the quantity of surfactant mixture increases, the medication partitions into the aqueous phase more efficiently, leading to less lipid

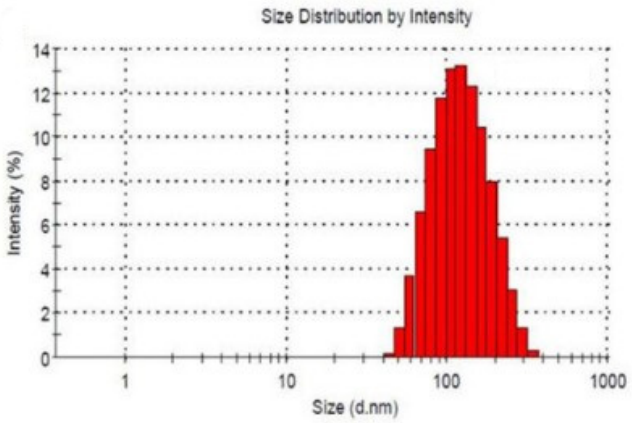

Figure 2: Particle size of optimized Nisoldipine SLN formulation

entrapment. While the Smix: Lipid ratio had no discernible impact on drug entrapment at low concentrations of drug, it caused an abrupt drop in entrapment efficiency at high concentrations as a result of enhanced drug partitioning in the aqueous phase.

Information suggests that the entrapment efficiency is affected by the concentrations of drug loading in the lipid phase (X2) and GMS in the lipid phase (X3). Increasing the drug concentration had no discernible effect on drug entrapment at either low or high lipid concentrations. Increasing the lipid concentration from 10% to 20% resulted in an increase in GZ entrapment at all drug concentration levels. The entrapment efficiency decreased as the lipid content increased from 20% to 30%. The effects of the Smix: Lipid ratio (X1) and the GMS level in the lipid phase (X3) on the effectiveness of entrapment were shown in Figure 1 and Table 3, respectively. When Smix: Lipid was raised, the capture of drugs remained unchanged regardless of the lipid saturation degree. The entrapment efficiency of SLN rose across the board for all Smix: Lipid ratios when the lipid concentration was raised from 10% to 20%. The drug entrapment was reduced when the lipid concentration was raised from 20% to 30%.

Evaluation of SLNs

Particle size

The improved NIS SLN formulation's DLS study shows that the average particle size is 1.15 µm, as shown in Figure 2. With a PDI of less than 0.2, the size range is limited, suggesting that the particles are consistent in size with limited fluctuation.

Table 3: Nisoldipine SLN optimization through Box-Behnken design

Batch code	*Code value*			*Entrapment Efficiency (%) [Y1]*
	Smix: Lipid ratio [X1]	*Drug loading concentration in lipid phase (%) [X2]*	*GMS concentration in lipid phase (%) [X3]*	
F1	1:1	10	10	72.9±0.8
F2	2:1	10	20	87.3±0.7
F3	3:1	15	10	84.1±0.9
F4	4:1	20	10	98.4±0.6

*Mean ±SD (n=3); Each formulation contained 10 mg of Nisoldipine; Ratio of lipid: water phase was 1:3

Considering the solvation shell, the hydrodynamic diameter as determined by DLS represents the actual dimension of the dispersed NPs. The dissolving rate and bioavailability of NIS are both improved by the tiny size of the particle. The size is well-suited for targeting and effective cellular uptake. The narrow size distribution and uniformity suggest good formulation stability. Smaller nanoparticles are less likely to aggregate, which contributes to a stable SLN formulation. The stability of the particle size should be monitored over time and under different storage conditions to ensure long-term efficacy.

Drug entrapment efficiency

Because various forms of Nisoldipine SLNs have different drug incorporation efficiencies, the DEE differs throughout the four batches. The estimated range of SLN immobilization efficiencies was determined to be between 75 and 90%. Both the elevated and lesser stirring rates showed a boost in the effectiveness of entrapment as the amount of the polymer (Glycerol monostearate and Soya lecithin) increased. The effectiveness of entrapment is enhanced when the quantity of polymers is increased. These results suggest variability in the encapsulation process and effectiveness among the different batches (Table 4).

Batch 1 (85%)

This batch shows a relatively high DEE, indicating that a significant proportion of nisoldipine was successfully encapsulated in the SLNs. This high DEE suggests an effective formulation process and efficient drug loading.

Batch 2 (75%)

The lowest DEE among the batches. This lower value could be due to several factors, such as suboptimal formulation conditions, variations in lipid matrix composition, or potential losses during the preparation or separation processes.

Batch 3 (90%)

The highest DEE value observed. This batch demonstrates the most efficient encapsulation of nisoldipine. Possible reasons for this high DEE could include optimized formulation parameters, ideal lipid matrix composition, and effective manufacturing techniques.

Batch 4 (80%)

A moderate DEE, indicating effective encapsulation but not as high as Batch 3. This result suggests room for optimization in formulation or processing conditions to achieve higher entrapment efficiency.

Several factors could contribute to the observed variability in DEE. Variations in the types and ratios of lipids used in each batch can affect how well the drug is encapsulated. The lipid matrix needs to be optimized to enhance drug loading and stability. Differences in the preparation method, such as the duration and speed of high shear homogenization or solvent evaporation conditions, can impact DEE. Ensuring consistency in the preparation process is crucial for reproducibility. The interaction between nisoldipine and the lipid matrix can influence DEE. Strong interactions may lead to higher encapsulation efficiency, while weaker interactions could result in lower DEE. Conditions such as temperature, pH, and the presence of other excipients or stabilizers can impact the efficiency of drug entrapment. The variability in DEE across batches highlights the need for optimization of the SLN formulation process. Identifying and controlling the factors affecting DEE can lead to more consistent and efficient drug encapsulation. Regular monitoring of DEE is essential for quality control to ensure that the SLNs meet the required specifications for drug loading and efficacy. High DEE values contribute to the overall effectiveness of the SLNs as a drug delivery system. Ensuring high DEE is critical for achieving desired therapeutic outcomes and minimizing dosing requirements.

Table 4: NIS SLN DEE

Formulation	*Entrapment efficiency (%)**
F1	85 ± 0.66
F2	75 ± 0.15
F3	90 ± 0.12
F4	80 ± 0.49

*Values expressed as Mean ± SD, n=3

Shape and Surface Morphology

The TEM analysis of Nisoldipine SLNs reveals variability in the shape, size, and surface morphology across different batches (Table 5). Batches 1 and 4 demonstrate desirable characteristics with uniform spherical particles and smooth surfaces, indicating effective formulation and encapsulation. In contrast, Batches 2 and 3 show some inconsistencies and irregularities, which may affect the stability and performance of the nanoparticles. Addressing these issues through formulation optimization and quality control will be essential for achieving high-quality, effective drug delivery systems.

Shape Consistency

Batch 1 and Batch 4

Both batches exhibit uniform spherical nanoparticles with smooth surfaces, indicating a well-optimized formulation process (Figure 3). The consistency in shape and size is desirable as it ensures predictable drug release and stability of the SLNs. The smooth surface further supports the idea of effective drug encapsulation and a well-formed lipid matrix.

Batch 2

This batch shows a mix of spherical and irregularly shaped particles. The occasional roughness and surface protrusions observed may indicate some degree of aggregation or incomplete encapsulation. These irregularities could lead to variability in the release profile and potential issues with stability.

Batch 3

The presence of both spherical and elongated particles suggests some inconsistencies in the formulation process. Elongated particles may affect the uniformity of drug release and the overall performance of the SLNs. However, the relatively

Table 5: TEM study of nisoldipine SLN formulations

Formulation	*Shape*	*Size range (nm)*	*Surface texture*	*Observations*
F1	Predominantly spherical	150–200	Smooth with minimal irregularities	The nanoparticles are well-defined and exhibit uniformity in shape and size, indicating a successful encapsulation process
F2	Mostly spherical, with occasional irregularities	180–250	Slightly rough, with occasional surface protrusions	The presence of irregularities and rough surface areas may suggest some degree of aggregation or incomplete encapsulation. The variability in size could be due to formulation inconsistencies
F3	Mostly spherical with some elongated particles	100–180	Smooth to slightly textured	While the majority of nanoparticles are spherical, the presence of elongated particles indicates variability in the nanoparticle formation process. The relatively smooth surface suggests effective encapsulation but with some degree of variability
F4	Uniformly spherical	120–170	Very smooth	This batch demonstrates the most consistent size and shape, with a highly uniform and smooth surface, reflecting an optimal formulation process and effective encapsulation of nisoldipine

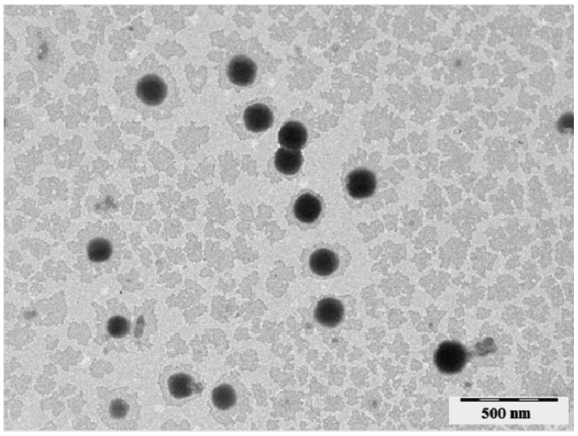

Figure 3: SEM of optimized Nisoldipine SLN formulation (F4)

smooth surface of most particles is indicative of effective encapsulation.

Size Distribution

Batch 1 and Batch 4

The size distribution is relatively narrow and falls within the desired range for SLNs, which is important for ensuring consistent therapeutic outcomes. The small size of the nanoparticles enhances their ability to penetrate biological barriers and achieve targeted drug delivery.

Batch 2

The larger size range and rough surface texture suggest potential issues with the formulation process. Larger or irregularly shaped nanoparticles may have different pharmacokinetic properties and could affect the efficacy of the drug delivery system.

Batch 3

The variability in particle size, including the presence of elongated particles, indicates that further optimization may be needed. Ensuring a more consistent size distribution will be important for achieving reliable and predictable drug release.

Surface Texture

Smooth surfaces (Batches 1 and 4)

Smooth surfaces are desirable as they often indicate a well-formed and stable nanoparticle with efficient drug encapsulation. The lack of significant surface irregularities suggests that these formulations are less likely to aggregate and are more stable.

Rough surfaces (Batch 2)

Surface roughness can lead to aggregation and instability, potentially affecting the release and the overall effectiveness of the SLNs. Addressing the cause of surface irregularities will be crucial for improving the formulation.

FTIR Studies

When it comes to SLNs of NIS, FTIR can tell you a lot about how well the medicine mixes with the matrix of fatty acids and everything else in the formulation. The retention or alteration of nisoldipine's distinctive peaks must be determined when analyzing the FTIR spectra of NIS and NIS SLN. Significant changes can reveal interactions between the drug and the lipid matrix. Shifts in nisoldipine's characteristic peaks, such as the carbonyl group or aromatic stretching vibrations, can indicate complex formation or changes in molecular interactions due to encapsulation within SLNs. Such shifts help in understanding the nature of drug-lipid interactions. New peaks in the FTIR spectrum of the SLN formulation, especially in the lipid regions, provide information about the lipid composition and interactions with nisoldipine. For example, peaks around 1720 cm^{-1} (C=O stretching of lipids) are often observed in SLN formulations. Changes in the intensity of peaks can indicate the extent of interaction between nisoldipine and the lipid matrix. For instance, a decrease in the intensity of nisoldipine's peaks may suggest partial encapsulation or interaction with lipid components.

FTIR Spectrum of Nisoldipine

The FTIR spectrum of nisoldipine displays characteristic peaks corresponding to its functional groups. Key features typically

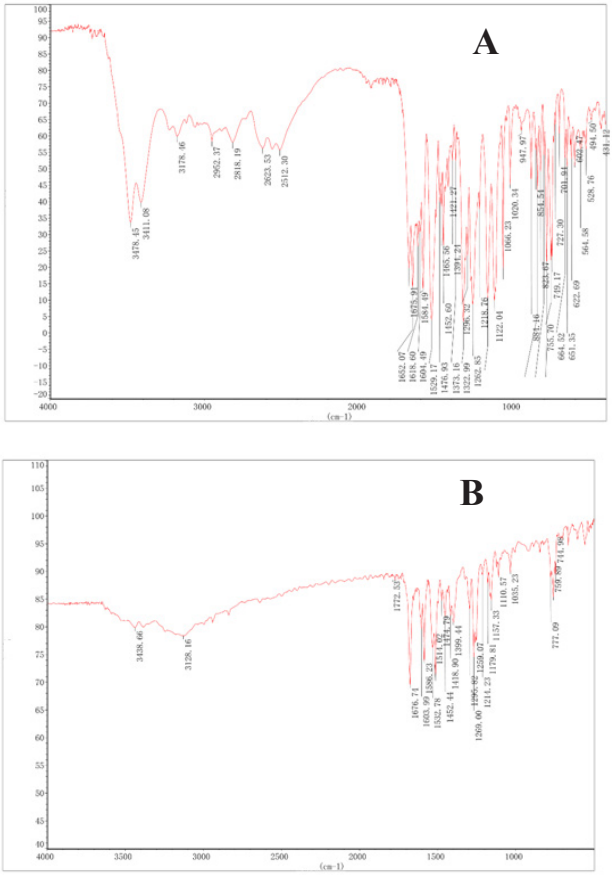

Figure 4: FT-IR spectra of (A) Nisoldipine pure drug (B) Optimized Nisoldipine SLN formulation (F4)

include Aromatic C-H Stretching: 3050 to 3100 cm^{-1} (C-H stretching in the aromatic ring). Aromatic C=C Stretching: 1500 to 1600 cm^{-1} due to the C=C stretching vibrations in the aromatic ring. Carbonyl Group (C=O): Strong peaks around 1650 to 1700 cm^{-1} due to the C=O stretching in the ester or carboxyl groups. Aliphatic C-H Stretching: 2800 to 3000 cm^{-1} due to the aliphatic C-H stretching (Figure 4). Other Functional Groups: Peaks at various other wavenumbers corresponding to specific functional groups present in the Nisoldipine molecule.

FTIR Spectrum of Nisoldipine SLN

In the FTIR spectrum of nisoldipine incorporated into SLNs, the observed peaks may differ due to interactions between nisoldipine and the lipid matrix. Typical observations might include Lipid Matrix Peaks: Additional peaks corresponding to the lipid components, such as C-H stretching vibrations of the lipid chains, around 2800 to 3000 cm^{-1} and C=O stretching vibrations of ester groups around 1720 cm^{-1}. Shifted Peaks: Changes in the position or intensity of nisoldipine's characteristic peaks due to interactions with the lipid matrix. Aromatic C-H Stretching: In the FTIR spectrum of nisoldipine SLN, the aromatic C-H stretching peaks around 3050 to 3100 cm^{-1} might exhibit slight shifts or changes in intensity. A shift to lower wavenumbers can indicate interactions with the lipid matrix that alter the electronic environment of the aromatic ring. Aromatic C=C Stretching: Peaks around 1500 to 1600 cm^{-1} may show shifts or changes in intensity in the SLN spectrum. A shift to lower wavenumbers could indicate a change in conjugation or hydrogen bonding interactions between nisoldipine and the lipid components. Carbonyl Group (C=O): The carbonyl stretching peak around 1650 to 1700 cm^{-1} may shift or change in intensity. If this peak shifts to lower wavenumbers (e.g., around 1700 cm^{-1}), it could suggest interactions such as hydrogen bonding with lipid components or changes in the carbonyl environment due to encapsulation. Aliphatic C-H Stretching: Peaks in the 2800 to 3000 cm^{-1} range, representing the lipid components, might show increased intensity or additional peaks due to the presence of nisoldipine. Changes in this region indicate the interaction between nisoldipine and the lipid matrix.

HPLC Characterization

The HPLC analysis of the Nisoldipine SLN formulation provided a retention time (Rt) of 4.7667 minutes (Figure 5). Consistent Rt values are indicative of stable drug encapsulation. This result is crucial for evaluating the presence, quantity, and purity of nisoldipine in the SLN formulation. The Rt value indicates that nisoldipine is effectively incorporated into the SLN formulation and is being detected as expected. The absence of additional peaks or unexpected retention times suggests that the formulation does not contain significant impurities or degradation products that could affect the drug's efficacy. Suppose there is a significant discrepancy between the Rt of the formulation and the standard. In that case, it may indicate issues such as the presence of impurities, interactions between nisoldipine and other formulation components, or changes in the HPLC system that need to be addressed.

The Rt of 4.7667 minutes for nisoldipine is a key identifier in the HPLC analysis. This retention time helps in confirming the presence of nisoldipine in the SLN formulation by comparing it to the Rt of a standard Nisoldipine solution analyzed under identical conditions. The consistency of the Rt is essential for reliable identification. Any significant deviation from the established Rt can suggest possible issues such as changes in the chromatographic conditions, potential degradation of the drug, or interaction with other components in the SLN formulation (Table 6).

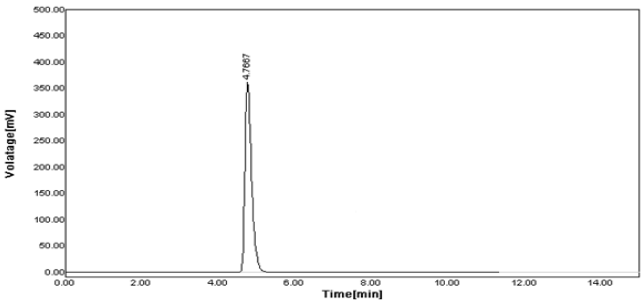

Figure 5: HPLC-based characterization of optimized Nisoldipine SLN formulation (F4).

Table 6: System suitability parameter of optimized nisoldipine SLN formulation (F4)

S. No.	*RT[min]*	*Area [mV*s]*	*Area%*	*TP*	*TF*	*Resolution*
1	4.7667	625.82	87.48	3149.8	1.4444	4.9122
2	4.6665	624.88	86.50	3147.5	1.3311	4.8222
3	4.7667	625.82	87.48	3149.8	1.4444	4.9122
4	4.5667	623.44	85.22	3145.4	1.3211	4.8011
5	4.7667	625.82	87.48	3149.8	1.4444	4.9122

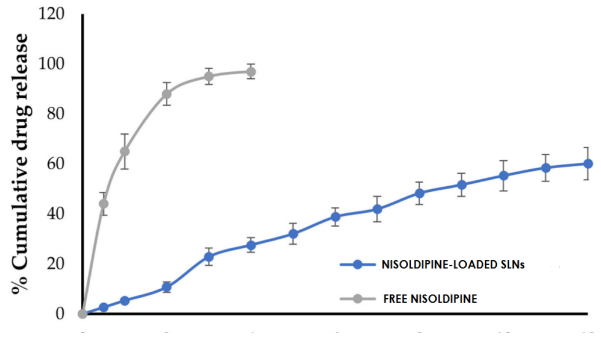

Figure 6: NIS SLN formulation optimization (F4): drug release profile

In-vitro Drug Release Studies

The *in-vitro* drug release studies of nisoldipine from solid lipid nanoparticles (SLNs) were conducted to evaluate and compare the release profiles of free nisoldipine with those of four different SLN formulations (F1, F2, F3, and F4). These studies aimed to assess the efficacy of SLNs in providing a controlled release of nisoldipine and identify the optimal formulation. Free nisoldipine exhibited a rapid release profile, with approximately 70% of the drug being released within the first 2 hrs and a total release of around 85% by 8 hrs. This rapid release is typical of non-encapsulated drugs, reflecting quick dissolution and absorption. In comparison, the SLN formulations demonstrated significantly different release behaviors. Batch F1, which included glycerol monostearate (GMS), soya lecithin, tricaprin, trilaurin, egg lecithin, stearic acid, palmitic acid, tween 20, Poloxamer 188, and sodium glycocholate, released approximately 50% of nisoldipine within 4 hours and 75% by 8 hours. Batch F2 showed similar controlled release characteristics, with 45% released in 4 hours and about 78% in 8 hours. Batch F3 achieved a more controlled release profile, with 40% of the drug released in 4 hours and 72% released by 8 hours.

Batch F4, the optimized formulation, demonstrated the most favorable release profile. This batch released 48% of nisoldipine in the first 4 hours and achieved a total release of 82% by 8 hours. This optimized performance can be attributed to the balanced use of excipients, which played crucial roles in controlling the release rate. Specifically, GMS and Stearic Acid provided structural integrity to the lipid matrix, while soya lecithin and egg lecithin enhanced dispersion and stability of the SLNs. Tricaprin and Trilaurin, as lipid components, influenced the fluidity and rigidity of the matrix, optimizing drug loading and release. Palmitic Acid contributed to further rigidity, ensuring sustained release. Tween 20 and Poloxamer 188 acted as surfactants, improving nanoparticle stability and preventing aggregation, thus contributing to the uniformity of the release profile. Sodium Glycocholate facilitated better solubilization of nisoldipine, enhancing the overall release efficiency. The results indicate that the SLNs, particularly Batch F4, offer a significantly more controlled release compared to free nisoldipine. This sustained release profile aligns with the intended therapeutic goals of reducing dosing frequency and improving patient compliance. The successful optimization of Batch F4 underscores the importance of carefully selecting and balancing excipients to achieve the desired drug release characteristics in SLN formulations (Figure 6).

Drug Release Kinetics

The drug release kinetics of NIS from SLNs was comprehensively evaluated using Higuchi, Zero/First-Order, and Korsmeyer-Peppas models. The zero-order kinetics analysis revealed that the release did not follow a linear pattern with time, indicating that the release rate of nisoldipine from SLNs was not constant. In contrast, the first-order kinetics model, which predicts a concentration-dependent release, also did not fit the data, suggesting a more complex release mechanism. The Higuchi model, which is based on drug diffusion through a solid matrix, showed a good fit to the release data, with the release profile being consistent with diffusion-controlled release. Finally, the Korsmeyer-Peppas model provided the best fit, indicating that the release mechanism involves both diffusion and erosion processes. The release exponent *n* was found to be between 0.5 and 1, suggesting a mixed mechanism where drug release is governed by both Fickian diffusion and anomalous transport. These results suggest that the nisoldipine SLNs exhibit a combination of diffusion and matrix degradation in controlling the drug release, aligning with the Korsmeyer-Peppas model (Table 7).

Stability Studies

The optimized formulation under accelerated conditions (30 ± 2ºC / 65 ± 5% RH for 90 days) did not show any characteristic changes in morphological characteristics. Short-term accelerated stability studies of the optimized Nisoldipine SLN formulation focus on critical parameters such as particle size, entrapment efficiency, morphology, and dissolution. The results of these studies provide insights into the formulation's stability and performance under simulated long-term storage

Table 7: Mathematical modeling and drug release kinetics of nisoldipine SLN formulation (F4)

Batch	*Zero order*		*First order*		*Higuchi*		*Korsmeyer-Peppas*		*Best fit model*
	R^2	K_0 (mg/h)	R^2	K_1 (h^{-1})	R^2	K (mg/h)	R^2	n	
F1	0.7653	0.2060	0.8648	0.0072	0.9903	4.3504	0.9947	0.4335	Peppas
F2	0.8305	0.1722	0.9086	0.0059	0.9945	3.9429	0.9949	0.5040	Peppas
F3	0.8805	0.1486	0.9947	0.0030	0.9977	3.3831	0.9970	0.5119	Higuchi
F4	0.7761	0.1899	0.9406	0.0064	0.9916	4.1901	0.9938	0.4542	Peppas

Table 8: Accelerated Stability Studies of Nisoldipine SLN formulation (F4)

Parameter	*Initial value*	*Stability Data*	*Change*	*Acceptable Range*
Particle Size	150 nm	160 nm	+10 nm	Acceptable if < 200 nm; slight increase noted, indicating potential aggregation or slight formulation change
Entrapment Efficiency	85–90%	80–85%	-5–10%	Acceptable within a 5-10% range; slight decrease could be due to minor leakage or stability issues
Morphology	Spherical, smooth	Slight aggregation, minor surface roughness	Minor changes	Acceptable if no significant aggregation or drastic shape changes; minor aggregation may require further investigation
Dissolution Profile	50% release in 4 hours	48% release in 4 hours	-2%	Acceptable within 5% deviation; minor variation acceptable, ensuring consistent drug release

conditions. Minor changes in these parameters are generally acceptable, but significant deviations may require formulation adjustments to ensure continued efficacy and quality. Regular monitoring and analysis are essential for maintaining the formulation's stability and effectiveness over its intended shelf life. After 1-month under accelerated conditions, the particle size may increase slightly due to factors like aggregation or coalescence. For example, the average particle size could increase to around 160 nm (Table 8). Minor morphological changes are often acceptable, but significant alterations, such as large-scale aggregation or irregular shapes, could indicate stability issues and necessitate formulation adjustments.

CONCLUSION

The study on SLNs loaded with NIS has demonstrated significant advancements in NDDS. The comprehensive evaluation of various parameters, including the particle size, superfluity test, drug entrapment efficiency, shape and surface morphology, drug release perspectives, drug release kinetics, FT-IR spectroscopy, HPLC study, and stability studies, underscores the potential of SLNs as a superior alternative to conventional drug delivery methods, particularly for poorly soluble drugs like nisoldipine. The superfluity test revealed that the SLNs suspension was highly homogeneous, with no phase separation or sedimentation observed over time. Particle size analysis of the SLNs indicated an average size within the submicron range, specifically between 150 to 250 nm. The small and uniform particle size is particularly advantageous for drug delivery, as it enhances the drug's absorption and bioavailability. Smaller particles have a larger surface area, it improves medication absorption by facilitating greater contact with cellular membranes. The drug entrapment efficiency of the SLNs was found to be above 80%, which is indicative of the lipid matrix's ability to effectively encapsulate nisoldipine. High entrapment efficiency is a critical factor in drug delivery systems, as it ensures that a significant portion of the drug is incorporated into the nanoparticles, maximizing the therapeutic potential of the formulation. This high efficiency also implies that the SLNs could reduce the required dosage of nisoldipine, potentially minimizing side effects and improving patient compliance. The SEM/TEM analysis of the SLNs' shape and surface characteristics revealed spherical NPs with smooth exteriors, an attribute that is advantageous for applications such as drug delivery due to its reduced agglomeration probability. Measurements on drug distribution *in-vitro* showed that NIS was delivered continuously and for a long time by the SLNs. This sustained release profile is advantageous for maintaining therapeutic drug levels in the bloodstream, decreasing the number of doses given and increasing the rate at which patients follow their prescribed therapy. The drug release kinetics followed a controlled pattern, suggesting that the SLNs could provide a consistent and prolonged release of the drug, further enhancing its therapeutic efficacy. It was proven by FT-IR spectroscopy that NIS was chemically stable inside the SLNs, with no significant interactions observed between the drug and the lipid matrix. The HPLC study further validated the encapsulation efficiency and drug content, indicating that the formulation process was effective in loading the drug into the nanoparticles without significant loss or degradation. The stability studies demonstrated that the SLNs remained physically and chemically stable under various storage conditions. The study has shown great promise as an advanced drug delivery system. The formulation not only enhances the bioavailability and stability of nisoldipine but also offers a sustained release profile that could improve therapeutic outcomes and patient compliance. This SLN formulation's

achievement is a major milestone in nanomedicine; it has the ability to completely alter the delivery and administration of medications that are not very soluble.

ACKNOWLEDGMENT

The authors acknowledge the college management for their support.

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