RESEARCH ARTICLE

Innovative Proniosomal Formulation of Cilnidipine, Optimization and In-Depth Characterization

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ABSTRACT

This study aims to enhance the solubility and bioavailability of cilnidipine as a dual L/N-type calcium channel blocker for hypertension using proniosomes. Proniosomes are dry powders that turn into niosomes when they come into contact with water, improving drug encapsulation and stability. Different concentrations of Span-60, cholesterol, and sorbitol were tested in the Box-Behnken design. The entrapment efficiency, particle size, zeta potential, thermal characteristics, crystalline structure and *in-vitro* drug release were evaluated for 17 formulations. Entrance effectivity was highest in F3 at 71.83%, while a controlled released rate of over 85.28% was observed within 12 hours. The median particle size showed a moderate stability of -11.2 mV with zeta potentials indicating this fact (902.7 nm). When, cilnidipine was inserted into proniosome systems, considerable changes occurred both thermally and crystallinity-wise according to DSC as well as XRD measurements were taken during an experiment where various mathematical models showed first-order kinetics dominated among other processes involved in drug release along Higuchi's Equation.

Keywords: Cilnidipine, Proniosomes, Hypertension, Optimization, Characterization.

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INTRODUCTION

Calcium channel blockers, as the name suggests, are chemical compounds that block calcium channels in the cells by altering intracellular levels of calcium ions.[1] It belongs to a family of dihydropyridine and is reported to have a molecular weight of 492.51 g/mol. Its structure comprises two functional groups constituting a nitrobenzene moiety and a 1,4-dihydropyridine ring[2] shown in Figure 1. This compound is sparingly soluble in water, with higher solubility values observed in methanol and ethanol, which are common organic solvents. The lipophilic nature of cilnidipine also contributes to its excellent bioavailability and efficient permeation through lipid membranes. However, it is chemically stable under normal conditions but has been reported to be light-sensitive, necessitating proper storage.[3] The result is not only effective treatment for high blood pressure but also prevention against organ damage, especially where there exist chronic kidney disease patients, among other cases that may arise from hypertension-related complications.[4,5]

Non-ionic surfactants, cholesterol, and a stabilizer comprise proniosomes, which hydrate to create a bilayer structure resembling liposomes.[6,7] Proniosomes are superior to traditional vesicular systems primarily because of their increased stability and ease of storage due to their less susceptibility to physical deterioration.[8] Proniosomes are also a desirable alternative for large-scale manufacturing as they may be made with easy and affordable techniques.[9,10] Proniosomes have also been shown in studies to be effective in targeted medication delivery, allowing pharmaceuticals to be delivered directly to specific tissues or cells, enhancing therapeutic efficacy while decreasing systemic toxicity.[11,12] To date, no comprehensive studies have been reported on the formulation and evaluation of Cilnidipine-loaded proniosomes using a systematic optimization approach. While various advanced drug delivery systems have been explored for other calcium channel blockers, cilnidipine proniosomes remain unexplored, presenting a unique innovation opportunity to enhance the drug's therapeutic profile.[13,14]

MATERIAL AND METHODS

Drugs and Reagents

The development of cilnidipine proniosomes involved sourcing critical materials from reputable suppliers. Cilnidipine, obtained from Hetero Labs, serves as the active pharmaceutical ingredient. Cholesterol, Span-60 (surfactant), sorbitol (carrier), ethanol, and phosphate buffer saline (pH 6.8) were all procured

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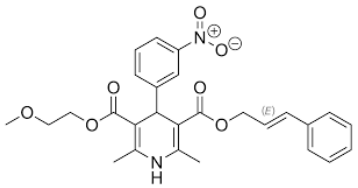

Figure 1: Chemical structure of clinidipine

from SD Chemicals, Mumbai. Cholesterol and Span-60 are essential for forming the bilayer structure of the proniosomes, enhancing stability and drug encapsulation. Sorbitol acts as a carrier, aiding in the formation and stability of the proniosomes. Ethanol is used as a solvent for dissolving cholesterol, diacetyl phosphate (DCP), and surfactant, while phosphate buffer saline (pH 6.8) is employed to dissolve Cilnidipine and sorbitol.

Zeta Potential, Surface Morphology and Particle Size

The surface shape, particle size, and zeta potential of proniosomes loaded with cilnidipine were described using various analytical methods. A limited number of proniosomes were affixed to a sample holder using double-sided adhesive tape to study surface morphology. The distribution curve, mean size, and polydispersity index (PI) were recorded. Pictures were taken by scanning electron microscopy at various amplifications to assess surface structure and form.

Differential Scanning Calorimetry

To analyse cilnidipine-loaded proniosomes, take about 1-mg of proniosomal powder for the differential scanning calorimetry (DSC) technique and put it into a sealed aluminum DSC pan. Use an empty aluminum pan as a reference. The DSC equipment heats the sample in an inert nitrogen environment up to 300°C at a rate of 10°C per minute after calibration with standard materials is done from 30°C. Heat flow is measured as a function of temperature to detect thermal events during the run, with the onset, peak, and end set temperatures and enthalpy changes noted. The generated thermograms are examined by contrasting them with the pure medication and excipients to evaluate cilnidipine's thermal behavior, stability, and interactions inside the proniosomal formulation.

X-ray Diffraction

The solid-state properties of proniosomes filled with cilnidipine were analyzed using X-ray diffraction. The XRD sample holder held 10 mg of the powdered proniosomal. Cu Kα radiation was used to operate a Phillips X-ray diffractometer scanning at 40 kV and 20 mA. Scanning speed was set at 2° per minute over a 2Theta range from 5° to 50°. The diffraction patterns were recorded and studied to identify crystalline peaks, which indicate Cilnidipine's crystalline or amorphous nature in niosomes. Comparing these against those produced by pure drug substances and other excipients used in formulation could give insight into changes brought about in structure and interactions within this system.

Entrapment Efficiency

An exact quantity of the niosomes (equal to 10 mg of Cilnidipine) was weighed, diluted in a small amount of ethanol, and thoroughly mixed to ascertain the entrapment effectiveness of Cilnidipine-loaded proniosomes. After that, the mixture was sonicated with an Ultrasonicator for around ten minutes. After being sonicated, the solution was sonicated again until it turned transparent. Then, a 100 mL volumetric flask was filled to the top with phosphate-buffered saline (pH 6.8). The drug concentration in the filtrate was measured using a UV-visible spectrophotometer set at 241 nm after filtering the dispersion through a Whatman membrane filter of 0.45 µm.

***In-vitro* Drug Release**

Cilnidipine was released from niosomes *in-vitro* using the Franz-diffusion cell method. Cellophane dialysis membranes soaked in distilled water for half a day were used as the diffusion cells. In the receptor compartment, 6.8 pH phosphate buffer saline (pH 6.8) of volume 10 mL was kept while stirring continuously at 37 ± 0.5°C. The donor compartment contained a Niosome solution sample of volume 2 mL. Every once in a while, 1-mL of aliquots was taken from the receptor section, and an equal amount of fresh buffer was replaced. These aliquots were then further diluted as required, and their quantities were read using a UV spectrophotometer set at 241 nm to determine how much drug had been released. This process offered a systematic and controlled way to assess the cilnidipine release profile from the niosomes.

RESULTS AND DISCUSSION

The study used a Box-Behnken design to optimize the formulation of cilnidipine-loaded proniosomes, focusing on three factors: Cholesterol (A), Span-60 (B), and sorbitol (C), across seventeen different formulations (F1-F17). The responses measured for each formulation included entrapment efficiency, drug release at 12 hours (%), and particle size (nm). Formulation F3, which contained the highest cholesterol (50%) and sorbitol (30%), achieved the highest entrapment efficiency. The greatest drug release at 12 hours (99.98%) was observed in formulation F14, which had the lowest cholesterol (5%) and the highest Span-60 (2%). Particle size varied widely across formulations, with the smallest size recorded in formulation F16 (486 nm) and the largest in formulation F3 (812 nm). These findings highlight how varying concentrations of cholesterol, Span-60, and sorbitol influence the entrapment efficiency, drug release, and particle size of the proniosomes.

Surface Morphology

Drug crystals and niosomes were distributed during the SEM investigation. The study also showed that niosomes had asymmetrical surfaces. Crystals formed from non-noisome drug crystals (Figure 2).

Particle Size and Distribution Study

The measurement findings for the formulation sample's particle size analysis (NX-Size) may show a wide range of particle sizes. The investigation, which was carried out at a holder temperature of 25.1°C and a scattering angle of 173 degrees, showed three separate peaks with mean diameters of 1508.5, 3.0, and 0.8 nm. The model is 1637.0 nm, while the median

Table 1: Variables and their ranges were used to optimize the formulations of proniosomes

Name	*Units*	*Type*	*Minimum*	*Maximum*	*Coded low*	*Coded high*	*Mean*	*Std. Dev.*
Cholesterol	%	Numeric	5.00	50.00	-1 ↔ 5.00	+1 ↔ 50.00	27.50	15.91
Span-60	%	Numeric	0.5000	2.00	-1 ↔ 0.50	+1 ↔ 2.00	1.25	0.5303
Sorbitol	%	Numeric	10.00	30.00	-1 ↔ 10.00	+1 ↔ 30.00	20.00	7.07

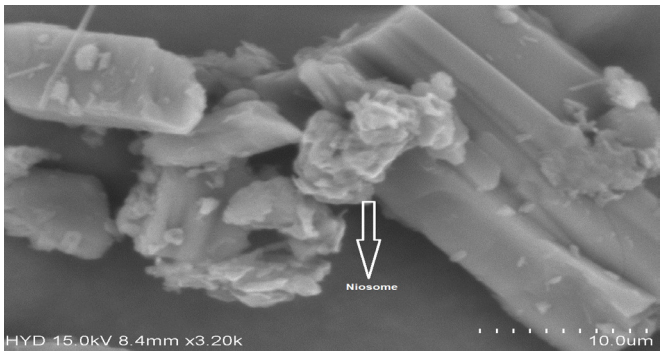

Figure 2: SEM study of optimized formulation of clinidipine

particle size is 902.7 nm. About 90% of the particles are less than 3864.3 nm, and 50% of the particles are smaller than 902.7 nm, according to the cumulative data. With a polydispersity index (PI) of 0.529 and a Z-average particle size of 829.7 nm, a heterogeneous distribution is suggested. The distribution and frequency of particle sizes are reflected in the histogram and cumulative curves, which draw attention to the substantial proportion of bigger particles in the sample. Particle size and dispersion analysis of the improved formulation is shown in Figure 3.

Zeta Potential Estimation

The zeta potential indicated the colloidal system's stability, which had a mean value of -11.2 mV and an electrophoretic mobility mean of -0.000087 cm²/V·s. At the time of the measurement, the electrode voltage was 3.3 V. The distribution of zeta potential values, centred around -11.2 mV, is depicted in the graphical representation at the bottom. This indicates that the system is moderately stable, with a minor inclination toward aggregation. The narrow apex in the graph shows that the zeta potential is distributed among the particles in a relatively uniform manner. The Zeta potential estimation of the optimized formulation is illustrated in Figure 4.

DSC of Study of Optimized Formulation

The study demonstrates a substantial endothermic peak with an onset temperature of 93.17°C, a peak temperature of 99.97°C, with an end set temperature of 103.81°C. With a normalized value of -160.13 J/g, the integral of the peak is -800.64 mJ, suggesting that melting occurred at the phase transition. This strong endothermic peak offers information about the formulation's thermal stability and heat-related behavior by pointing to a clearly defined phase transition unique to the constituent parts. Figure 5 shows the optimized formulation's DSC thermogram.

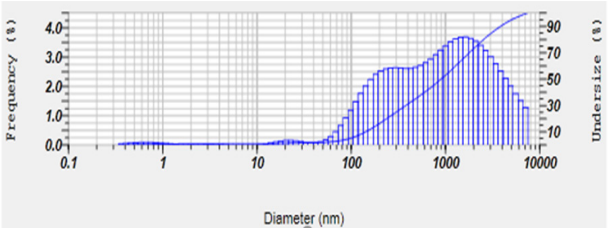

Figure 3: Particle size and distribution study of optimized formulation

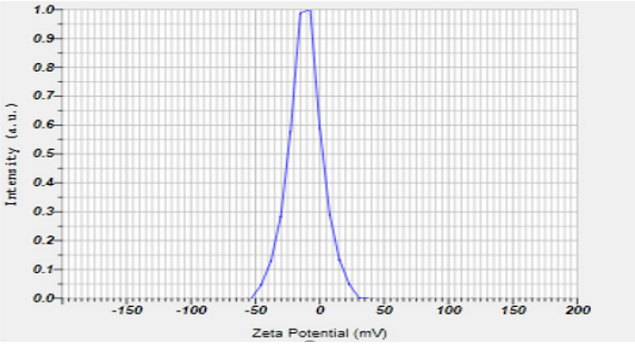

Figure 4: Zeta potential estimation of optimized formulation

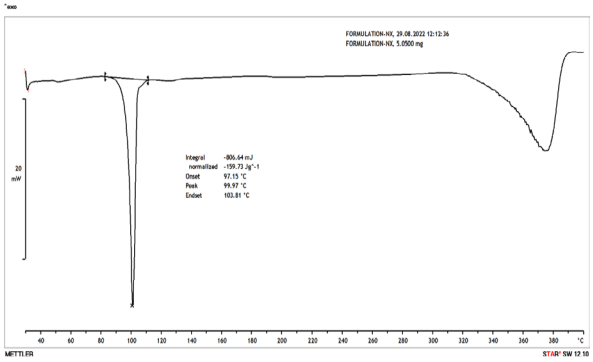

Figure 5: DSC thermogram of optimized formulation

X-ray Diffraction

The graph plots count versus the 2Theta angle (Coupled Two Theta/Theta) with a wavelength of 1.54060 Å. Key peaks are observed at 2Theta values of 11.61, 13.886, 18.68, 22.62, and 25.49°, with the most intense peak at 18.68° reaching nearly 50,000 counts. The pattern also shows numerous smaller peaks throughout the range, suggesting a complex crystalline structure with multiple phases. This detailed diffraction pattern provides crucial information on the crystallographic structure of the formulation, which is essential for understanding its physical properties and stability. Figure 6 illustrates the XRD spectra of the optimized formulation.

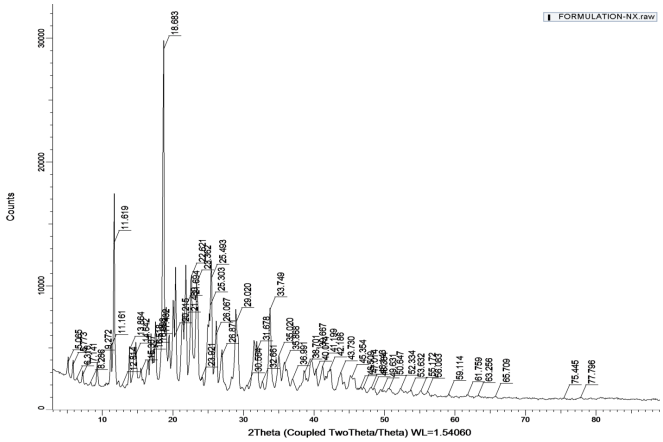

Figure 6: Optimal formulation XRD spectra

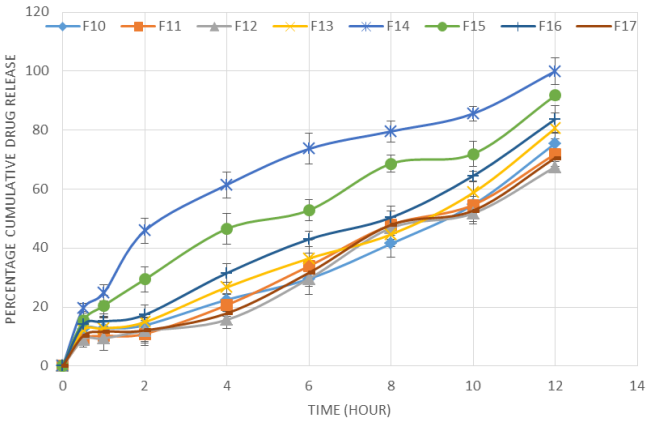

Figure 8: *In-vitro* evaluation of niosomes F1-F9 was conducted to assess their performance and characteristics

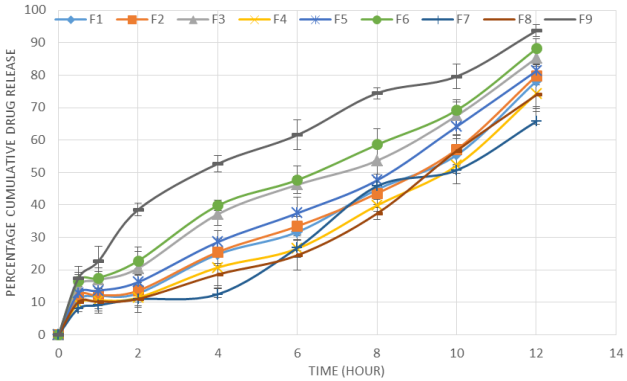

Figure 7: *In-vitro* evaluation of niosomes F1-F9 was conducted to assess their performance and characteristics

Entrapment Efficiency

Entrapment efficiency (EE) percentages for seventeen different formulations (F1-F17) of cilnidipine-loaded proniosomes. Entrapment efficiency measures the effectiveness of the proniosomes in encapsulating the drug. The values range from 45.58 to 71.83%. The highest EE is observed in formulation F3 (71.83%), suggesting optimal drug encapsulation conditions among the tested variables. Conversely, the lowest EE is found in formulation F14 (45.58%), indicating less efficient drug encapsulation. Several formulations, such as F5 (62.28%), F6 (61.36%), and F12 (63.35%), also show relatively high EE, demonstrating exemplary performance in encapsulating cilnidipine.

In-vitro Drug Release

For 12 hours, the in-vitro release of drugs from nine different forms (F1-F9) of Cilnidipine-loaded proniosomes. Each time point shows the percentage of drug released (mean ± standard deviation) at specific intervals: 0, 0.5, 1, 2, 4, 6, 8, 10, and 12 hours. Initially, all formulations have no drug release (0%). At 0.5 hours, F9 shows the highest release (17.46 ± 3.6%), while F7 has the lowest (8.04 ± 2.5%). As time progresses, the drug release increases for all formulations, with F9 consistently demonstrating the highest release rates, reaching 93.68 ± 1.8% at 12 hours. In contrast, F7 shows the slowest release, reaching 65.74 ± 4.7% at 12 hours. Formulation F3 also exhibits a high release profile, achieving 85.28 ± 1.9% by the end of the 12-hour period. These results highlight significant differences in the drug release rates among the formulations, influenced by their varying compositions**.** Figure 7 displays the evaluation study of niosomes F1-F 9 *in-vitro.*

Over 12 hours, this study analyses *in-vitro* drug release profiles for eight cilnidipine-loaded proniosome formulations (F10-F17). Each time point shows the percentage of drug released (mean ± standard deviation) at specific intervals: 0, 0.5, 1, 2, 4, 6, 8, 10, and 12 hours. Initially, no drug release exists for any formulation (0%). By 0.5 hours, formulation F14 shows the highest release (19.53 ± 1.7%), while F12 has the lowest (8.99 ± 1.7%). As time progresses, the drug release increases for all formulations, with F14 consistently demonstrating the highest release rates, reaching nearly complete release at 12 hours (99.98 ± 4.5%). Formulation F15 also shows a high release profile, achieving 91.77 ± 1.6% at 12 hours. On the other hand, F17 and F11 exhibit slower release profiles, reaching 70.58 ± 2.9% and 71.93 ± 5.2%, respectively, by the end of the 12 hours. These results highlight the differences in drug release rates among the formulations, influenced by their distinct compositions, with some formulations achieving rapid release and others providing a more sustained release over the 12 hours. Figure 8 displays the evaluation study of niosomes F10-F17 *in-vitro.*

Kinetics Study

Different mathematical models were employed to evaluate the release kinetics of a number of formulations (F1-F17) for Cilnidipine-loaded proniosomes. The models used were Higuchi, Korsmeyer-Peppas, First-order, Zero-order, and Hixson-Crowell. The R^2 values indicate the goodness of fit for each model. Most formulations exhibit higher R^2 values for the First-order and Higuchi models, suggesting that these models better describe the drug release kinetics. Specifically, formulations F9 and F14 show the highest R^2 values in the Higuchi model (0.995 and 0.988, respectively), indicating a strong correlation with this model. The Hixson-Crowell model

also shows high R^2 values for most formulations, indicating surface area and diameter changes over time. The Korsmeyer-Peppas model provides the n value, representing the release mechanism, with formulations such as F9 and F14 showing higher n values (0.249 and 0.248, respectively), indicating a combination of diffusion and erosion mechanisms. These results suggest that the drug release from the proniosomes is primarily governed by First-order and Higuchi kinetics, with contributions from other mechanisms as described by the Hixson-Crowell and Korsmeyer-Peppas models.

CONCLUSION

The study successfully developed and characterized cilnidipine-loaded proniosomes, demonstrating significant improvements in drug solubility, stability, and controlled release profiles. The Box-Behnken design optimization led to formulations with high entrapment efficiencies and favorable release kinetics, particularly the optimized formulation F3, which showed the highest entrapment efficiency and a sustained release profile. The particle size analysis, zeta potential, thermal properties, and crystalline structure confirmed cilnidipine's stability and effective encapsulation in the proniosomes. *In-vitro* release studies highlighted the ability of these proniosomes to provide a controlled and sustained drug release, making them a viable alternative to conventional drug delivery systems. These findings indicate that cilnidipine proniosomes can significantly enhance drug delivery and therapeutic efficacy for hypertension management. Future research should focus on scaling up production and conducting clinical trials to validate these promising results and further establish the clinical benefits of cilnidipine proniosomes in treating hypertension and related cardiovascular disorders.

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