Letters in Applied NanoBioScience
Open-Access Journal (ISSN: 2284-6808)

Article
Volume 14, Issue 3, 2025, 136
https://doi.org/10.33263/LIANBS143.136

Development and Evaluation of a Proniosomal Gel for Enhanced Transdermal Delivery of Nebivolol Hydrochloride

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Received: 7.03.2024; Accepted: 30.06.2024; Published: 6.09.2025

Abstract: This research introduces a cutting-edge generation of pro-vesicular drug delivery systems known as proniosomes, which are crafted from a blend of non-ionic surfactants, lecithin, and cholesterol. These components ingeniously transform into niosomes upon hydration, with the objective of establishing a reliable and enduring transdermal delivery system for nebivolol hydrochloride. The project entailed the development, optimization, and examination of a proniosomal gel designed for topical application aimed at enhancing the transdermal delivery of nebivolol hydrochloride. The concoction process incorporated Lutrol F68 and lecithin for surfactant purposes, cholesterol to aid in stabilization, and was completed with minimal additions of ethanol and water. The proniosomal gel, imbued with nebivolol hydrochloride, underwent stability testing, and the niosomes that emerged from this formulation were scrutinized for their morphological, size, zeta potential, and entrapment efficiency characteristics. These analyses verified their compatibility with skin applications. The creation of the proniosomal gel utilized the coacervation phase separation approach, followed by in vitro evaluations to measure nebivolol hydrochloride's permeation through freshly obtained rat skin samples. Findings from these permeation studies revealed a pronounced improvement in nebivolol hydrochloride delivery using the proniosomal gel as opposed to a conventional 1.0% pure gel. Remarkably, the NPG5 batch showcased the most significant entrapment efficiency, achieving up to 95.5%, and displayed sustained drug release over a 24-hour timeframe. The proniosomal gel's skin permeation rate was quantified at 75.5%. These results received further validation through Scanning Electron Microscopy (SEM) and zeta potential analysis, reinforcing the efficacy of the proniosomal gel as a transdermal drug delivery vehicle.

Keywords: proniosomes; nebivolol hydrochloride; lecithin; entrapment efficiency; gel.

1. Introduction

Nebivolol hydrochloride (NH) is a unique pharmacological entity among beta-blockers, distinguished by its dual mechanism of action. It combines beta-1 adrenergic receptor blockade with nitric oxide (NO)-mediated vasodilation, thereby offering a therapeutic profile that is beneficial for hypertensive patients with comorbid conditions [1-3]. NH is extensively prescribed for the management of hypertension and is valued for its efficacy in lowering blood pressure, improving endothelial function, and offering cardiovascular protection. Its distinct mechanism contributes to hemodynamic advantages and minimizes the adverse effects typically associated with conventional beta-blockers, such as reduced cardiac output and bronchoconstriction [4-6].

The primary objective in the formulation of a proniosomal gel for NH is to create a system that enhances the drug's bioavailability through the skin, offering a sustained release profile that could potentially minimize systemic side effects and bypass hepatic first-pass metabolism. This involves selecting appropriate non-ionic surfactants, co-surfactants, and cholesterol to form stable proniosomes that can effectively encapsulate NH, ensuring its stability and permeability through the skin [7-8].

Formulating proniosomes as a dry preparation, necessitating rehydration before the application, is an effective strategy for maintaining their physical stability and the integrity of the vesicles [9]. For transdermal drug delivery, proniosomes are engineered into a gel format that is appropriate for topical use. Upon application, this proniosomal gel utilizes the water present in the skin to reconstitute into niosomes [10-12]. The bilayer constituents, comprising phospholipids and non-ionic surfactants within the proniosomes, function as penetration enhancers, facilitating the effective delivery of the drug through the skin barrier [13-14].

In this study, efforts were made to create a nebivolol hydrochloride transdermal proniosomal gel designed to sustain the medication's release to targeted tissues. The goal is to enhance patient adherence to the treatment regimen and mitigate the potential gastrointestinal side effects associated with nebivolol hydrochloride.

2. Materials and Methods

2.1. Materials.

The active pharmaceutical ingredient, nebivolol hydrochloride, was generously provided as a sample by MSN Laboratories Ltd., located in Hyderabad, India. The excipients used, Lutrol F68 (Poloxamer 188) and Lipoid-80 H (Lecithin: soybean phosphatidylcholine), were sourced from UV Scientific, also in Hyderabad. Additionally, Carbopol 974P NF was acquired from BASF, Mumbai, India. All other chemicals and reagents utilized in this research were of analytical grade, ensuring the precision and reliability of the study outcomes.

2.2. Methods.

2.2.1. Preparation of nebivolol hydrochloride-loaded proniosomal gel.

Proniosomes loaded with nebivolol hydrochloride were synthesized using the coacervation phase separation technique as outlined by Vora and colleagues. Initially, a mixture of the non-ionic surfactant Lutrol F68, Lipoid-80 H, and cholesterol was prepared in a 9:9:6 weight ratio, accompanied by an appropriate quantity of ethanol, within a wide-mouthed

vial. To this blend, 1% w/w nebivolol hydrochloride was introduced, and the vial was securely sealed to inhibit the evaporation of the solvent. The mixture was then gently heated in a water bath maintained at a temperature of 65 ± 2°C for a duration of 10 minutes or until the components were fully dissolved. Following this, the mixture was allowed to return to ambient temperature. Subsequently, an aqueous phase, specifically a phosphate buffer with a pH of 6.8, was incrementally added to the vial, which was then placed back in the water bath for an additional 5 minutes or until a clear solution was achieved. This solution was left to cool at room temperature, ultimately forming the proniosomal gel [15-19].

2.2.2.1. Formulation of pure nebivolol hydrochloride-loaded topical gel (PG).

Carbopol-974P was carefully blended with approximately 25 ml of distilled water, employing mechanical agitation to ensure a homogenous mixture. To this mixture, methylparaben (0.2%) and propylparaben (0.02%) were first dissolved in ethanol and subsequently integrated into the Carbopol solution under continuous stirring. This concoction was then allowed to rest overnight to facilitate the full hydration of the Carbopol polymer. Following the hydration period, nebivolol hydrochloride was introduced into the mixture at a concentration of 1% w/w, with stirring maintained to promote uniform drug dispersion within the gel. The pH of the polymer mixture was adjusted to the desired level through the cautious, dropwise addition of triethanolamine. After complete mixing, the preparation yielded a finely textured gel.

2.2.2.2. Drug diffusion and drug entrapment effect of bilayer composition.

To investigate the effect of various factors on the characteristics of proniosomes, several batches were prepared, with the concentration of the non-ionic surfactant Lutrol F68 and cholesterol being the primary variables under study. The research design was based on a 3^2-factorial methodology, as outlined in Table 1. The level of Lipoid-80 H was kept constant in all formulations, as specified in Table 2. Entrapment efficiency (EE) and drug diffusion rate were selected as the dependent variables for this study. This approach enabled an evaluation of how changes in the quantities of Lutrol F68 and cholesterol affect these crucial aspects of proniosome performance [20-21].

Lutrol F68 and cholesterol were combined with alcohol and an aqueous solution to create a semi-solid gel formulation that encapsulates nebivolol hydrochloride, known as a proniosomal gel. This gel is capable of transforming into a niosomal dispersion when diluted with an ample aqueous phase and subjected to mild agitation. The process leading to the proniosomal gel's formation involves the initial creation of a lamellar liquid crystal phase. This phase emerges from the interaction between the non-ionic surfactant (Lutrol F68) and lecithin (Lipoid-80 H) upon reaching the kraft temperature, facilitated by a small amount of alcohol. Upon the introduction of excess water, this crystalline structure transitions into a niosomal dispersion.

The proniosomal gel is designed to draw moisture directly from the skin, initiating instant hydration. This unique hydration mechanism helps circumvent common stability challenges faced by aqueous niosome dispersions, such as vesicle fusion, aggregation, and content leakage. This approach not only enhances the stability of the drug delivery system but also ensures a more efficient and controlled release of the active pharmaceutical ingredient onto the skin.

Table 1. Experimental design layout.

Formulation code	X_1	X_2
NPG-1	-1	+1
NPG-2	-1	0
NPG-3	-1	-1
NPG-4	0	+1
NPG-5	0	0
NPG-6	0	-1
NPG-7	+1	+1
NPG-8	+1	0
NPG-9	+1	-1
CCNPG-1	-0.5	-0.5
CCNPG-2	+0.5	+0.5

Table 2. Formulae for nebivolol hydrochloride proniosomal gel (NPG) batches.

Formulation	Nebivolol hydrochloride in mg	Lutrol F68 in mg	Cholesterol in mg	% entrapment efficiency (%EE)	% drug diffused	Vesicle size (nm)	Polydispersity index (PDI)
NPG-1	8	80	80	65.36 ±0.89	96.73±0.23	652.4±20.41	1.152 ± 0.20
NPG-2	8	80	60	82.12±0.91	92.52±1.83	445.1±30.15	1.326 ± 0.10
NPG-3	8	80	40	84.25± 1.36	94.12±0.22	712.2±20.22	1.834 ± 0.15
NPG-4	8	90	80	89.53± 1.02	95.23±0.56	703.5±24.50	0.934± 0.12
NPG-5	8	90	60	98.41± 0.34	98.72 ± 1.15	308.0±10.32	0.918± 0.12
NPG-6	8	90	40	94.67± 1.07	97.52 ± 0.43	449.1±26.41	0.921± 0.10
NPG-7	8	100	80	81.12±0.82	92.52±1.83	445.1±30.15	1.326 ± 0.10
NPG-8	8	100	60	83.25± 1.31	94.12±0.22	712.2±20.22	1.834 ± 0.15
NPG-9	8	100	40	80.22± 1.00	95.23±0.56	703.5±24.50	0.934± 0.12
CCNPG-1	8	85	50	78.89±0.18	95.14±0.61	645.4±5.16	1.291±0.61
CCNPG-2	8	95	70	77.18±0.41	94.49±0.92	643.3±15.11	1.209±0.05

*Data expressed as Mean ± SD; n=3.

2.3. Characterization of nebivolol hydrochloride proniosomal gel.

2.3.1. Entrapment efficiency.

To accurately prepare a sample for analysis, a precise amount of 0.1 g of the proniosomal gel is measured and transferred into a glass tube. To this, 10 ml of an aqueous solution, specifically a phosphate buffer with a pH of 6.8, is added. This mixture is then subjected to sonication for a duration of 5 minutes using an ultrasonicator, a step crucial for ensuring the thorough dispersion of the gel and facilitating the transformation of the proniosomal gel into niosomes. Following the sonication process, the mixture, now containing nebivolol-loaded niosomes, undergoes centrifugation at 10,000 rpm for 45 minutes using a REMI centrifuge. This centrifugation is designed to segregate the niosomes encapsulating nebivolol from any non-encapsulated drug remaining in the suspension [22-25].

The supernatant, which holds the unentrapped nebivolol, is then carefully extracted for further analysis. The concentration of nebivolol within this supernatant is quantitatively determined utilizing a UV spectrophotometer (Merck, Thermoscientific Evolution 201, Shanghai, China), set to a wavelength of 282 nm, with the phosphate buffer pH 6.8 serving as the reference blank [27-30]. The calculation of the drug's entrapment efficiency (EE) within the niosomes is subsequently performed by employing the specified equation.

$$\text{EE}\ (\%) = \frac{\text{Entrapped drug}}{\text{Total drug added}} \text{X}\ 100 \qquad (1)$$

2.3.2. Percentage drug diffusion.

For the diffusion studies, a Franz diffusion cell apparatus was utilized. A dialysis membrane was securely affixed onto the diffusion cell, creating a barrier for the test substance. A predetermined quantity of the proniosomal gel was carefully placed on one side of this membrane. The opposite side, known as the receptor compartment, was filled with 10 ml of phosphate buffer saline with a pH of 6.8 (PBS). This arrangement ensures a controlled environment for the diffusion study.

To maintain a consistent mixing and facilitate the diffusion process, the solution within the receptor compartment was stirred at a constant speed of 100 rpm using a Teflon-coated magnetic bead. Over a duration of 24 hours, 1 ml samples were systematically extracted from the receptor compartment every 60 minutes. Each of these samples was then subjected to analysis using a UV spectrophotometer, specifically set to a wavelength of 282 nm, to determine the concentration of the diffused substance.

To compensate for the volume of solution removed during sampling, fresh phosphate buffer saline (pH 6.8) was added back into the receptor compartment, ensuring the volume within the compartment remained constant throughout the experiment. This meticulous replacement process is crucial for maintaining the integrity of the diffusion conditions over the 24-hour observation period [26-33].

2.3.2. Particle size and polydispersity index.

Photon correlation spectroscopy (PCS), employing a Nanophox device at ambient temperature, was utilized to ascertain the mean particle size and size distribution of the drug-laden proniosomal gel following its hydration with phosphate buffer saline (PBS) at pH 6.8. The resultant nebivolol niosomal dispersion was diluted with filtered double-distilled water. This dilution step is essential to mitigate Multiple Scattering events that could otherwise skew the accuracy of particle size measurements. The breadth of the size distribution, an important parameter for characterizing the uniformity of particle sizes within the dispersion, was quantified by the Polydispersity Index (PDI) [34-36]. The PDI is calculated using a specific formula that reflects the degree of size variation within the sample, offering insights into the consistency and stability of the niosomal formulation.

$$\mathrm{PDI} = \frac{(\mathrm{X90}-\mathrm{X10})}{\mathrm{X50}} \qquad (2)$$

2.3.4. Scanning electron microscopy.

The morphology of the proniosomes, post-hydration with phosphate buffer saline (PBS) at pH 6.8, was meticulously analyzed through a preparation process involving gold ion coating for a duration of 5 minutes. This coating enhances the conductivity of the samples, making them suitable for high-resolution imaging. Following this preparatory step, images were captured using a scanning electron microscope (SEM), specifically the Hitachi S-3700N model located in Tokyo, Japan. The SEM was operated at an accelerating voltage of 20 kV, enabling the detailed visualization of the surface structures and morphological characteristics of the hydrated proniosomes. This imaging technique provides critical insights into the physical attributes of the proniosomes, including their shape, size, and surface topology, which are essential for understanding their behavior in biological systems.

2.3.5. Zeta potential analysis.

The surface charge of drug-loaded vesicles was accurately measured using a Zeta potential analyzer, specifically the Horiba SZ-100 and Malvern Instruments Nano ZS from the U.K. To ensure precision in the measurement, the analysis duration was set to 60 seconds. This timeframe was chosen to provide a balance between acquiring a stable measurement and minimizing potential changes in the sample during the analysis. The average zeta potential, which is a key indicator of the surface charge and stability of the vesicular system, was determined after hydrating the proniosome preparation with phosphate buffer saline (PBS) at pH 6.8. The measurements were conducted at a controlled temperature of 25°C to ensure consistency and repeatability of the results. To enhance the reliability of the data, three separate runs were performed, allowing for the calculation of an average value and an assessment of the variability within the measurements. This methodological approach ensures a comprehensive understanding of the electrostatic properties of the proniosomes, which are critical for predicting their behavior in biological environments.

2.3.6. Differential scanning calorimetry.

The thermal behavior of nebivolol hydrochloride proniosomes, post-hydration with PBS pH 6.8, was analyzed through differential scanning calorimetry (DSC) using a Shimadzu DSC 60 apparatus. This procedure involved examining 10 mg samples of the proniosomal gel, which were encapsulated in conventional aluminum pans, to ensure a consistent analysis environment. The thermal profiles, or thermograms, for both the hydrated nebivolol proniosomes and the raw nebivolol powder were recorded. This was executed at a uniform heating rate of 10°C/min, spanning a broad temperature spectrum from 0 to 400°C, under a steady flow of nitrogen at 20 ml/min.

This DSC analysis is instrumental in revealing the thermal transitions, such as melting points and crystallization behavior, of the proniosomes compared to the unformulated drug. The introduction of a nitrogen atmosphere is a critical step in avoiding oxidation and other reactions influenced by air, thereby ensuring the integrity of the data collected. This investigation aids in understanding the stability and the physicochemical interactions within the proniosomal system, which are vital for optimizing formulation strategies and predicting shelf life.

2.3.7. X-ray diffraction.

The solid-state properties of nebivolol hydrochloride proniosomes, following hydration with phosphate buffer saline (PBS), were scrutinized using X-ray diffraction (XRD) analysis. This technique provides insights into the crystalline structure and phase purity of materials. The investigation encompassed both the bulk nebivolol hydrochloride and the drug-encapsulated proniosomal dispersion. A Shimadzu XRD-7000 X-ray diffractometer, fitted with an X-ray generator set to operate at 45 kV and 4 mA, was employed for this purpose.

The scanning procedure was conducted at a speed of 4° per minute. This careful and deliberate scanning pace allows for the detailed observation of diffraction patterns, which are pivotal for identifying the crystalline or amorphous nature of the substances under study. By comparing the XRD patterns of the hydrated proniosomes with those of the bulk drug, researchers can determine any alterations in the drug's solid-state form induced by the

proniosomal formulation process. Such information is crucial for understanding the stability, solubility, and bioavailability of the drug within the proniosomal system.

2.3.8. Ex vivo skin permeation studies.

Ex vivo skin permeation studies were meticulously conducted utilizing a modified Franz diffusion cell setup, focusing on the abdominal skin of male albino Wistar rats, each weighing approximately 250 ± 20 g. The experimental design ensured that the skin was secured in such a manner that its dermal side faced the receptor medium directly. This medium, phosphate buffer saline (PBS) with a pH of 6.8, filled the receptor chamber, which had a cross-sectional area of 4.32 cm^2.

The proniosomal gel was evenly applied to the dorsal side of the rat skin. Following this, the donor compartment was carefully positioned, and the entire setup was maintained at a constant temperature of 37°C ± 0.5°C, with a stirring speed set to 100 rpm. This controlled environment is essential for closely simulating human skin conditions.

After a specific period of 18 hours, a 1 ml sample was drawn from the receptor phase for analysis. This sample was then evaluated for the percentage of drugs that had permeated through the skin from the applied formulations. The analysis was performed using a UV spectrophotometer set at a wavelength of 282 nm. This process provides valuable insights into the efficiency of the proniosomal formulation in facilitating the transdermal delivery of the drug.

3. Results and Discussion

3.1. Effect of bilayer composition on drug diffusion and drug entrapment.

Surfactants play a pivotal role in the formation of niosomal vesicles, with their concentration significantly impacting the efficiency with which drugs are entrapped within these structures. Research indicates that altering the concentration of the surfactant Lutrol F68 affects the entrapment efficiency of the niosomes. Specifically, increasing the surfactant concentration from 80 to 90 mg results in a notable enhancement in entrapment efficiency. This improvement can be attributed to the increased formation of niosomes, leading to a larger hydrophobic domain within which drugs can be encapsulated, thereby elevating entrapment efficiency.

Contrarily, a subsequent increase in surfactant concentration from 90 to 100 mg (noted in formulation F9) causes a reduction in entrapment efficiency, which drops to 80.22 ± 1.00%. This decline is theorized to stem from the formation of mixed micelles alongside niosomal vesicles at higher surfactant concentrations. These micelles, typically smaller than 10 nm, have a reduced capacity for drug encapsulation compared to niosomes, which likely contributes to the observed decrease in entrapment efficiency.

Given these observations, it becomes evident that a surfactant concentration threshold exists, beyond which the formation of mixed micelles diminishes the overall entrapment efficiency of the niosomal system. Therefore, based on the data, a surfactant quantity of 90 mg is identified as the optimal concentration for achieving maximum entrapment efficiency in the proniosomal gel formulation. This finding underscores the importance of carefully optimizing surfactant concentrations in the development of niosomal drug delivery systems to ensure efficient drug encapsulation and subsequent therapeutic efficacy.

The concentration of cholesterol significantly influences the drug entrapment efficiency within vesicles, highlighting its critical role in the formation and stability of niosomal structures. An increase in cholesterol content from 40 mg (in formulation F6, with an entrapment efficiency of 94.67%) to 60 mg (in batch NPG5, achieving an entrapment efficiency of 98.41%) markedly enhances the entrapment efficiency. This improvement can be attributed to cholesterol's function as a stabilizing agent within the surfactant bilayer, effectively minimizing the transition from gel to sol states and thus resulting in vesicles that are less prone to leakage. This results in the formation of a more rigid vesicular structure, which improves entrapment efficiency and decreases the permeability of the medication that is encapsulated.

Nevertheless, the entrapment efficiency drops to 89.53% in formulation F4 when the cholesterol concentration is raised to 80 mg. This decrease is probably the result of the medication and cholesterol molecules competing for space inside the bilayer. In addition to displacing the medication from the bilayer, too much cholesterol can also compromise the vesicular membrane's structural integrity.

Furthermore, it was found that, especially in the NPG5 batch, the diffusion rate of nebivolol hydrochloride through a dialysis membrane rises with the surfactant content. The presence of non-ionic surfactant, which modifies the stratum corneum's structural makeup and improves the drug's thermodynamic activity and skin-vesicle partitioning, can be used to explain this occurrence. These results lead to the conclusion that the proniosomal gel formulation's ideal cholesterol concentration of 60 mg optimizes drug entrapment efficiency. With this particular cholesterol level, the NPG5 batch stands out as the best formulation because it provides a balance between permeability and structural integrity, which is essential for efficient drug administration.

3.2. Characterization of nebivolol hydrochloride proniosomal gel (NPG).

3.2.1. Entrapment efficiency.

At 98.41±0.34%, the percentage entrapment efficiency was consistently high across several proniosomal batches. This consistency across all evaluated batches suggests a dependable formulation method that ensures effective drug encapsulation. It is indicative of a robust and optimized formulation process capable of achieving consistently high levels of drug encapsulation within the proniosomal vesicles.

3.2.2. Percentage drug diffusion.

The comparative in vitro drug diffusion study between nebivolol hydrochloride proniosomal gel (NPG5) and the pure drug-loaded gel (PG) was meticulously conducted using a dialysis membrane and a Franz diffusion cell setup. The core objective of an ideal topical formulation is to ensure a prolonged release of the active ingredient. Such a characteristic minimizes the need for frequent application, thereby enhancing patient compliance. According to the results, which are detailed in Table 3, the plain drug-loaded gel (PG) exhibited a quicker diffusion rate of the drug compared to the proniosomal gel.

This observation underscores the slower diffusion rate of the drug when delivered via the nebivolol hydrochloride proniosomal gel (NPG5), indicating a more controlled and sustained release profile. This sustained release mechanism is particularly advantageous for topical applications, as it ensures a continuous delivery of the drug over an extended period.

Therefore, the nebivolol hydrochloride proniosomal gel (NPG5) stands out as an optimized formulation, effectively balancing drug release rates to meet therapeutic needs while potentially improving user adherence to the treatment regimen.

Table 3. Comparative *In-vitro* drug diffusion study through the dialysis membrane.

Time (h)	NPG5 batch (% diffused)	Pure drug-loaded gel
0	**0**	**0**
1	10.2±0.85	22.14
2	19.85±0.49	45.84
3	24.1±0.71	61.22
4	31.85±0.64	81.63
5	36.15±0.64	88.55
6	42.05±0.35	98.66
7	44.95±0.49	-
8	51.95±0.92	-
12	57.3±071	-
18	63.05±0.21	-
24	65.5±0.71	-

3.2.3. Particle size and polydispersity index.

Table 4 and Figure 1 present the measurements of vesicle dimensions and the diversity in size distribution (polydispersity index) for the NPG5 proniosomal gel batch following its reconstitution with phosphate buffer saline (PBS). These metrics are pivotal for evaluating the uniformity and physical attributes of the niosomes produced upon the gel's hydration.

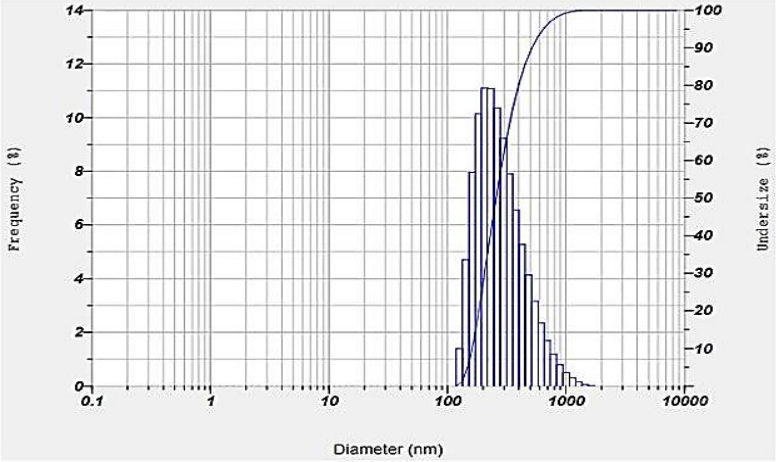

Figure 1. Vesicle size distribution curve of optimized batch (NPG5).

3.2.4. Scanning electron microscopy.

The scanning electron micrograph (SEM) of the proniosomal dispersion from the NPG5 batch, as depicted in Figure 2, illustrates that the vesicles exhibit a spherical shape and are within the nanometer scale in size. This visual evidence is crucial for confirming the nano-scale morphology of the proniosomal vesicles, which is indicative of their potential for enhanced cellular uptake and efficient drug delivery through biological barriers. The spherical nature of the vesicles is typical of well-formed niosomes, suggesting an optimal formulation process that could Favor consistent drug release profiles and stability.

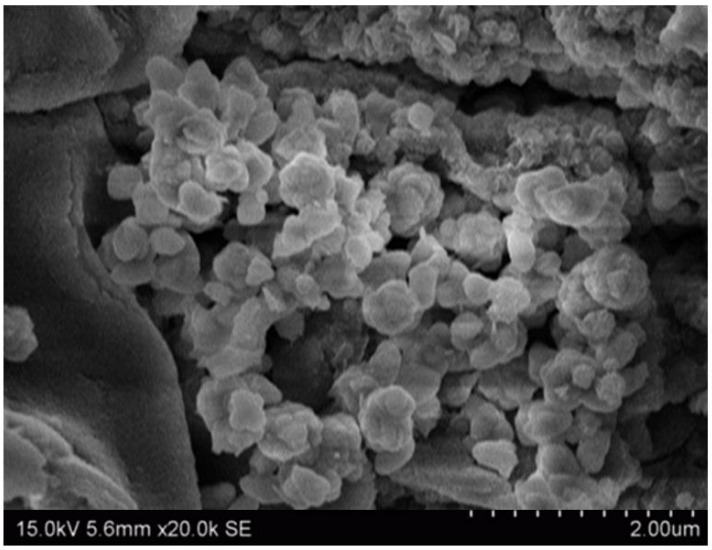

Figure 2. SEM image of optimized formulation batch (NPG5).

3.2.5. Zeta potential analysis.

As shown in Figure 3, the optimized batch NPG5's zeta potential measurement was -30.4 ± 7.68 mV By avoiding aggregation, this value indicates that the generated proniosomes have a significant surface charge, which is essential for preserving vesicular stability. This size of negative zeta potential usually indicates that the vesicles are sufficiently electrostatically repelled to prevent coalescence and provide a steady dispersion. This property contributes to the steady release rates and bioavailability of the encapsulated medication and is crucial for the durability and effectiveness of proniosomal formulations in drug delivery applications.

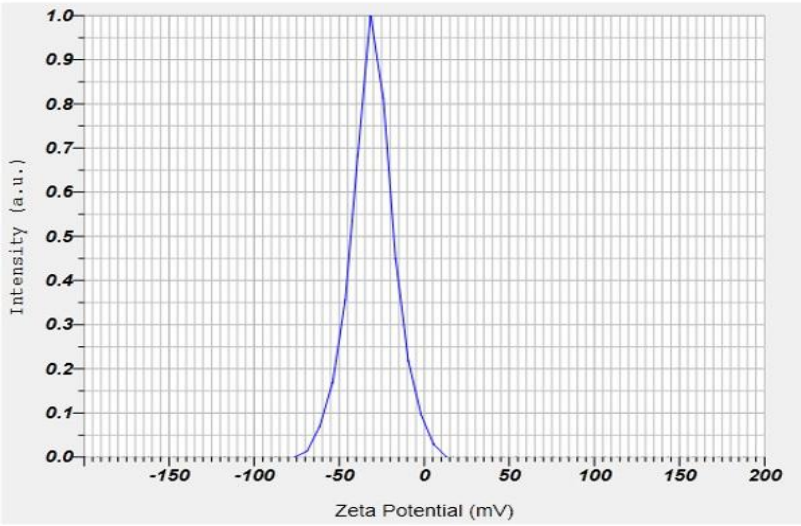

Figure 3. Zeta potential graph of optimized formulation batch NPG5

3.2.6. Differential scanning calorimetry.

The proniosomal dispersion containing nebivolol hydrochloride was subjected to thermal examination using differential scanning calorimetry (DSC). Nebivolol hydrochloride's crystalline structure was demonstrated by its strong melting point of 229.31°C in its pure form. However, as seen in Figures 4a and b, this particular thermal signature was not present in the drug's DSC thermograms following its formation into the proniosomal gel. The sharp endothermic peak linked to nebivolol hydrochloride's melting point in the gel form vanishes, indicating that the medication

changes into an amorphous state inside the proniosomal matrix. This change suggests that the drug molecules' ordered, structured organization is upset, resulting in a dispersion that, when embedded in the proniosomal gel, lacks a distinct crystalline shape.

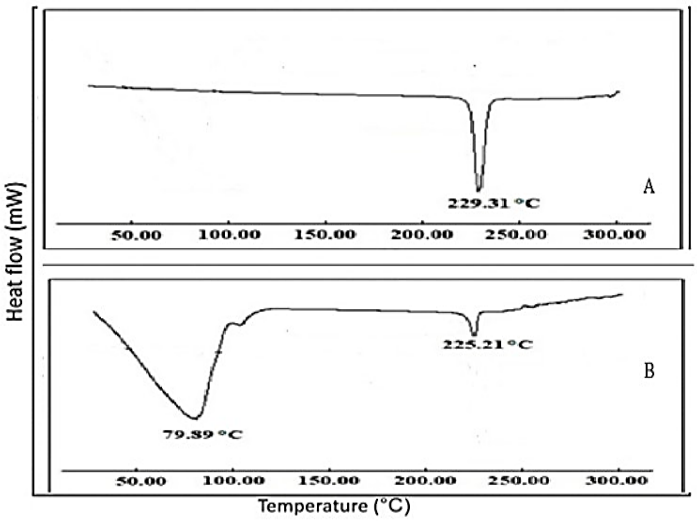

Figure 4. (a) DSC thermogram of pure drug nebivolol hydrochloride; **(b)** optimized formulation batch NPG5.

3.2.7. X-ray diffraction (XRD).

The findings from the study align with the outcomes of the differential scanning calorimetry (DSC) analysis, confirming the crystalline nature of nebivolol hydrochloride. This crystalline state is characterized by distinct peaks observed at 13.6°, 17.8°, 22.7°, 27.9°, 33.3°, and 41.2°, as depicted in Figures 5a and b. Such peaks are indicative of the structured, regular arrangement of molecules typical of a crystalline substance.

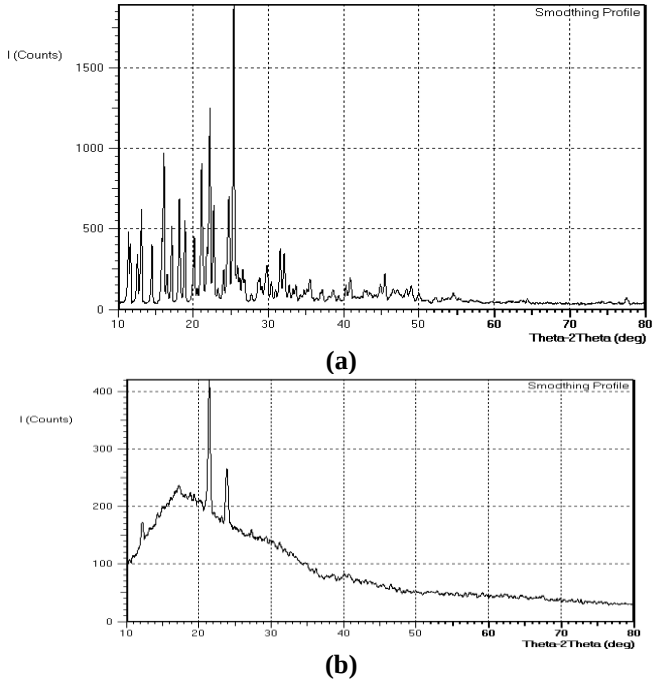

Figure 5. XRD graph of **(a)** pure drug nebivolol hydrochloride; **(b)** in formulation batch NPG5.

Table 4. Characterization of nebivolol hydrochloride-loaded proniosomal gel (Batch NNP5).

Appearance	Size (nm)	Zeta potential (mV)	Polydispersity index	(%) entrapment efficiency
Clear gel	308.0±10.32	-30.4 ± 7.68	0.918± 0.12	98.41± 0.34

Data represent mean ± SD (n = 3).

3.2.8. Ex vivo skin permeation studies.

The study focused on comparing the skin permeation capabilities of two formulations: the proniosomal gel (specifically batch NPG5) and a gel loaded with the pure drug. Both formulations were applied to the dorsal skin surface, which was then positioned against the donor compartment for permeation testing over a 24-hour period. The outcomes from the ex vivo skin permeation studies indicated that the gel containing the pure drug-facilitated a 66.97% drug permeation through rat skin within the first 6 hours, showcasing a higher rate of permeation and the greatest cumulative percentage of drug release (% CDR) into the receptor medium. In contrast, the optimized nebivolol hydrochloride proniosomal gel formulation (NPG5) exhibited a slower permeation rate, achieving 58.73% drug permeation through the skin by the 24-hour mark. A graphical comparison of the drug permeation rates between these two formulations is presented in Figure 6, illustrating the differential permeation profiles and efficacy of each formulation in delivering the drug through the skin.

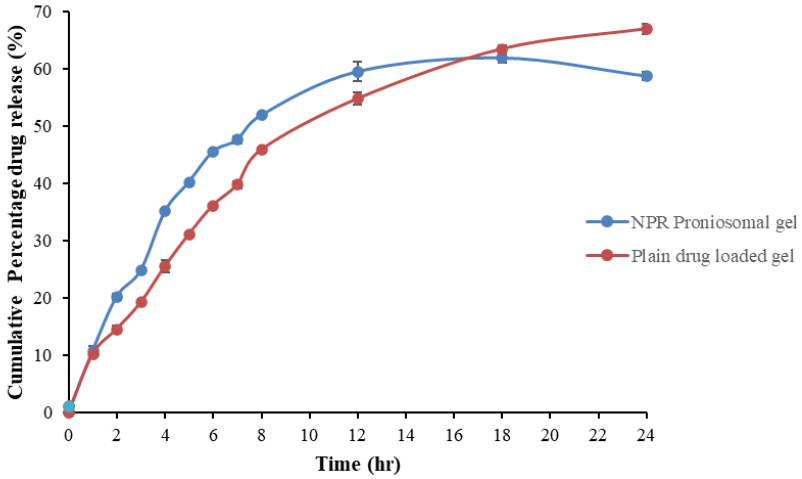

Figure 6. *Ex vivo* skin permeation study of nebivolol hydrochloride proniosomal gel (NPG5) and pure drug-loaded gel.

3.3. Discussion.

For the development of a proniosomal gel designed for topical application, excipients known for their compatibility with the skin were chosen. Surfactants such as Lutrol F68 and lecithin were selected due to their proven skin-friendliness. Additionally, cholesterol, a key structural element of cellular membranes, was added to improve the vesicles' permeability and stability. This addition notably increases the vesicles' entrapment capability for the drug while simultaneously elevating the viscosity of the niosomal dispersion and adding structural integrity to the otherwise flexible bilayers, resulting in more orderly and structurally coherent vesicles.

Cholesterol's integration into the bilayer not only facilitates better drug partitioning but also optimizes its encapsulation within the vesicles. This structural enhancement decreases the vesicles' permeability, contributing to a higher entrapment efficiency. Nevertheless, a cholesterol content exceeding 80 mg tends to diminish drug entrapment levels, as excessive cholesterol may displace the drug molecules within the bilayer, potentially leading to the expulsion of the drug during vesicle formation. Additionally, it can disrupt the consistent bilayer structure, which could result in the medicine being enclosed leaking.

The distinctive crystalline peaks of nebivolol hydrochloride vanished after the drug was integrated into the vesicles, according to solid-state examinations of the gel using differential

scanning calorimetry (DSC) and X-ray diffraction (XRD). This indicated that the drug had changed to an amorphous state. This modification offers benefits for efficient topical administration by greatly improving the drug's penetration and retention properties.

Applying the gel to the skin is made easier by its pseudoplastic qualities, which allow it to realign with applied shear and exhibit a decrease in viscosity with shear stress. By changing the crystallinity of the lipid layers of the skin, non-ionic surfactants also improve penetration and boost the effectiveness of medication administration. Increased skin penetration rates are also a result of nebivolol hydrochloride's better solubility in this formulation.

The proniosomal formulation changes into a vesicular structure when hydrated, greatly increasing the drug's ability to penetrate the skin. This feature sets it apart from pure nebivolol hydrochloride gel, which mostly functions via a hydrophilic mechanism and doesn't contain any lipidic or oily components. Vesicles of nanometric size are produced when hydrophobic surfactants and cholesterol are added to the NPG formulation. These vesicles provide the skin with a large surface area and an efficient occlusive layer. This layer improves the skin's moisture content and stops transepidermal water loss, which allows the medication to penetrate deeper and permeate better.

The smaller particle size inherent to these vesicles and the reduced potential for drug degradation contributes to a sustained release profile. Moreover, the transformation of the drug into an amorphous state further optimizes skin moisture retention and enhances the drug's pharmacodynamic effects upon topical application. This multifaceted approach leverages the vesicular nature of proniosomes to improve drug delivery through the skin, offering an advanced alternative to conventional hydrophilic gel systems.

Polynomial equations for dependent variables were derived, and their validity was checked by formulation counter-check formulations (CCNPG-1, CCNPG-2).

$$Y1 = 84.326 + 2.143\,X1 - 3.855\,X2 - 4.948\,X1X2 - 14.817\,X12 - 5.402\,X22\ (\%\,EE) \quad (3)$$

$$Y2 = 95.19 - 0.25\,X1 - 0.398\,X2 + 1.33\,X1X2 - 2.95\,X12 + 0.105\,X22\ (\%\,DD) \quad (4)$$

$$Y3 = 570.122 + 8.517\,X1 - 10.634\,X2 + 49.65\,X1X2 + 124.883\,X12 + 122.533\,X22\ (VS) \quad (5)$$

$$Y4 = 1.242 - 0.036\,X1 - 0.0462\,X2 - 0.269\,X1X2 + 0.477\,X12 - 0.176\,X22\ (PDI) \quad (6)$$

X_1, X_2, X_1X_2, X_1^2, and X^2 were tested for their effects on %EE, %DD, VS, and PDI using the factor tool. The results of the study claimed that two variable factors, X_1, X_2, and X_1^2, X_2^2, show the curve in an additive fashion and parallel to one another. In addition to that, the coded factor claims that a synergistic effect was observed in binate amount of constrained independent variables such as X12 and X22, X1 and X2 alone could not effectively prolong the dependent variables. It was asserted by respective p-value and coded equation. Furthermore, the coded factor claims that a positive effect (Synergistic effect) was observed in amounts of constrained independent variables X1X2 (1.33, 49.65) for % drug diffuses and vesicular size. The coded factor claims that a negative effect (antagonistic effect) was observed in amounts of constrained independent variables X1X2 (–4.948 and -0.269) for % entrapment

efficiency and polydispersity index, respectively. The combination of X1 and X2 in the value of 90 mg and 60 mg, respectively (mid-level), provides an appropriate release of the drug compared to the other level of formulations. The interaction between Lutrol and cholesterol is responsible for predictive-dependent responses. The same has been witnessed in RSM Figure 7-10.

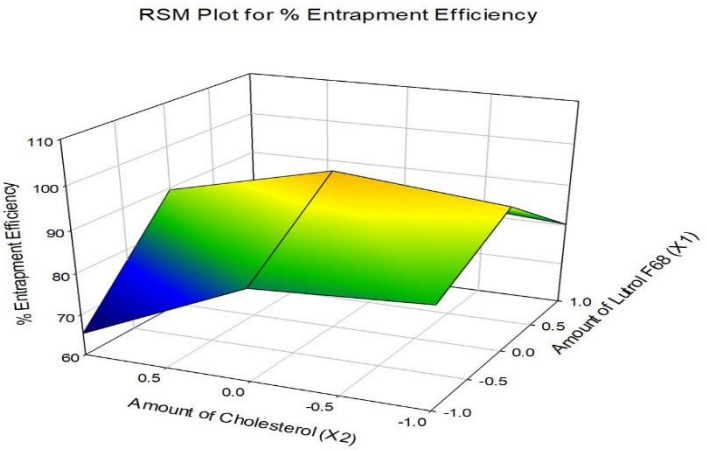

Figure 7. Response surface morphological plot for % entrapment efficiency.

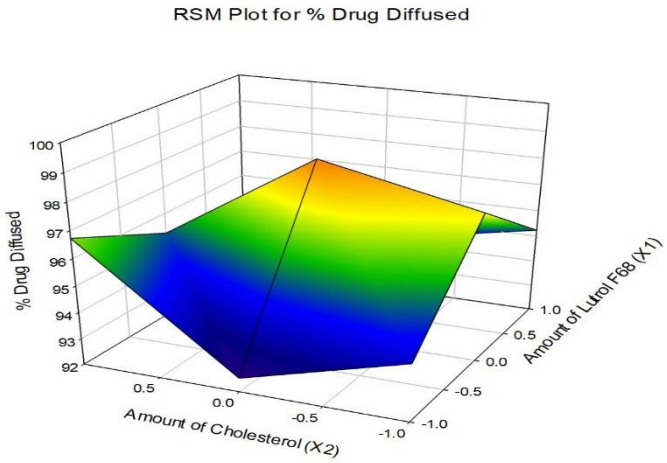

Figure 8. Response surface morphological plot for % drug diffused.

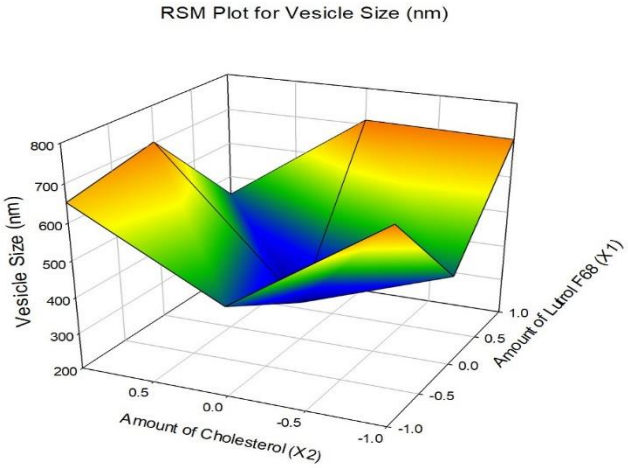

Figure 9. Response surface morphological plot for vesicle size (nm).

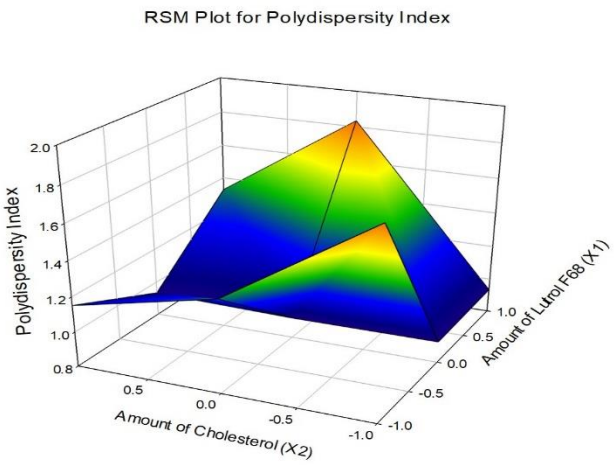

Figure 10. Response surface morphological plot for polydispersity index.

4. Conclusion

The findings from this study suggest that employing the coacervation phase separation technique for crafting a proniosomal gel presents an efficient avenue for creating stable nebivolol hydrochloride proniosomal gel. Among various batches tested, the NPG5 batch stood out by showcasing superior entrapment efficiency and presenting vesicles with an ideal size. The morphological characteristics of these vesicles, including their spherical and uniform appearance, were verified through scanning electron microscopy (SEM) studies. Additionally, the zeta potential measurements indicated a high degree of stability for this formulation, minimizing the risk of vesicle aggregation.

The optimized formulation of the proniosomal gel was also evaluated for its physical attributes, including clarity, appearance, and overall consistency, all of which met the desired standards. The performance of this formulation was further assessed through in vitro drug diffusion and skin permeation studies, which indicated an extended-release of the drug, underscoring the potential of proniosomes for enhancing transdermal delivery of nebivolol hydrochloride. These advantages mark a significant improvement over traditional gel-based drug delivery systems. Ultimately, the research concluded with the development of a viable and effective transdermal delivery system for nebivolol, demonstrating the promising application of proniosomal gels in medicinal formulations.

Author Contributions

All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

Data Availability Statement

Data supporting the findings of this study are available upon reasonable request from the corresponding author.

Funding

This research received no external funding.

Acknowledgments

The management and staff of all participating Institutes are gratefully acknowledged by the authors for the facilities provided and ongoing support for the completion of the current study.

Conflicts of Interest

The authors declare no conflict of interest.

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