Formulation of Enalapril Maleate Nanoproniosomal Gels and Their Pharmacokinetic Evaluations in Hypertensive Albino Wistar Rats: Ex Vivo and In Vivo Approaches

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Abstract

Proniosomes are drug-encapsulated, nanoscale vesicular structures that generate multilamellar niosomal dispersions upon hydration. This study aimed to develop enalapril maleate nanoproniosomal gels and assess their effectiveness in experimental hypertensive rat models. The gels were synthesized based on the coacervation phase separation method, using lecithin, cholesterol, and various nonionic surfactants as formulation components, along with the drug. The developed gels were then subjected to various analyses, such as pH, viscosity, rate of spontaneity, entrapment efficiency, vesicle size, ex vivo permeation, skin irritation, scanning electron microscopy, stability, in vivo bio-availability, in vivo antihypertensive activity, and in vitro–in vivo correlation studies. Results revealed that all synthesized gel formulations maintained good physical characteristics, within permissible limits. The results of the ex vivo skin permeation analysis revealed non-Fickian release kinetics and zero-order penetration behaviors of the drug formulations with diffusion, achieving a cumulative permeation rate of 58.75%–89.72% through albino rat skin over 24 h. Moreover, skin irritation tests revealed that the topical application of the drug formulations did not cause any signs of irritation, indicating their safety. Furthermore, in vivo bio-availability studies revealed that one particular formulation, EMNP7, demonstrated an approximately 188.99-fold greater bio-availability compared to the Vasotec tablet. Additionally, in vivo antihypertensive analysis revealed that this formulation effectively restored elevated rat blood pressures to the normal range. Furthermore, the in vitro–in vivo correlation analysis suggested that the ex vivo (in vitro) data could accurately replicate in vivo physiological conditions. Overall, our findings indicate that enalapril maleate encapsulated within nanoproniosomal gels can effectively function as controlled drug delivery systems, releasing the drug once per day for effective hypertension management.

Keywords: enalapril maleate; proniosomes; ex vivo permeation; in vivo antihypertensive research; in vivo bio-availability investigation; pharmacokinetic research; in vitro–in vivo correlation
Introduction

Transdermal administration stands out as the most effective and profitable method of drug delivery today. Interestingly, this innovative systemic drug delivery approach has found widespread applications in drug delivery systems designed to deliver medications into the systemic circulatory system at a controlled rate. Within these systems, the skin serves as a barrier, allowing medications to enter the bloodstream and undergo absorption. Some key benefits of transdermal delivery include its noninvasive nature, avoidance of the gastrointestinal tract, along with the prevention of gas-induced discomfort and gastrointestinal degradation. Furthermore, transdermal delivery simplifies medication regimes by requiring fewer doses and facilitating self-administration. It also enhances the therapeutic efficacy and safety of medicines, thus mitigating their adverse impacts and allowing for easy discontinuation of any given medicine when necessary.

Although individual drug delivery systems cannot simultaneously satisfy all requirements to ensure the safest drug administration, cutting-edge techniques for controlled or targeted drug administration have been introduced. These sophisticated administration methods are designed to minimize adverse effects while enhancing patient compliance and maintaining consistent and effective drug concentrations in the bloodstream. With developments in nanotechnology, various biocompatible or biodegradable nanovesicular carriers capable of shielding pharmaceuticals from cleavage and degradation have been introduced. These carriers are compatible with both hydrophilic and hydrophobic drugs, which can selectively accumulate at target sites and regulate drug release rates, thus acting as drug reservoirs. Accordingly, various vesicular carriers, including liposomes, niosomes, ethosomes, transferosomes, enzymosomes, virosomes, sphingosomes, archaeosomes, and pharmacosomes, are commonly employed in vesicular techniques, facilitating targeted and regulated drug administration. However, these vesicles suffer from physical challenges, such as sedimentation, aggregation, or fusion during storage, along with certain chemical degradation challenges related to hydrolysis or oxidation. Nevertheless, the provesicular concept (proniosomes) is capable of addressing the stability issues of traditional vesicular systems [1–3].

Proniosomes are drug-encapsulated, nanoscale vesicular structures available as free-flowing, dry powders or gels. Upon hydration, these gels undergo multilamellar niosomal dispersion. Before administration, a niosomal suspension is first prepared by briefly agitating proniosomal powder in a hot aqueous medium. The resulting multi-lamellar niosome suspension is then easily administered either orally or through other methods. In the gel form, proniosomes are first converted into niosomes in situ by absorbing water from the skin following topical application. These species then interact with the stratum corneum (SC) via strong hydrogen bonding interactions, thus loosening and reversibly perturbing the highly dense structure of the lipid lamellae matrix and thereby increasing the fluidity and permeability of the skin. Notably, these proniosomes offer numerous advantages, including reduced toxicity, improved drug penetration, and regulated drug release. Furthermore, they provide additional benefits in terms of transport, distribution, storage, dosage, and design while also addressing physical stability issues associated with niosomes, such as aggregation, fusion, and leakage [4–6].

The process of coacervation phase separation (CPS), which involves the hydration of a mixture containing cholesterol and a nonionic surfactant in a small amount of alcohol such as ethanol or isopropanol, yields proniosomal gel preparations as semisolid products. These semisolid gels can be clear, transparent, or translucent, ensuring their physical stability during storage. The surfactant bilayer, as the structural backbone of proniosomes, features outward facing hydrophilic ends and inward facing hydrophobic chains, forming a bilayer structure [7–9].

Proniosomes have garnered widespread attention in transdermal drug delivery owing to the penetration-enhancing characteristics of their nonionic surfactant and phospholipid components. Furthermore, these components are bio-degradable, nontoxic, and amphiphilic, capable of encapsulating both hydrophilic and lipophilic drugs without necessitating sophisticated storage conditions. Modulations of the ratio between the nonionic surfactants and cholesterol molecules can help tailor the release properties and
encapsulation effectiveness of entrapped drugs [4, 10].

Notably, proniosomal gels have gained popularity over their semisolid counterparts owing to their superior percutaneous penetration properties and ease of application. Furthermore, these gels can facilitate controlled drug release, conform to the shape of the application area, and withstand physiological stress caused by skin flexion, blinking, or mucociliary movements. Consequently, proniosomes are frequently formulated as gels [11].

The proniosome-based transdermal drug delivery system regulates the rate of drug release and enhances the penetration and release of active substances through the skin. Here, interactions between vesicles and the skin significantly influence the passage of drugs through the skin. Upon application, proniosomes hydrate to form niosome vesicles. At the interface between each vesicle and the SC, a high thermodynamic activity gradient (the driving force underlying drug permeation through the skin) emerges, thus increasing the diffusion pressure and permeability of the drug through the SC. Eventually, all generated niosome vesicles clump, fuse, and adhere to the surface of the skin, releasing drugs into the bloodstream through endocytosis and membrane breakdown by lysozymes. Upon penetrating the SC, the drug molecules can easily permeate through the epidermal and dermal layers, ultimately reaching the systemic circulatory system through capillaries [6].

Conventionally, angiotensin-converting enzyme (ACE) inhibitors such as enalapril maleate (EM) are commonly prescribed for treating congestive heart failure and hypertension. Notably, EM is particularly favored for anti-hypertensive therapy owing to its high efficacy and minimal toxicity. Although EM demonstrates a bio-availability of 50%–60%, its oral absorption can be reduced by 40%–50% when consumed with food. In particular, with advantageous features such as a low molecular weight of 492.53 (less than 600 daltons), narrow dosing range (2.5–20 mg), brief plasma half-life (1.3 h), and poor oral bio-availability, EM serves as an ideal transdermal formulation. However, to further maintain effective therapeutic drug levels for extended periods and improve its bio-availability during transdermal delivery, EM is often prepared as a nanoproniosomal transdermal formulation, facilitating drug delivery at a controlled pace across intact skin [12–14].

At present, ACE inhibitors are often adopted as the first-line treatment for hypertension. A key advantage of these inhibitors over other anti-hypersensitive agents is their ability to prevent renal and coronary heart failure in individuals with type 2 diabetes. In particular, EM stands out as an ACE inhibitor owing to its effectiveness, lipophilicity, therapeutic dosage, and molecular size.

The primary objectives of this study were as follows: creating a nanoproniosomal gel formulation of EM for treating hypertension; improving the drug’s permeability through the skin; enhancing its bio-availability; and ensuring efficient and sustained drug delivery through the transdermal route while circumventing the first-pass metabolism.

**Experimental**

**Materials**

EM was sourced as a complimentary sample from Lee Pharma Limited, Hyderabad, India. Furthermore, nonionic surfactants such as Tween 60, Tween 80, Soy lecithin, Span 20, and Span 40 and Cholesterol were obtained from Himedia Laboratories Pvt. Ltd., Mumbai, India. Meanwhile, Brij 30 and Brij 72 were purchased from Sigma–Aldrich, Hyderabad, India. Additionally, Pfizer’s methylprednisolone acetate (MPA) injection (Depo-MedrolTM) was purchased from a local medical supply store. All other substances and reagents used were of research-grade quality.

**Preparation of the nanoproniosomal gel**

The preparation of the nanoproniosomal gel formulations followed the CPS procedure, utilizing nonionic surfactants (such as Span 20, Span 40, Tween 60, Tween 80, Brij 72, and Brij 30), cholesterol, lecithin, an ethanol solvent, and an aqueous 0.1% glycerol solution for hydration. Specifically, for each gel formulation, a wide-mouth glass vial with a tightly sealed cap was first filled with weighed amounts of the nonionic surfactants. Subsequently, precise quantities of the drug, cholesterol, and lecithin were introduced into the glass vial. Thereafter, ethanol was added under continuous stirring, and the resulting mixture was heated until the drug, cholesterol, and lecithin completely dissolved in the surfactant, yielding a clear gel solution (note: soy lecithin typically
required 20–25 min for complete dissolution in the surfactant). Following this, a 0.1% glycerol solution, acting as an aqueous phase, was added to this transparent-gel-like solution and gradually heated until a clear solution was obtained (typically within approximately 10 min). The solution was then cooled under constant stirring with a glass rod, resulting in the formation of a nanoproniosomal gel solution [15, 16]. Table 1 details all the prepared nanoproniosomal formulations.

**Property assessments of the nanoproniosomal gel formulations**

**pH values**

A calibrated digital pH meter (Globe Scientific Instruments) was utilized to measure the pH values of the proniosomal gel formulations in triplicate [17]. Notably, the pH meter was calibrated using at least two buffer solutions, such as pH 7 and pH 4. The specific calibration steps were as follows:

1. Initially, the electrode of the pH meter was immersed in a pH 7 solution. After allowing approximately 1 min for the reading to stabilize, the meter displayed a pH value of 7. If not, the pH meter was adjusted to this value.

2. Subsequently, the electrode was thoroughly rinsed with demineralized water and immersed in the pH 4 buffer solution.

3. The above steps were repeated until a reliable measurement was obtained. To test the accuracy of the calibration, the pH meter was again immersed in the pH 4 solution. Any deviations from pH 4 in this case implied calibration discrepancies.

**Viscosity**

A Brookfield viscometer (DV-E) was employed to measure the viscosities of the formulations. Approximately 10 g of each gel formulation was added to a beaker, and the spindle of the viscometer was immersed into the gel. The viscosity value was then recorded by rotating spindle 06 at 100 r·min⁻¹ [18].

**Vesicle size determination (microscopic evaluation)**

Each proniosomal gel (100 mg) formulation was hydrated in a small vial containing 10 mL of phosphate-buffered saline and shaken manually for 5 min. The resulting niosomes were microscopically examined using an Olympus BX51 Fluorescence Microscope to measure their average vesicle sizes [8, 19, 20].

**Rate of spontaneity**

The rate of spontaneity refers to the number of niosomes that spontaneously form within 15–20 min of proniosome hydration. A specific amount of each proniosomal gel (20 mg) formulation was evenly spread across the walls of a clean, stoppered glass container using a glass rod. After adding 2 mL of a 0.9% NaCl saline solution, the glass container was allowed to stand for 20 min. Later, a drop of this glass container solution was added to a Neubauer’s chamber to count the number of vesicles (niosomes) formed [8, 21].

**Table 1** Formulations of the EM nanoproniosomal gel

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>EMNP1</th>
<th>EMNP2</th>
<th>EMNP3</th>
<th>EMNP4</th>
<th>EMNP5</th>
<th>EMNP6</th>
<th>EMNP7</th>
<th>EMNP8</th>
<th>EMNP9</th>
<th>EMNP10</th>
<th>EMNP11</th>
<th>EMNP12</th>
<th>EMNP13</th>
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<tr>
<td>Drug (mg)</td>
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<td>Lecithin (mg)</td>
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<td>100</td>
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<td>Cholesterol (mg)</td>
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<tr>
<td>Span 20 (mg)</td>
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<td>Span 40 (mg)</td>
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<td>Tween 60 (mg)</td>
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<td>Tween 80 (mg)</td>
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<td>Brij 72 (mg)</td>
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<td>Brij 30 (mg)</td>
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<td>1 000</td>
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<td>500</td>
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<td>500</td>
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<tr>
<td>Alcohol (mL)</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
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<td>0.5</td>
<td>0.5</td>
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<td>0.5</td>
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<tr>
<td>0.1% glycerol solution (mL)</td>
<td>QS</td>
<td>QS</td>
<td>QS</td>
<td>QS</td>
<td>QS</td>
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<td>QS</td>
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<td>QS</td>
<td>QS</td>
<td>QS</td>
<td>QS</td>
<td>QS</td>
</tr>
</tbody>
</table>

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Entrapment efficiency

For entrapment efficiency assessments, 100 mg of each proniosomal gel formulation was added to an aqueous refined phase and slightly heated to promote niosomes formation. The resulting mixture was then centrifuged (Remi CPR-24 axis) at 18000 r·min⁻¹ for 40 min at 5 °C. The supernatant was then analyzed using ultraviolet (UV) spectrophotometry at 206 nm for drug concentration evaluations [8, 22]. The entrapment efficiency was then calculated as follows:

Encapsulation efficiency (%) = \[1 - \frac{(\text{unencapsulated drug/total drug})}{\times 100}\]

Morphological scanning electron microscopy (SEM) evaluations

The morphologies of the produced niosomes were characterized using SEM analysis. Specifically, a given amount of each proniosomal gel formulation was diluted with phosphate-buffered saline (pH 7.2) in a glass test tube. Subsequently, the formed vesicles were dispersed on an adhesive carbon tape placed on an aluminum stub. The samples were then coated with gold using a vacuum evaporator (3 × 10⁻¹ atm) and examined under an SE microscope, equipped with a digital camera, at an accelerating voltage of 25 kV [15,17].

Experimental animals

Healthy albino Wistar male rats, weighing approximately (250 ± 25) g, were selected for dermal irritation, in vivo bio-availability, and in vivo antihypertensive investigations. Throughout these investigations, all rats remained healthy. They were placed in a controlled laboratory environment, ensuring 100% fresh air exchange, a temperature of (25 ± 1) °C, and a relative humidity of (55 ± 5)%. Additionally, the rats were housed in polypropylene cages and provided with unlimited access to water and a normal diet (Lipton feed). Drug dosages were determined based on individual body weights and surface area ratios.

Institutional Animal Ethical Committee (IAEC) granted ethical approval to use testing animals in the assessment of characteristics, including in vivo bio-availability studies, skin irritation investigation, ex vivo skin penetration examination, and in vivo antihypertensive studies. Protocol No. SVCP/IAEC/II-012/2019-20 dt 18.11.19 was approved by IAEC for the animal studies. The experiment was conducted in accordance with the CPCSEA criteria.

Ex vivo skin permeation studies

For ex vivo skin permeation studies, albino rats weighing 150–200 g were selected. These rats were then euthanized by administering anesthetic ether, and their fur was clipped to a length of 2 mm using scissors. Subsequently, skin samples of approximately 4.5–5.0 cm² were removed from the shaved abdominal areas. Following cutaneous fat excision, the dermal specimens were cleaned with isotonic phosphate buffer and distilled water. The samples were then segmented as required, wrapped in aluminum foil, and stored at −20 °C in a deep freezer over two weeks.

For the actual skin permeation studies, a modified Franz-diffusion cell was employed. This cell featured a receptor chamber with a surface area of approximately 3.14 cm² for diffusion, along with an internal volume of ~ 60 mL. Each prepared animal skin sample was sandwiched between the donor and receptor portions. Subsequently, a specific amount of each proniosomal gel formulation was placed on top of the epidermis facing the donor component, while phosphate buffer (pH 7.2) was added to the receptor chamber. Notably, a thermostatic hotplate equipped with a magnetic stirrer was used to heat the system, maintaining a temperature of (37 ± 0.5) °C. Furthermore, a Teflon-coated magnetic bead placed in the receptor chamber agitated the receptor diffusion medium. Samples were then collected at designated intervals, replaced with equal amounts of fresh receptor fluids each time, and subjected to spectrophotometric analysis at 207 nm [8, 23–27].

Skin irritation studies

In accordance with the recommendations of the Organization for Economic Co-operation and Development (OECD), skin irritation testing was performed on three healthy albino rats. One day before testing, the back sides of the animals were shaved. Following this, 0.5 g of each proniosomal gel formulation was applied to the exposed skin region measuring approximately 2.54 cm × 2.54 cm, and the rats were then placed in separate cages. Following 24 h of exposure, the proniosomal gels were removed, and any residual material was washed off using tap water. One rat underwent initial skin irritation testing, while two rats were subjected to confirmatory skin irritation testing. The test spots were then evaluated
for erythema and edema using a visual scoring scale (Table 2) [25–28].

### Table 2 Visual scoring scale

<table>
<thead>
<tr>
<th>Score</th>
<th>Erythema scale</th>
<th>Edema scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Nothing</td>
<td>Nothing</td>
</tr>
<tr>
<td>1</td>
<td>Slightly</td>
<td>Slightly</td>
</tr>
<tr>
<td>2</td>
<td>Clearly identified</td>
<td>Clearly identified</td>
</tr>
<tr>
<td>3</td>
<td>Medium</td>
<td>Medium</td>
</tr>
<tr>
<td>4</td>
<td>Development of scars</td>
<td>Development of scars</td>
</tr>
</tbody>
</table>

### Stability studies

Stability studies were aimed at assessing product stability, defined as the ability of a particular dosage formulation, placed within an appropriate container, to retain its physical, chemical, microbiological, therapeutic, and toxicological properties from the time of its manufacture. In line with the International Council for Harmonisation of Technical Requirements of Pharmaceuticals for Human Use guidelines, we investigated the degradation of the proniosomal gel formulations over a specific storage period. Specifically, the developed formulations were stored at three temperatures (refrigeration temperature (4–8 °C), room temperature ((25 ± 2 °C), and oven temperature ((40 ± 2 °C)) for 45 days. During these periods, the proniosomal gel formulations were stored in glass vials sealed with aluminum foil. Samples were periodically collected and subjected to drug concentration, viscosity, pH, homogeneity, physical appearance, and vesicle size assessments [8, 29].

### In vivo anti-hypertensive investigations

For antihypertensive studies, healthy male albino Wistar rats weighing (250 ± 25) g were selected. Throughout the experiment, all rats were housed in six-cell polypropylene cages under suitable circumstances, with unlimited access to water and a standard laboratory diet of Lipton feed. In total, 30 rats were divided into 5 groups (Groups A–E), each consisting of 6 rats. Subsequently, to induce hypertension, MPA (20 mg·kg⁻¹·w⁻¹) was subcutaneously injected into the rats in Groups B–E over two weeks. Group A served as the control group. Table 3 lists the treatments administered to each group.

Before applying the gel formulations, the skin of each rat was examined to ensure its normal condition. The dorsal fur of each rat was then carefully shaved or trimmed to avoid damaging the skin, following which the exposed skin surface was gently cleansed using hot water and dried using an alcoholic pad. Subsequently, the selected EMNP7 formulation was applied to the skin of the rats and covered with microporous adhesive tape to ensure stability at the application site. Thereafter, the rats were allowed unrestricted access to food and water within their enclosures. Later, a rat BP measuring device (Biopac system, USA) was used to record BP readings every 24 h from the rats’ tails. This device comprised an animal holder, a tail-cuff, and a scanner connected to the primary instrument, equipped with a digital BP display panel [25, 26, 30–32].

### Statistical analysis

The collected data were analyzed using the one-way analysis of variance (ANOVA) technique. Furthermore, for assessing the different gel formulations, paired t-test and Dunnette’s multiple comparison test were performed using the GRAPHPAD INSTAT 3 software. A significance threshold of $p < 0.05$ was set across all tests.

### In vivo bio-availability and pharmacokinetic investigations

For in vivo bio-availability and pharmacokinetic investigations, 12 albino Wistar male rats were selected and divided into two groups, Group A and Group B, each containing 6 rats. Group A was transdermally treated with the proniosomal gels. The
gels applied at the treatment site were secured using microporous adhesive tape. Before treatment, the dorsal fur of the rats was carefully shaved. Subsequently, the exposed skin regions were cleaned with distilled water and dried with alcohol swabs before topical gel application. Conversely, Group B was treated with an oral antihypertensive medication (Vasotec tablet). The medicated rats were then housed in their chambers and provided with food and water. Blood samples (0.5 mL) were then collected from the tail veins of the rats at 0.5, 1, 2, 4, 8, 12, 18, and 24 h in micro tubes containing an anticoagulant. These blood samples were then centrifuged at 4 500 r·min⁻¹ for 5 min to separate plasma samples from the blood. Acetonitrile was then added to deproteinize the supernatant, which was then separated and subjected to high-performance liquid chromatography (HPLC) analysis.

**HPLC analysis**

Each drug formulation was quantified using an Agilent HPLC spectrophotometer, equipped with a C18 column (4.6 mm/250 mm, reversed-phase 5 μm) and a UV detector. The mobile phase (solvent), comprising acetonitrile and the phosphate buffer with a pH of 7.2 (65%:35%), was ultrasonically sonicated and filtered through a 0.45 μm membrane filter. Subsequently, chromatographic separation and analysis were performed at a flow rate of 0.8 mL·min⁻¹. The temperature within the column was maintained at 25 °C, and 20 μL of samples were added to it.

**Sample preparation**

Based on the protein precipitation technique, 0.5 mL of plasma was added to 0.5 mL of acetonitrile, following which the mixture was vortexed for 5 min and centrifuged at 10 000 r·min⁻¹ for 3 min. The supernatant was then collected and dried under nitrogen gas. Following vortexing, the residue was added to the mobile phase (100 μL) and centrifuged at 10 000 r·min⁻¹ for 2 min. Subsequently, 20 μL of each sample was introduced into the HPLC apparatus. Numerous pharmacokinetic parameters, including the elimination half-life (t₁/₂), mean residence time (MRT), maximum plasma drug concentration (Cmax), time required to reach the maximum plasma concentration (Tmax), absorption rate constant (Kₐ), elimination rate constant (Kₑ), area under the curve (AUC)₀–t, area under the first moment curve (AUMC)₀–t, and relative bio-availability (F%), were derived from the results. Among these, Cmax and Tmax were directly obtained from the plasma profiles (plasma concentration–time data). Later, using the residual approach, Kₐ and Kₑ were derived. For AUC and AUMC calculations, the trapezoidal method was employed. The MRT was determined based on the following relation: MRT = AUMC/AUC. Meanwhile, the elimination half-life (t₁/₂) was calculated using t₁/₂ = ln2/Kₑ [21, 22, 26, 33, 34] Statistical assessments were conducted using ANOVA on GRAPHPAD INSTAT 3, and a significance threshold of p < 0.05 was set.

**In vitro–in vivo correlation (IVIVC) study**

An IVIVC study was conducted to assess the therapeutic efficacy of each formulation and establish a correlation between the in vivo and ex vivo findings. Specifically, IVIVC plots of the EM proniosomal gels were created with the in vivo percentage absorption of each formulation on the y-axis and in vitro percentage permeation on the x-axis [26].

**Results and Discussion**

Notably, proniosomes are drug-encapsulated, nanoscale vesicular structures that generate multilamellar niosomal dispersions upon hydration. In this study, proniosomal gel preparations were formulated as semisolid products and comprised nonionic surfactants, cholesterol, lecithin, a solvent, and an aqueous solution for hydration. The CPS technique was employed to synthesize the nanoproniosomal gels, which were then subjected to physicochemical investigations, ex vivo penetration testing, SEM analyses, dermal irritation testing, stability studies, in vivo bio-availability examinations, and in vivo anti-hypertensive analyses.

**Physicochemical characterization of the proniosomal gels**

The physicochemical properties of the EM nanoproniosomal gel formulations were characterized, as detailed in Table 4. All prepared formulations demonstrated acceptable physicochemical properties, within specified tolerances. The pH values of all formulations ranged 6.7–7.3, ensuring their safe transdermal administration. Furthermore, the vesicle sizes of the formulations ranged 5.2–21.6 μm, indicating that the span surfactants (Span 20, Span 40) generated smaller
vesicles and demonstrated higher encapsulation efficacies compared to the tween and brij surfactants. The spontaneity rate, indicating the total number of niosomes produced from proniosomes after hydration, was determined to be between 8 and 16. Moreover, the encapsulation efficiencies of the drug formulations were observed to be between 53.36% and 85.23%, while their viscosities were measured to be between 8751 and 12340 cps. Overall, all the synthesized formulations demonstrated acceptable physicochemical properties, lying within tolerance ranges.

**Microscopic evaluations**

Microscopic evaluations revealed that the prepared drug formulations maintained good physical dimensions. Figures 1(a)–1(c) display the microscopic images of a few samples.

**Ex vivo skin permeation studies**

All prepared proniosomal gel formulations, as well as the drug suspension of the marketed Vasotec tablet, were subjected to *ex vivo* permeation studies. All formulations demonstrated good permeation (60.27%–89.72%) across the skin membranes of albino rats. Among all prepared formulations, EMNP7 exhibited the highest percentage of drug penetration, approximately 89.72%, compared to the drug suspension of the marketed tablet (46.54%). Consequently, the proniosomal formulation demonstrated enhanced drug permeation across the skin membranes of all albino rats. Notably, this increase in the drug diffusion rate and extent could be attributed to the decrease in vesicle size. Figure 2 depicts the skin penetration data for all formulations.

**Skin irritation studies**

The results of the skin irritation test, performed according to the OECD guidelines, revealed no signs of edema or erythema on the skins of the rats following proniosomal gel treatment for a period of 72 h. Consequently, all formulations cleared the skin irritation test and were deemed safe for use on human skin. Table 5 and Fig. 3 present the data and images

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**Table 4 Physicochemical characterizations of the prepared drug formulations**

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>pH*</th>
<th>Vesicle size* (μm)</th>
<th>Rate of spontaneity*</th>
<th>Entrapment efficiency (%) *</th>
<th>Viscosity (cps)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>EMNP1</td>
<td>6.7 ± 0.45</td>
<td>8.3 ± 0.80</td>
<td>10 ± 3.07</td>
<td>80.61 ± 0.28</td>
<td>10496 ± 1.72</td>
</tr>
<tr>
<td>EMNP2</td>
<td>6.8 ± 0.03</td>
<td>7.5 ± 0.42</td>
<td>13 ± 0.73</td>
<td>82.46 ± 0.47</td>
<td>11307 ± 1.59</td>
</tr>
<tr>
<td>EMNP3</td>
<td>7.0 ± 0.63</td>
<td>20.2 ± 1.34</td>
<td>9 ± 2.58</td>
<td>53.36 ± 0.41</td>
<td>11824 ± 3.96</td>
</tr>
<tr>
<td>EMNP4</td>
<td>7.2 ± 0.57</td>
<td>21.6 ± 1.05</td>
<td>8 ± 1.62</td>
<td>55.52 ± 0.98</td>
<td>12067 ± 2.58</td>
</tr>
<tr>
<td>EMNP5</td>
<td>6.9 ± 0.48</td>
<td>12.7 ± 2.83</td>
<td>14 ± 1.04</td>
<td>74.94 ± 0.57</td>
<td>8751 ± 1.97</td>
</tr>
<tr>
<td>EMNP6</td>
<td>7.1 ± 0.52</td>
<td>16.4 ± 1.72</td>
<td>12 ± 2.95</td>
<td>73.65 ± 0.51</td>
<td>9374 ± 3.86</td>
</tr>
<tr>
<td>EMNP7</td>
<td>6.9 ± 0.74</td>
<td>5.2 ± 0.64</td>
<td>16 ± 0.21</td>
<td>85.23 ± 0.78</td>
<td>10786 ± 2.05</td>
</tr>
<tr>
<td>EMNP8</td>
<td>7.2 ± 0.34</td>
<td>17.7 ± 0.36</td>
<td>10 ± 3.46</td>
<td>62.81 ± 0.73</td>
<td>9793 ± 2.82</td>
</tr>
<tr>
<td>EMNP9</td>
<td>6.8 ± 0.61</td>
<td>14.8 ± 3.61</td>
<td>11 ± 2.84</td>
<td>71.27 ± 0.75</td>
<td>10583 ± 2.62</td>
</tr>
<tr>
<td>EMNP10</td>
<td>7.1 ± 0.06</td>
<td>18.5 ± 2.95</td>
<td>15 ± 1.76</td>
<td>66.19 ± 0.82</td>
<td>10220 ± 1.63</td>
</tr>
<tr>
<td>EMNP11</td>
<td>7.2 ± 0.32</td>
<td>11.2 ± 2.24</td>
<td>12 ± 2.08</td>
<td>75.83 ± 0.46</td>
<td>10748 ± 2.95</td>
</tr>
<tr>
<td>EMNP12</td>
<td>6.8 ± 0.05</td>
<td>15.5 ± 3.02</td>
<td>10 ± 3.65</td>
<td>77.68 ± 0.13</td>
<td>11526 ± 3.74</td>
</tr>
<tr>
<td>EMNP13</td>
<td>7.3 ± 0.08</td>
<td>12.4 ± 1.87</td>
<td>13 ± 1.94</td>
<td>76.29 ± 0.35</td>
<td>12340 ± 1.85</td>
</tr>
</tbody>
</table>

*Mean ± standard deviation (n = 3).
obtained from the skin irritation tests, respectively.

**SEM evaluations**

The optimal formulation, EMNP7, was selected for the SEM analysis. As depicted in Fig. 4, the formulation demonstrated good surface morphology.

**Stability studies**

The results of the stability analysis revealed that all formulations maintained their homogeneity and physical appearance throughout the storage period. In particular, the EMNP7 formulation was observed to be highly stable under various temperature conditions over the specified timeframe. Consequently, all formulations passed the stability tests, and their stability data are summarized in Table 6.

**In vivo bio-availability and pharmacokinetic investigations**

This analysis was aimed at assessing the bio-availability and efficacy of each proniosomal formulation compared to the corresponding metrics of the Vasotec tablet. Results revealed that the EMNP7 formulation achieved an approximately 188.99-fold higher bio-availability, in terms of sustained plasma concentrations for up to 24 h, compared to the commercial tablet. Table 7 and Fig. 5 summarize the in vivo characteristics of the EM proniosomal gel formulations injected into Wistar rats. As depicted, the plasma concentration–time profile graph presented extended drug release for up to 24 h. The $C_{\text{max}}$ value of the proniosomal gel formulation was 28.83 ± 3.17 ng·mL$^{-1}$, whereas that of the marketed tablet was 36.88 ± 2.42 ng·mL$^{-1}$. Furthermore, the proniosomal gel formulation and the marketed tablet exhibited $T_{\text{max}}$ values of 4.57 ± 0.63 h and 2.11 ± 0.58 h, respectively. The AUC was employed as a key metric for evaluating drug bio-availability. Notably, the AUC metric reflects the total area under the plasma concentration–time plot and reflects the total amount of drug absorbed into the bloodstream following its administration. The AUC$_{0-t}$ value for the proniosomal gel formulation was higher (363.93 ± 11.33 ng·h·mL$^{-1}$) than that of the marketed tablet (192.56 ± 8.14 ng·h·mL$^{-1}$), with a statistically significant difference ($p < 0.05$). These findings demonstrated that while the AUC and $t_{1/2}$ metrics of the proniosomal gel formulation increased compared to the marketed tablet, the AUC$_{0-t}$ value was significantly higher, indicating superior bio-availability.
to those of the commercial tablet, its $C_{\text{max}}$ dramatically decreased.

**In vivo antihypertensive studies**

In the antihypertensive studies, MPA injections were administered to induce hypertension in normal, healthy rats. Notably, hypertension conditions persisted for 72 h following the termination of MPA treatments. The proniosomal gel formulation EMNP7 significantly ($p < 0.001$) reduced BPs, bringing them closer to the normal value, and this effect persisted for 24 h (Table 7). This suggested that the EMNP7 formulation was retained in the rats’ systemic circulation systems and continuously delivered for up to 24 h. However, the post-treatment BP readings for Group D were similar to those of the control group (Group A). When comparing the effects of all drug formulations, the EMNP7 formulation was observed to more effectively lower the mean BPs of rats (by 26.03%) compared to the Vasotec tablet (12.80%) (Tables 8 and 9). The EMNP7 formulation eventually restored the BPs of the rats to normal levels. These findings suggest that the EMNP7 formulation holds...
promise for the treatment of hypertension; however, its applicability must be validated through clinical trials.

**IVIVC**

As stated, IVIVC plots were created by plotting \textit{in vitro} percentage permeations on the x-axis and \textit{in vivo} percentage absorptions on the y-axis. As depicted in Fig. 6, the IVIVC plot of the proniosomal gel formulation EMNP7 exhibited a linear trend, with a regression value of $R^2 = 0.9995$, indicating excellent agreement between the \textit{in vitro} and \textit{in vivo} findings. This trend also indicated that the \textit{ex vivo} (\textit{in vitro}) data could accurately model the \textit{in vivo} physiological conditions. Furthermore, Table 10 summarizes the IVIVC data (\textit{in vitro} permeation (%) vs. \textit{in vivo} absorption (%)) of the proniosomal gel formulation EMNP7.

**Conclusion**

This study prepared various EM nanoproniosomal gel formulations to boost the bioavailability and permeation of EM. Specifically, the enhancement in drug penetration and bioavailability was achieved by entrapping the drug within nanoproniosomal vesicles. Results revealed that the physical characteristics of the produced drug formulations were well maintained, within permissible limits. The findings of \textit{ex vivo} dermal penetration investigations indicated non-Fickian release and zero-order drug penetration with diffusion, with a cumulative permeation rate of 58.75\%–89.72\% through albino rat skin within 24 h. Among all developed formulations, EMNP7

<p>| Table 8 Effects of EM proniosomal gel formulations on the mean BPs of MPA-induced hypertensive rats |</p>
<table>
<thead>
<tr>
<th>Group</th>
<th>Treatments</th>
<th>Mean BP (mm Hg)*</th>
<th>Reduction in BP*</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Control</td>
<td>121.32 ± 10.05</td>
<td>—</td>
</tr>
<tr>
<td>B</td>
<td>Only MPA</td>
<td>122.15 ± 8.27</td>
<td>163.45 ± 11.32</td>
</tr>
<tr>
<td>C</td>
<td>MPA + placebo EMNP7</td>
<td>122.46 ± 12.36</td>
<td>162.37 ± 12.15</td>
</tr>
<tr>
<td>D</td>
<td>MPA + EMNP7</td>
<td>121.53 ± 11.08</td>
<td>163.76 ± 13.21</td>
</tr>
<tr>
<td>E</td>
<td>MPA + Vasotec tablet (marketed tablet)</td>
<td>122.71 ± 6.82</td>
<td>161.28 ± 11.18</td>
</tr>
</tbody>
</table>

* Mean ± standard deviation ($n = 6$).

<p>| Table 9 \textit{In vivo} antihypertensive effects of the proniosomal gel formulation (EMNP7) in hypertensive rats at different time intervals |</p>
<table>
<thead>
<tr>
<th>No.</th>
<th>Time (h)</th>
<th>Mean BP (mm Hg)*</th>
<th>Reduction in BP*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Post-MPA treatment</td>
<td>Post proniosomal gel treatment (EMNP7)</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>162.37 ± 12.15</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>162.37 ± 12.15</td>
<td>155.48 ± 4.56</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>162.37 ± 12.15</td>
<td>146.37 ± 5.21</td>
</tr>
<tr>
<td>4</td>
<td>15</td>
<td>162.37 ± 12.15</td>
<td>137.12 ± 4.72</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>162.37 ± 12.15</td>
<td>128.29 ± 6.68</td>
</tr>
<tr>
<td>6</td>
<td>24</td>
<td>162.37 ± 12.15</td>
<td>121.12 ± 8.79</td>
</tr>
</tbody>
</table>

* Mean ± standard deviation ($n = 6$).
demonstrated the highest percentage of drug penetration, approximately 89.72%, in \textit{ex vivo} studies. Furthermore, it demonstrated optimal performance across various analyses, including physico-chemical property tests, \textit{ex vivo} release tests, SEM analysis, stability studies, skin irritation tests, \textit{in vivo} bio-availability studies, and \textit{in vivo} antihypertensive investigations. Skin irritation tests revealed that the topical application of the drug formulations did not cause any irritation, signifying their safety for use in humans. Moreover, SEM analysis revealed good surface morphologies of vesicles. Furthermore, stability studies indicated that the formulations remained stable throughout the stability period. Notably, the EMNP7 formulation achieved an approximately 188.99-fold higher bio-availability, in terms of sustained plasma concentrations for up to 24 h, compared to the Vasotec tablet (12.80%).

Thus, nanoproniosomal gels can serve as effective controlled release drug delivery systems, functioning as efficient formulations that can be applied topically to administer drugs once per day to improve hypertensive management. The following key conclusions can be derived from our research outcomes: Nanoproniosomal gels encapsulating EM can be applied topically, offering a promising alternative to traditional dosage forms for the therapeutic management of hypertension. In particular, the EMNP7 formulation demonstrates significant application potential in hypertension treatments. However, its clinical applicability must be further validated through clinical trials. Furthermore, additional research is necessary to overcome the limited permeability of the drug through the SC using varying permeation enhancers and technologies.

Overall, the proposed nanoproniosomal carrier systems demonstrate potential for the successful transdermal administration of bioactive drugs and complex therapeutic molecules aimed at treating various diseases and ailments. Future research efforts must focus on the delivery of other drugs to gain insights into their local and systemic effects.

\section*{CRediT Author Statement}

\textbf{Mekala Sabareesh}: conceptualization, investigation, methodology data curation, writing original draft, writing–review, and editing. \textbf{Jayaraman Rajangam}: supervision, visualization, validation, writing–review, and editing. \textbf{Janapati Pedda Yanadaiah}: data curation, formal analysis, investigation, and resources.

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\section*{Conflict of Interests}

The authors declare that no competing interest exists.
References


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