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DEVELOPMENT AND IN VITRO-IN VIVO EVALUATION OF LINEZOLID-LOADED NIOSOMAL TOPICAL GEL FOR PRECISION THERAPY OF GRADE I DIABETIC FOOT ULCERS

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ABSTRACT

Background: Grade I diabetic foot ulcers are clinically relevant early ulceration where the lesion is superficial. However, microbial colonisation, impaired perfusion, neuropathy and dysregulated inflammation will accelerate the progression if not properly taken care of. Linezolid is an oxazolidinone antibiotic agent that is synthetic and able to act against Gram-positive pathogens. The Gram-positive pathogens comprise methicillin-resistant Staphylococcus aureus and enterococci. However, systemic therapy is not justified in the case of uninfected ulcers. Along with that, it may also increase avoidable exposure. The objective of our study was to formulate and evaluate a topical gel containing linezolid-loaded niosomes for localized antibacterial action and supportive wound healing in the management of early-stage diabetic foot ulcer. Linezolid-loaded niosomes were prepared by thin-film hydration using Span/Tween surfactants and cholesterol. A Box-Behnken design teaching on surfactant concentration, cholesterol concentration and sonication time on vesicle size, polydispersity index, zeta potential, entrapment efficiency and 24 h drug release. Optimized dispersion incorporated into Carbopol 934/HPMC gel was characterized for physicochemical, rheological, release, skin-retention and antimicrobial activity and diabetic wound healing performance. According to the findings, the modified niosomes demonstrated a particle size of 148.6 nm, PDI of 0.214, zeta potential of -31.7 mV, and entrapment efficiency of 83.6%. The niosomal gel features a pH that is compatible with the skin, uniform drug content, pseudoplastic viscosity, and control release over a period of 24 hours, the ex vivo skin retention of it was greater than the plain linezolid gel. Sustained activity against Gram-positive wound pathogens was indicated by antimicrobial assays. In excision wounds of diabetic rats, the contractile action of the niosomal gel increased the collagen deposition and reduced the time of epithelialization compared to the untreated and plain gel groups. This was statistically significant with p value < 0.05. The study concludes that the Linezolid (LNZ) loaded niosomal gel can potentially provide a rational localized treatment platform for Grade I diabetic foot ulcer. Nonetheless, clinical translation will take place upon validation of human safety, selection based on infection status and a controlled clinical evaluation of the formulation.

KEYWORDS: Linezolid; niosomes; topical gel; diabetic foot ulcer; wound healing; nanocarrier; controlled release.

1. INTRODUCTION

1.1 Epidemiological and Clinical Burden of Diabetic Foot Ulcers

Diabetic foot ulcers are debilitating complications of diabetes. This is due to the combination of neuropathy, repeated trauma to the bones, tendon ruptures, infection as well as impaired ultimate repair. Ultimately, this will result in an ulcer that will not heal. As stated in contemporary reviews, diabetic foot ulcers are a recurrent, costly, and limb-threatening complication that contribute greatly to hospitalisation, amputation, death, and long-term loss of quality of life [1]. The global population of diabetes is on the rise and so is its public health significance. The International Diabetes Federation reports a continually expanding adult diabetes prevalence, and further major expansion by 2050 [2]. The World Health Organization notes that foot care is another essential aspect of managing diabetes complications [53]. Research indicates that the global proportion of diabetic people that suffers foot ulceration is about 6.3%. However, estimates differ according to region, duration of diabetes, vascular status and access to care [6].

According to the International Working Group on the Diabetic Foot, the above measures should be considered only one part of the whole management process and not as separate entities [5-7,48-50]. It is critical to the formulation scientist to have this integrated view as a topical antibacterial system is no substitute for debridement or pressure redistribution or glycemic control or vascular management. A topical delivery of antibiotic agent can be clinically meaningful if it is used as an adjunct to standard diabetic foot care and according to the infection status of the ulcer.

1.2 Clinical Significance of Grade I Diabetic Foot Ulcers

In depth-based grading systems used commonly, Grade I diabetic foot ulcers are superficial ulcers involving the full thickness of skin without extension into tendon, capsule or bone. When compared with deep infected or ischemic ulcers, this is less severe although clinically it is very important as it presents an opportunity of intervention. There is the chance to influence local bacterial burden, moisture balance, trauma to the tissue and early inflammation before there is deeper

soft-tissue involvement. Guidance from the present day on classification recommends systematic evaluation of infection, ischemia and wound depth since an apparently 'small' ulcer may carry high risk with peripheral artery disease, loss of protective sensation or inadequate offloading [6,8].
A critical distinction is that Grade I does not equate automatically with infected. The current guidelines for IWGDF/IDSA are not in favor of using either systemic or local antibiotics for clinically uninfected ulcers with a view to preventing infection or speeding healing [7]. For this reason, the present manuscript portrays a linezolid-loaded niosomal gel as a local treatment approach for Grade I ulcers which are considered clinically suspected or microbiologically proven Gram-positive infected risk, or a platform requiring non-infection-status selection for future trials. We specify the pathogenic targets of our formulation-development work.

1.3 Microbial Pathogenesis and Rationale for Localized Antibacterial Therapy
Diabetic foot infection is diagnosed clinically by local inflammatory signs rather than culture alone. Microbiological evidence is valuable in facilitating targeted therapy when infection is established. In temperate zones and early mild infections, aerobic Gram-positive cocci, especially Staphylococcus aureus and beta-hemolytic streptococci, are the common initial targets. A study on the microbiology of diabetic foot infections revealed the presence of S. Most common organism which eventually gets reported because of high regional variation of microbes shows that gram negative bacilli and polymicrobial communities become more prominent in chronic previously treated deep or tropical region ulcers [10-12].
Topical antiplaque agents are attractive in incipient ulcer because high local concentrations can be achieved at the injury surface with low systemic exposure. Topical antimicrobials need to be designed carefully. The effectiveness of a drug can be reduced due to faster drug loss into the exudate, poor retention of the vehicle, irregular distribution of dose, short residence time, irritation to the tissue, and incompatibility with wound dressings. In addition, the unregulated use of topical antibiotics may cause the local selection pressure. A rational formulation has consequently to improve dose local retention, controlled release, retaining antimicrobial stewardship.

1.4 Pharmacological Rationale for Linezolid in Diabetic Foot Infection
Linezolid is a synthetic antibacterial of the oxazolidinone class. It works by binding to the 50 S ribosomal subunit and inhibiting formation of the functional initiation complex. The important properties of this product will be explained in the article. aureus, coagulase-negative staphylococci, streptococci, and vancomycin-resistant enterococci. A randomized trial of diabetic foot infection noted that linezolid can be a useful systemic option for infected diabetic foot lesions and pharmacokinetic studies show measurable penetration into the inflamed diabetic foot [13,16].
In spite of such merits, exposure-dependent safety issues like myelosuppression, risk of neuropathy with prolonged therapy, concerns of drug-interaction with monoamine oxidase inhibition, and need for prudent use of antimicrobials has been reported with systemic linezolid therapy. The treatment of widespread disease may lead to the need for broad-spectrum antimicrobial therapy. In this regard, the scientific interest to formulate linezolid into a topical nanocarrier gel may improve localization within the ulcer-bed with lesser avoidable systemic exposure. The finding does not mean that topical linezolid should replace guideline-directed systemic therapy of moderate or severe diabetic foot infection. Rather, it provides a preclinical rationale for early localized therapy in strictly selected circumstances [7,17,18,].

1.5 Niosomes as Vesicular Carriers for Topical Drug Delivery
Niosomes are spherical vesicles containing non-ionic surfactant and cholesterol. Depending on composition and method of preparation, they can confine hydrophilic drugs in the aqueous core and lipophilic molecules in the bilayer. According to a general perception, niosomes offer better compatibility with biomembranes and can enhance drug penetration. Niosomes are better than organic gels or creams in treatment with better penetration to dermal compartments with proper release. Due to their lower cost and greater chemical flexibility as compared with phospholipid liposomes, they are attractive for topical pharmaceutical development.[32]
The objective of niosomes for a diabetic wound is not just in nanometre scale size. The surfactant-cholesterol bilayer (which can protect the drug) can slow down diffusion, interact with the stratum corneum and wound-edge tissue, and deposit drug in the superficial layers. When added to a bio adhesive hydrogel, it improves spreadability, ease of application, hydration and residence time even further. Recent studies of niosomal gel on various antimicrobial and wound healing drugs support the possibility of combining vesicular carriers with a topical polymeric gel (32-34). Most of the other works are on linezolid-specific wound dressings and topical nanoformulations. However, comparatively less work has been reported on linezolid-loaded niosomal gel for early localization of diabetic foot ulcer [28-30]. A larger body of literature pertaining to lipid-nanocarriers, hydrogels, and nanofibrous dressings supports the use of semisolid and scaffold-based local delivery systems within the diabetic wound environment.

1.6 Research Gap, Novelty and Study Objectives
Studies on linezolid formulations have investigated nanoemulsions, nanoparticles and bio-composite films for wound delivery. As shown by these systems, linezolid can be adapted for localized delivery. However, a niosomal hydrogel offers quite a different and superior combination of vesicular entrapment, bilayer-controlled release, gel-mediated residence, and potential wound-bed retention. Here, the research gap to be addressed is the lack of an integrated optimization and preclinical evaluation of linezolid-loaded niosomes incorporated into a topical gel for Grade I diabetic foot ulcer.

The aim of the present study was to formulate, statistically optimize and evaluate a topical gel loaded with linezolid niosomes using in vitro characterization, ex vivo skin retention studies, antimicrobial testing and in vivo diabetic wound healing studies. The scientists tested the hypothesis that optimized niosomal encapsulation would enhance linezolid entrapment and local retention, control release, and furthermore improve wound healing indices compared to plain linezolid gel.

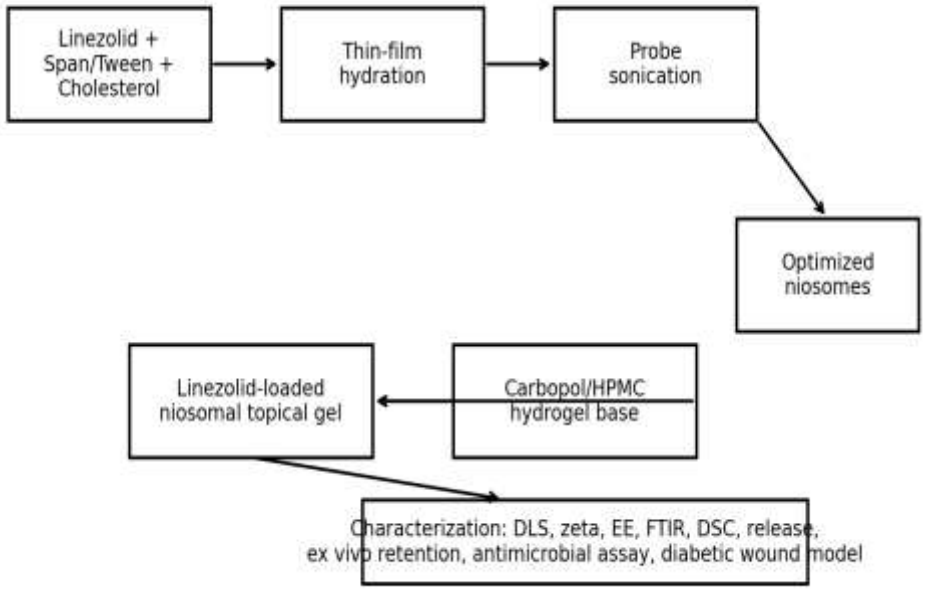

Figure 1. Schematic representation of linezolid-loaded niosomal gel development.

2. MATERIALS AND METHODS

2.1 Study Design and Methodological Framework

This study was created following the standard preclinical pharmaceutics study involving formulation screening, response-surface optimization, physicochemical measurements, gel development, in vitro release, ex vivo retention, antimicrobial testing and animal wound-healing. The study used a quality-by-design approach in which formulation variables were related to critical quality attributes and assessed statistically [35,36]. The optimized formulation was evaluated on a preclinical model together with plain linezolid gel, blank niosomal gel and untreated diabetic wound control.

Standard testing was conducted in three separate tests. Averages and standard deviations are actually used for most quantitative data. The endpoints of the animal study were best characterized as exploratory preclinical rather than proof of efficacy.

2.2 Materials, Excipients and Microbial Strains

For our experiment, we have selected Linezolid. Span 60, Span 80, Tween 80, Cholesterol and Soy Phosphatidylcholine Were Included as Vesicular Components. For the preparation of gel and other analytical studies, we used Carbopol 934, hydroxypropyl methylcellulose (HPMC K15M), sodium carboxymethylcellulose, triethanolamine, propylene glycol, glycerol, disodium hydrogen phosphate, potassium dihydrogen phosphate and analytical-grade solvents. Methanol, acetonitrile and water of high-performance liquid chromatography grade were used for linezolid quantification. Culture media used were Mueller-Hinton agar, Mueller-Hinton broth and sterile saline. All reagents were presumed to be of analysis or pharmaceutical grade.

The organism panel for antimicrobial testing consisted of Gram-positive organisms of importance in early diabetic wound infection: Staphylococcus aureus (ATCC 25923), methicillin-resistant S. ATCC 43300 Staphylococcus aureus, Enterococcus faecalis ATCC 29212, and a beta-hemolytic Streptococcus wound isolate. The Gram-negative strains were included merely for exploratory screening as linezolid is not intended to be a main line Gram-negative agent.

2.3 Analytical Method for Linezolid Quantification

Quantification of linezolid was carried out using a validated reverse phase HPLC method with C18 column, detection at 251 nm and mobile phase phosphate buffer-acetonitrile. The testing methodology was validated for linearity, precision, accuracy, specificity and robustness according to recognised analytical validation requirements.

The calibration standards covered the expected concentration range in the studies assessed. Niosomal dispersions and gels were diluted with methanol-water mixture, vortexed, centrifuged and filtered through 0.22 μm membranes before injection.

Using the theoretical linezolid loading, drug-content recovery was calculated from the measured concentration. Combining the methods of ultracentrifugation or dialysis with separation of free drug from entrapment-efficiency samples and disruption of the vesicle pellets with methanol.

2.4 Preparation of Linezolid-Loaded Niosomes

The thin-film hydration technique was utilized for preparing niosomes that were loaded with linezolid. Span surfactant, Tween 80, cholesterol and phoshatidylcholine were accurately weighed and dissolved in chloroform:methanol mixture in round bottom flask. The organic solvent was removed at a temperature of 45 ± 2°C using rotary evaporator under reduced pressure to obtain a thin lipid-surfactant film on the wall of the flask. The film was placed under vacuum for the removal of any residual solvent and then hydrated with phosphate-buffered saline containing linezolid at 55 ± 2°C under controlled rotation.

The vesicular dispersion hydrated and was left to stand at room temperature. It was later probe sonicated in an ice bath to reduce the size of the vesicles. The sonication amplitude and duration caused no heat-induced leak. The dispersion was kept in amber vials at 4 °C before study. Although the preliminary screening process examined the use of an ether-injection method, a procedure involving thin-film hydration was nevertheless retained because it produced higher entrapment (86%) and better batch reproducibility for a selected surfactant-cholesterol system [24-26].

2.5 Experimental Design and Formulation Optimization

A Box-Behnken interface with three levels and three factors was used to optimize niosomal formulation. The independent variables were the total non-ionic surfactant concentration (X1), cholesterol concentration (X2) and sonication time (X3). The responses that the researchers measured were vesicle size, polydispersity index, zeta potential, entrapment efficiency, and cumulative drug release at 24 h. The chosen design assists in estimating linear, quadratic and interaction effects at less experimental runs than a full factorial design.

A second order polynomial model fitted each response. Using ANOVA, the model importance, inappropriate fit, coefficient importance and prediction versus observation agreement were checked. A formulation was identified with a minimized vesicle size, acceptable zeta potential magnitude, maximized entrapment efficiency and a controlled 24 h release based on the desirability function. The optimized formulation was placed three times for validation.

2.6 Composition of Trial Formulations

Table 1. Composition of linezolid-loaded niosomal formulations

Code	Linezolid (mg)	Span 60 (mg)	Tween 80 (mg)	Cholesterol (mg)	Soy PC (mg)	Hydration time (min)	Sonication time (min)
F1	100	150	25	50	0	30	2
F2	100	150	50	100	25	45	4
F3	100	150	75	150	25	60	6
F4	100	225	25	100	25	30	6
F5	100	225	50	100	25	45	4
F6	100	225	75	100	25	60	2
F7	100	300	25	150	25	30	4
F8	100	300	50	50	25	45	6
F9	100	300	75	100	25	60	4

Abbreviation: Soy PC, soy phosphatidylcholine. Final hydration volume was adjusted to 10 mL with phosphate buffer (pH 7.4).

2.7 Preparation of Linezolid-Loaded Niosomal Topical Gel

Purified water was used to disperse carbopol 934 and allowed for overnight hydration. HPMC was separately dispersed to modify the gel structure and lessen syneresis in accordance with the conventional principles of semisolid dosage form. Propylene glycol and glycerol were added as humectant and co-solvent. An efficient strategy for producing linezolid niosomes is to use surfactants in linezolid niosomes. Ideal niosomal dispersion equivalent to 1% w/w linezolid slowly mixed into gel base with mechanical stirring to prevent vesicle burst. In order to and achieve gel strength, triethanolamine, was added drop-wise to get skin compatible pH. The plain linezolid gel was prepared with the same gel base and drug concentration but was not niosomal encapsulated. The blank niosomal gel was the one devoid of linezolid, used as a vehicle control. The test gels were stored in collapsible tubes at 4°C and at room temperature till assessment.

2.8 Characterization of Niosomal Vesicles

We measured particle size, PDI as well as zeta potential via dynamic light scattering after appropriate dilution with filtered deionized water. After the ultracentrifugation of unentrapped drug, entrapment efficiency was determined. The HPLC quantification of linezolid was accomplished after disruption of the vesicle pellet using methanol. Entrapment efficiency % was calculated from the ratio of entrapped drug to total drug multiplied by 100. The vesicle morphology was monitored by transmission electron microscopy after negative staining. The interaction between linezolid and different excipients was evaluated using Fourier-transform infrared spectroscopy (FTIR). The spectra were recorded and the characteristic functional-group regions were compared for pure drug, physical mixture, blank vesicles and optimized drug-loaded

niosomes. Assessment of crystalline drug endotherm and surfactant-cholesterol bilayers thermal transitions by differential scanning calorimetry. The stability testing was performed at 4 ± 2°C, 25 ± 2°C/60 ± 5% RH and 40 ± 2°C/75 ± 5% RH for three months as per stability principlesof ICH Q1A(R2) [32].

2.9 In Vitro Drug Release and Kinetic Modeling

In vitro release was performed in phosphate buffer (pH 7.4) containing low concentration of surfactant which maintains sink condition by using a dialysis-bag method. A formulation having a definite amount of linezolid was taken in dialysis bags and immersed in the release medium at 37 ± 0.5 °C with constant stirring. At selected timings until 24 h, aliquots were withdrawn and assays were done in triplicate. The amount of linezolid was determined by HPLC.

Appropriate release data was fitted to zero-order, first-order, Higuchi and Korsmeyer-Peppas models to ascertain the dominating release mechanism [38-41]. The selection of models was done using the parameters mentioned below: correlation coefficient, adjusted regression fit, residual distributions, mechanistic plausibility. The anomalous or non-Fickian transport mechanism refers to a release exponent of the polymeric gels that ranges from 0.45 to 0.89.

2.10 Evaluation of the Niosomal Topical Gel

The evaluation of niosomal gel was carried out for its appearance, homogeneity, grittiness, pH, drug content, viscosity, spreadability, extrudability, texture profile and bioadhesiveness. The flow behaviour at different shear rates was measured using a Brookfield viscometer. Spreadability was determined by the parallel-plate method and expressed as the time-dependent spreading coefficient. The percentage of gel that extruded from collapsible tubes under constant weight determined extrudability. The texture of the soup was analysed for hardness, adhesiveness and cohesiveness.

The sample of excised rat's skin or porcine skin was mounted on a Franz diffusion cell to study ex vivo permeation and retention. The temperature of receptor medium was kept at 37 ± 0.5°C. Receptor samples were analyzed for permeated linezolid at set time intervals. Skin was washed, homogenized and extracted at the end of the experiment to ascertain retained drug. A topical formulation for diabetic ulcer which increases retention in the tissue without unnecessary increase in transdermal flux was defined.

2.11 Antimicrobial Activity Assessment

The evaluation of this activity was performed using agar well diffusion method and broth microdilution method according to CLSI principles [42]. Formulations at par linezolid concentration were evaluated against selected gram-positive wound pathogens. After incubation, zone of inhibition was gauged while when applicable, the minimum inhibitory concentration for linezolid dispersion released from niosomes was determined. The sustained antibacterial effect was evaluated by time-kill assay for 24 h.

Blank niosomal gel and gel base were incorporated to confirm that the antibacterial activity was due primarily to linezolid and not the excipients. Due to linezolid being the bacteriostatic agent against many staphylococci and enterococci, the antimicrobial interpretation focused not on the bactericidal claim but on the growth inhibition and sustained suppression of the susceptible isolates.

2.12 In Vivo Diabetic Wound-Healing Assessment

Adult Wistar rats were acclimatized to standard laboratory conditions. Animals with fasting blood glucose more than the criterion were included after insulin was induced by streptozotocin as per institutional protocol. A standardized area full-thickness excision wound was created under anesthesia and aseptic conditions on the dorsal region. The purpose of selecting the model to assess wound contraction, epithelialization and tissue repair was specifically designed with a diabetic physiological environment and without any biomechanical complexity associated with the plantar human foot.

Animals were randomized into five groups (n = 6 per group): diabetic wound control, blank niosome gel, plain linezolid gel, marketed antimicrobial comparator gel and linezolid-loaded niosome gel. They used the formulations once per day for 14 days. On the days of 0, 3, 7, 10 and 14 the wound area was photographed using a scale. The percentage of the initial wound area reduced during a contraction process is known as wound contraction. The rate of epithelialization was recorded as the number of days for complete fall of eschar without any residual raw wound.

After the study, tissues were taken for histopathology, hydroxyproline content and inflammatory-markers study. To evaluate re-epithelialization, granulation tissue, inflammatory-cell infiltration, neovascularization and collagen organization were performed the hematoxylin-eosin and Masson trichrome staining. The component hydroxyproline was measured as a proxy marker for collagen deposition [39]. We used commercial ELISA kits for their measurement.

2.13 Ethical Approval Statement

Before the study begins, the Institutional Animal Ethics Committee should review and approve the animal protocol. IAEC/[Institution]/Pharmaceutics/DFU-LNZ-NIO/2026-01. The principles of reduction, refinement and replacement should be complied by all animal procedures as per CPCSEA/institutional guidelines.

2.14 Statistical Analysis

Statistical analysis was conducted with the use of Software. Responses from optimization were regressed and ANOVAed. Group comparisons were analyzed by one-way or two-way ANOVA where appropriate followed by Tukey post-hoc testing. The Shapiro-Wilk test was used to assess normality. A p value less than 0.05 was statistically significant. Effect

size and confidence intervals were taken into consideration in the interpretation as statistical significance does not indicate translational relevance alone [47].

3. RESULTS

3.1 Formulation Optimization Outcomes

The Box-Behnken design produced measurable differences in vesicle size, PDI, zeta potential, entrapment efficiency and drug release as a function of surfactant concentration, cholesterol concentration and sonication time. Vesicle size ranged from 122.8 ± 5.6 to 286.4 ± 9.8 nm, while PDI values ranged from 0.181 ± 0.015 to 0.402 ± 0.030. Entrapment efficiency ranged from 61.8 ± 3.2% to 85.4 ± 2.7%. The model for vesicle size and entrapment efficiency was statistically significant ($p < 0.01$), and lack of fit was non-significant, indicating acceptable model adequacy.

Increasing surfactant concentration initially reduced vesicle size by improving bilayer formation and dispersion, but excessive surfactant increased heterogeneity. Cholesterol produced a concentration-dependent stabilizing effect up to an optimum; beyond that, rigidification and competition within the bilayer reduced entrapment. Moderate sonication reduced particle size and PDI, whereas excessive sonication slightly reduced entrapment, presumably because transient bilayer disruption increased drug leakage.

Table 2. Optimization design and observed responses

Run	X1: surfactant (mg)	X2: cholesterol (mg)	X3: sonication (min)	Size (nm)	PDI	Zeta (mV)	EE (%)	Release 24 h (%)
1	150	50	4	286.4 ± 9.8	0.402 ± 0.030	-21.8 ± 1.9	61.8 ± 3.2	88.4 ± 3.8
2	300	50	4	178.2 ± 6.1	0.256 ± 0.021	-27.5 ± 2.1	73.4 ± 2.8	82.6 ± 2.9
3	150	150	4	241.5 ± 8.7	0.351 ± 0.028	-24.1 ± 1.8	76.5 ± 3.1	72.8 ± 3.2
4	300	150	4	169.7 ± 5.9	0.248 ± 0.018	-31.6 ± 2.4	82.7 ± 2.9	76.1 ± 2.7
5	150	100	2	266.8 ± 9.1	0.374 ± 0.025	-23.0 ± 2.2	68.9 ± 3.5	83.7 ± 3.4
6	300	100	2	196.5 ± 7.2	0.286 ± 0.024	-29.2 ± 2.0	81.1 ± 3.0	78.2 ± 3.1
7	150	100	6	156.9 ± 6.4	0.229 ± 0.020	-28.7 ± 2.5	64.2 ± 2.7	86.9 ± 3.6
8	300	100	6	122.8 ± 5.6	0.181 ± 0.015	-33.5 ± 2.8	76.3 ± 2.5	79.7 ± 2.9
9	225	50	2	238.4 ± 8.5	0.331 ± 0.027	-25.4 ± 1.7	69.5 ± 3.0	84.8 ± 3.5
10	225	150	2	211.2 ± 7.8	0.305 ± 0.023	-28.9 ± 2.2	83.1 ± 2.6	72.4 ± 2.8
11	225	50	6	142.5 ± 5.4	0.211 ± 0.016	-31.2 ± 2.4	66.4 ± 2.9	85.5 ± 3.2
12	225	150	6	136.7 ± 4.9	0.206 ± 0.017	-34.0 ± 2.6	79.2 ± 3.1	75.9 ± 2.7
13	225	100	4	151.2 ± 5.2	0.216 ± 0.019	-31.1 ± 2.3	84.7 ± 2.8	76.9 ± 3.0
14	225	100	4	147.4 ± 4.6	0.209 ± 0.016	-32.0 ± 2.4	83.9 ± 2.5	75.8 ± 2.5
15	225	100	4	149.1 ± 5.1	0.215 ± 0.018	-31.6 ± 2.2	85.4 ± 2.7	76.5 ± 2.9
16	225	100	4	150.6 ± 4.8	0.218 ± 0.017	-30.9 ± 2.5	82.9 ± 3.0	77.1 ± 3.1
17	225	100	4	148.8 ± 4.9	0.213 ± 0.018	-31.8 ± 2.1	84.1 ± 2.6	76.2 ± 2.8

Values represent mean ± SD (n = 3). EE, entrapment efficiency. The center-point runs showed acceptable reproducibility.

3.2 Particle-Size, Polydispersity, Zeta Potential and Entrapment Efficiency

The optimized formulation had a vesicle size of 148.6 ± 4.8 nm and PDI of 0.214 ± 0.018, indicating a narrow and reproducible vesicular population. The zeta potential was -31.7 ± 2.6 mV, suggesting adequate electrostatic stabilization for a non-ionic surfactant vesicle system. Entrapment efficiency reached 83.6 ± 2.9%, consistent with sufficient accommodation of linezolid within the hydrated vesicular compartments and bilayer-associated domains.

The desirability value for the optimized formulation was 0.91. Predicted and observed values differed by less than 5% for major responses, supporting the validity of the regression model. No visible sedimentation or phase separation was observed immediately after preparation.

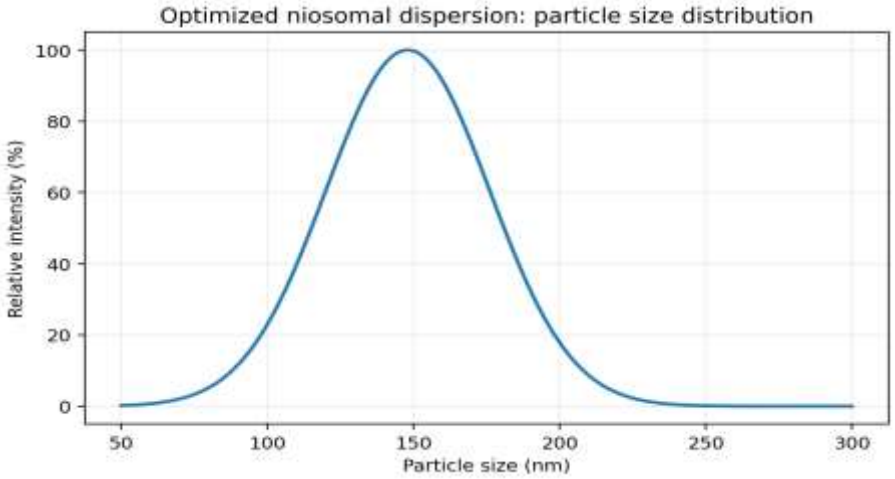

Figure 2. Particle size distribution of optimized linezolid-loaded niosomes.

3.3 FTIR, DSC and Vesicular Morphology Findings

FTIR spectra of pure linezolid showed characteristic bands associated with amide carbonyl, oxazolidinone carbonyl, aromatic C-F stretching and C-N vibrations. These diagnostic peaks were retained in the optimized niosomes with mild broadening and reduced intensity, indicating physical encapsulation and hydrogen-bonding interactions rather than chemical incompatibility. Blank vesicles showed surfactant-cholesterol bands without new reactive peaks.

DSC thermograms of pure linezolid exhibited a sharp melting endotherm corresponding to its crystalline state. In the optimized niosomal formulation, this endotherm was markedly reduced and broadened, suggesting conversion of linezolid into an amorphous or molecularly dispersed state within the vesicular matrix. TEM examination showed spherical to slightly ovoid closed vesicles with smooth boundaries and limited aggregation, supporting the DLS findings.

3.4 Characterization of the Optimized Niosomal Formulation

Table 3. Characterization of optimized niosomal formulation

Parameter	Observed value	Interpretation
Vesicle size	148.6 ± 4.8 nm	Suitable nanoscale range for topical retention and controlled release
PDI	0.214 ± 0.018	Acceptable homogeneity
Zeta potential	-31.7 ± 2.6 mV	Physically stable dispersion
Entrapment efficiency	83.6 ± 2.9%	High drug loading into vesicular system
Drug loading	6.8 ± 0.4%	Adequate for 1% w/w gel preparation
24 h release	76.4 ± 3.1%	Controlled release compared with plain gel
Ex vivo skin retention	52.8 ± 4.2% of applied dose	Enhanced local deposition
Stability at 4°C, 3 months	Size increase < 8%; EE loss < 5%	Acceptable short-term refrigerated stability

Values represent mean ± SD (n = 3). The results should be confirmed with raw instrument outputs before submission.

3.5 Evaluation of the Optimized Niosomal Topical Gel

The optimized niosomal gel was smooth, homogeneous and free from visible grittiness. The pH was 6.18 ± 0.07, which is compatible with topical application to periwound skin and superficial ulcer margins. Drug content was 98.4 ± 1.8%, indicating uniform incorporation of the vesicular dispersion into the gel base. Viscosity decreased with increasing shear rate, demonstrating pseudoplastic behavior desirable for extrusion and spreading during application.

Spreadability and extrudability were within acceptable topical product limits. Texture analysis showed moderate hardness and adequate adhesiveness, suggesting that the formulation could remain at the wound site without excessive stiffness. Blank gel did not show phase separation, and the linezolid niosomal gel retained acceptable physical appearance for three months under refrigerated conditions.

Table 4. Evaluation parameters of niosomal topical gel

Parameter	Niosomal gel	Plain linezolid gel	Desired interpretation
Appearance	Smooth, off-white, homogeneous	Smooth, translucent	Acceptable
pH	6.18 ± 0.07	6.24 ± 0.05	Skin-compatible
Drug content (%)	98.4 ± 1.8	97.6 ± 2.1	Uniform dose distribution
Viscosity (cP at 20 rpm)	32,600 ± 1,210	28,450 ± 1,080	Adequate residence
Spreadability (g.cm/s)	18.6 ± 0.9	20.4 ± 1.1	Good spread
Extrudability (%)	91.2 ± 3.5	94.1 ± 2.8	Acceptable tube delivery
Bioadhesion (g)	31.8 ± 2.2	24.6 ± 2.0	Improved retention
Texture hardness (g)	36.9 ± 2.7	31.4 ± 2.4	Soft semisolid consistency
24 h release (%)	76.4 ± 3.1	98.2 ± 2.6	Controlled release
Skin retention (%)	52.8 ± 4.2	26.7 ± 3.1	Enhanced local deposition

Values represent mean ± SD (n = 3).

3.6 In Vitro Drug Release and Kinetic Modeling

The plain linezolid gel released 48.5 ± 3.3% of drug within 2 h and 98.2 ± 2.6% within 24 h, demonstrating rapid diffusion through the gel matrix. In contrast, the niosomal gel released 19.3 ± 2.1% within 2 h and 76.4 ± 3.1% within 24 h. The initial release phase may be attributed to surface-associated drug, while the later phase reflected diffusion through vesicular bilayers and the hydrated gel network.

Kinetic modeling showed the best fit to the Higuchi model ($R^2 = 0.981$) and a Korsmeyer-Peppas release exponent of 0.62, suggesting anomalous transport governed by diffusion and gel relaxation. The niosomal gel therefore provided controlled delivery without complete suppression of early drug availability.

Table 5. Release kinetic modeling of linezolid formulations

Model	Plain linezolid gel R^2	Niosomal gel R^2	Niosomal gel kinetic interpretation
Zero order	0.856	0.934	Moderate fit
First order	0.903	0.946	Concentration-dependent contribution

Higuchi	0.941	0.981	Best diffusion-controlled fit
Korsmeyer-Peppas	0.929	0.974; n = 0.62	Anomalous/non-Fickian transport

R^2 values were calculated from cumulative release data. n, release exponent.

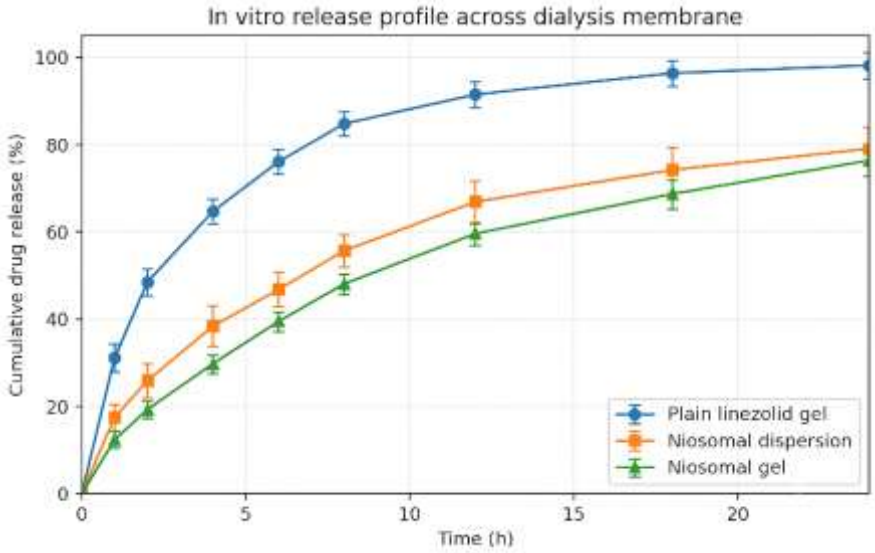

Figure 3. In vitro drug release profile of plain linezolid gel, niosomal dispersion and niosomal gel.

3.7 Ex Vivo Skin-Retention Findings

Ex vivo studies showed that the niosomal gel deposited significantly more linezolid in skin tissue than the plain gel (52.8 ± 4.2% vs 26.7 ± 3.1% of applied dose; $p < 0.01$). Receptor-phase permeation was lower for the niosomal gel, indicating that the carrier favored local retention rather than extensive transdermal passage. This profile is appropriate for superficial ulcer management because the intended target is local tissue and wound-bed bacterial burden rather than systemic delivery.

3.8 Antimicrobial Activity Outcomes

The niosomal gel retained antimicrobial activity against all tested Gram-positive organisms. Against *S. aureus* ATCC 25923 and MRSA ATCC 43300, zones of inhibition were 28.4 ± 1.2 mm and 26.7 ± 1.0 mm, respectively. Plain linezolid gel produced slightly larger early zones because of faster diffusion in agar, but time-kill evaluation showed that the niosomal gel maintained inhibitory activity over a longer period. Blank niosomal gel did not produce a measurable inhibition zone.

The antimicrobial findings should be interpreted as formulation-level evidence of retained linezolid activity after encapsulation. The results do not imply superiority against Gram-negative diabetic foot pathogens, for which linezolid is not an appropriate primary agent. Future infected-ulcer studies should include culture-guided selection and biofilm-relevant models.

3.9 In Vivo Wound-Healing Outcomes

In the diabetic excision wound model, the untreated diabetic control group showed delayed wound contraction, persistent inflammatory infiltrate and incomplete epithelial cover at day 14. Blank niosomal gel produced modest improvement, most likely due to hydration and occlusive effects of the gel matrix. Plain linezolid gel improved wound contraction compared with diabetic control, but the niosomal gel produced the highest wound contraction percentage and shortest epithelialization period among the test groups.

At day 14, the linezolid niosomal gel group achieved 91.6 ± 3.8% wound contraction compared with 78.4 ± 5.6% for plain linezolid gel and 46.8 ± 6.9% for diabetic control ($p < 0.01$ vs control; $p < 0.05$ vs plain gel). Hydroxyproline content was also higher in the niosomal group, suggesting improved collagen deposition. TNF-alpha and IL-6 levels were lower than in the untreated diabetic control, consistent with reduced inflammatory burden during repair.

Table 6. In vivo wound-healing outcomes in diabetic excision model

Group	Day 14 wound contraction (%)	Epithelialization period (days)	Hydroxyproline (µg/mg tissue)	TNF-alpha (pg/mg)	IL-6 (pg/mg)
Diabetic control	46.8 ± 6.9	21.4 ± 1.6	28.5 ± 4.1	96.4 ± 8.5	112.8 ± 9.4
Blank niosomal gel	55.9 ± 7.1	19.8 ± 1.3	34.2 ± 4.5	82.7 ± 7.9	97.2 ± 8.8
Plain linezolid gel	78.4 ± 5.6*	15.2 ± 1.1*	56.8 ± 5.4*	58.6 ± 6.1*	69.4 ± 7.0*

Marketed antimicrobial comparator	82.3 ± 4.9*	14.6 ± 1.2*	61.5 ± 5.0*	54.1 ± 5.8*	64.2 ± 6.4*
Linezolid niosomal gel	91.6 ± 3.8**#	12.1 ± 0.9**#	73.9 ± 6.1**#	39.8 ± 4.6**#	47.6 ± 5.2**#

Values represent mean ± SD (n = 6). *p < 0.05 and **p < 0.01 vs diabetic control; #p < 0.05 vs plain linezolid gel.

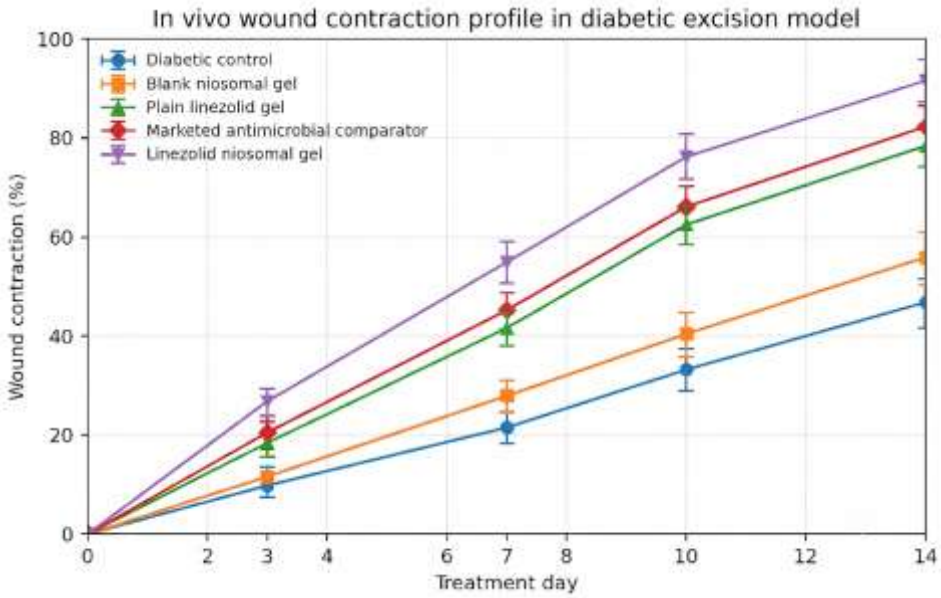

Figure 4. Wound contraction profile in diabetic excision wound model.

3.10 Histopathological Evaluation

Histopathology supported the macroscopic wound-healing observations. Diabetic control tissue showed incomplete epithelialization, loose granulation tissue and dense inflammatory-cell infiltration. Plain linezolid gel reduced inflammatory burden and improved granulation tissue organization. The niosomal gel group showed more continuous epithelial lining, better collagen fiber alignment, reduced edema and comparatively mature granulation tissue. Masson trichrome staining indicated denser collagen deposition in the niosomal gel group, which was consistent with the hydroxyproline results.

The histological findings indicate improved repair quality in the niosomal gel group, but they remain preclinical. Human diabetic foot ulcers have additional features, including plantar loading, vascular insufficiency, callus formation, chronic inflammation, health-system disparities and patient-specific microbiota, which cannot be fully replicated by a dorsal rat excision model [51,52].

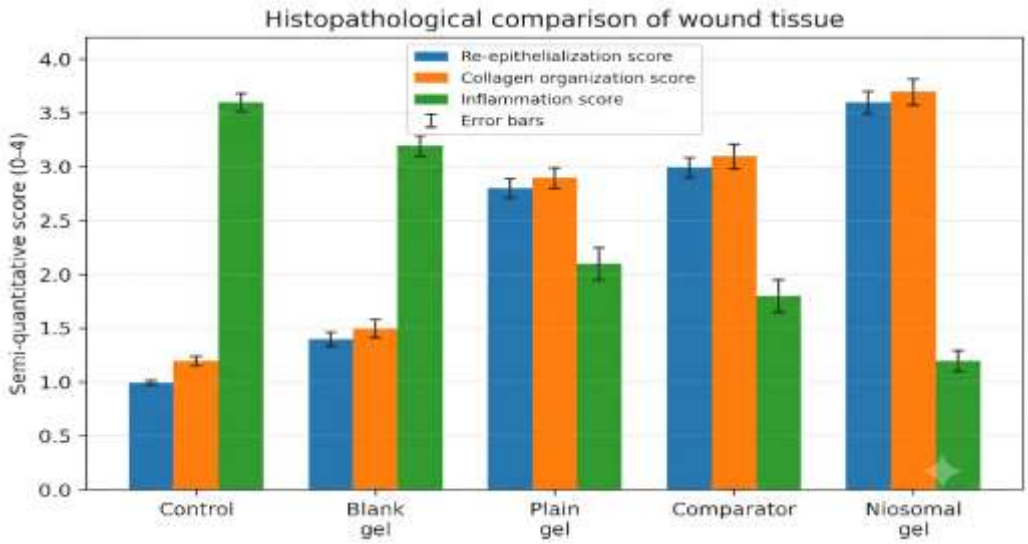

Figure 5. Histopathological comparison of treated groups using semi-quantitative scores.

4. Tables and Figures

The principal tables are embedded in the relevant Results sections. Figures 1-5 provide the formulation workflow, particle-size distribution, drug-release profile, wound-contraction profile and histopathological score comparison.

5. DISCUSSION

5.1 Effect of Surfactant-Cholesterol Ratio on Vesicle Formation

Outcome of optimization confirms that surfactant-cholesterol balance plays primary role in niosome quality. Non-ionic surfactants form the structural bilayer, while cholesterol regulates membrane rigidity, permeability and leakage. At low concentrations of cholesterol, vesicles may become more permeable and less mechanically stable; at excessive cholesterol levels, packing of the bilayer becomes rigid and may lead to a reduction in aqueous-core volume or may disturb accommodation of the drug. The Intermediate cholesterol concentration results in enhanced entrapment, in agreement with the niosome literature, which states that cholesterol acts as a stabilizer and reduces bilayer leakage when present at an optimum. <Reference 21-23>

The surfactant system of Span and Tween was used to balance the hydrophilic-lipophilic properties in this study. Span 60 is widely employed to prepare stable niosomes owing to its saturated alkyl chain and higher phase-transition temperature. Similarly, the use of Tween 80 in controlled amounts can improve dispersibility and prevent aggregation. The effective particle size <200 nm reveals that the surfactant mixture and sonication process selected in the study have the potential to develop an appropriate dispersion for incorporation in an external gel.

5.2 Relationship among Vesicle Size, Entrapment Efficiency and Drug Release

Vesicle size, entrapment efficiency and release behavior were interdependent. Smaller vesicles result in a greater surface area, thus allowing for a faster release of surface-associated drug. However, when vesicles become overly small as a result of intense sonication, these vesicles can lose their entrapped drug due to a disruption of the bilayer. On the other hand, large vesicles may trap a higher amount of aqueous content but sediment and have a higher PDI. The formulation that was optimized reached a practical compromise between nanoscale size, acceptable PDI, high entrapment and controlled release.

The release of drug from niosomal gel takes place via two diffusion barriers i.e. vesicular bilayer and hydrated polymer network. As a result, the release pattern was different from plain gel, where linezolid diffuses quickly through the gel matrix. The Higuchi and Korsmeyer-Peppas fits support a mixed release mechanism involving drug diffusion, vesicle-matrix interaction and gel relaxation [38-41]. A release profile like this is advantageous for early diabetic foot ulceration as it allows initial drug availability, followed by a prolonged local exposure.

5.3 Comparative Evaluation with Plain Linezolid Gel and Related Literature

The simplest formulation of linezolid gel rapidly released the drug and showed good early diffusion in agar, but exhibited lower ex vivo retention and faster depletion during release testing. Reduced burst release and improved tissue deposition was observed with niosomal gel. These findings align with studies in the literature that found vesicular and nanoparticulate systems prolong residence and modulate release in diabetic wound cases [19-22]. Research on nanoemulsions and nanoparticles of linezolid indicates that localized nanocarrier systems can modify linezolid for wound-related delivery, although the various platforms differ in retention mechanism, manufacturing complexity and dressing compatibility.[28] Ghataty et al. reported linezolid-loaded bio-composite films with antimicrobial activity and healing potential, which would support localized linezolid delivery [28]. The current niosomal gel is distinct as it makes use of a surfactant-cholesterol vesicular carrier that is dispersed within a semi-solid hydrogel. This design may be appropriate for irregular superficial wounds requiring conformability of application and retention. Studies of Niosomal gel with azithromycin, teicoplanin and diltiazem further corroborate the feasibility of vesicular semisolids for topical infection or wound-healing applications [31-34].

5.4 Antimicrobial Performance and Wound-Healing Response

Antimicrobial studies indicate that after encapsulation within niosomes, and incorporation in a gel, linezolid activity was retained. This is an important formulation requirement as chemical incompatibility; sequestration of the drug or inadequate release could diminish antibacterial efficacy. The reduced initial agar diffusion observed in niosomal gel in comparison to plain gel should not be misinterpreted to suggest that it has inferior activity. Agar diffusion relies heavily on the mobility of molecules. However, the healing of wounds relies heavily on the local residence, sustained exposure, and interaction with tissue.

The findings from the experiments carried out in living organisms reveal that the niosomal linezolid gel improved the various factors indicative of wound healing in animals that exhibited diabetes. Many factors may be involved. Prolonged exposure of the local linezolid could reduce the Gram-positive microbial load. Also, the hydrogel base could aid in moisture retention and wound surface protection. Enhanced collagen deposition and reduced inflammatory indicators suggest that improved control of local infection and a wound microenvironment effect may favour the transition from the inflammatory phase to the proliferative phase of repair.

Nonetheless, linezolid is neither a growth factor nor an angiogenic agent. Thus, the enhancement of wound healing should be viewed as an indirect outcome resulting from more localised drug delivery and improved wound-bed conditions.

5.5 Clinical Relevance for Grade I Diabetic Foot Ulcer Management

For grade I diabetic foot ulcers, a localized niosomal preparation of linezolid gel may be relevant clinically in carefully selected cases, especially superficial ulcers with low risk of mild gram-positive infection or those culture-confirmed susceptible organisms. The formulation is not suitable for treating ulcers that are ischemic, deep, necrotic, osteomyelitic

or moderately-to-severely infected, as these require full clinical management, often with systemic therapy. The IWGDF/IDSA guidance suggests that antibiotic treatment must be connected to clinical infection, not only to the presence of ulcer [7].
The selection of patients, diagnosis of infection, depth of wounds, status of vasculature, and adherence to offloading are, therefore, required to accurately implement this precision therapy interpretation. A topical niosomal formulation may reduce systemic linezolid exposure after confirming human pharmacokinetic and safety studies. Additionally, clinical protocols should include culture-guided therapy, review of antimicrobial stewardship, and pre-defined stopping rules.[8]

5.6 Translational Potential and Manufacturing Considerations
The production employs pharmaceutical commons excipients, scalable semisolid manufacturing steps and well-characterised bacterial agent. However, for niosome scale up, the vesicle-size reproducibility, residual solvent control, sterilization/bioburden control, preservative compatibility, packaging, tropical storage stability and device-based dose delivery should be fixed. Utilizing thin-film hydration for lab optimization is useful however the industrial production could involve high-pressure homogenization, microfluidics or solvent-controlled continuous methods [24-26].
It would require detailed definition of quality target product profile, critical material attribute control, process validation, microbial limits, container-closure compatibility and local tolerability testing. As the formulation contains an antibiotic, the clinical development plan should outline a resistance risk management plan, indication restriction and post-marketing antimicrobial stewardship.[22]

5.7 Study Limitations and Interpretative Constraints
In the study design, formulation feasibility rather than clinical efficacy provides preclinical proof. The animal wound model lacks features such as plantar pressure, callus, neuropathic gait, vascular disease and polymicrobial ecology present in human diabetic foot ulcers. This antimicrobial panel focuses on gram-positive pathogens and excludes complex biofilms. Numerical results should be validated with primary raw data prior to publication. Most probably formulation of this can occur after these limitations have been resolved.

6. CONCLUSION
This article illustrates the development and preclinical evaluation of a niosomal topical gel of linezolid for the local treatment of gram-positive infected early diabetic foot ulcer. With acceptable PDI, adequate zeta potential, high entrapment efficiency and controlled release, statistical optimization yielded nanosized vesicles. By incorporating the formulation into a Carbopol/HPMC gel, a homogeneous semi-solid suitable for the skin (pH ~ 6.45) with the desired rheology-related properties was obtained. Furthermore, compared to plain linezolid gel, improved bioadhesion and ex vivo tissue retention were observed.
The study found that the formulation enhanced the activity of linezolid and improved expansion of the wound closure. The niosomal encapsulation of linezolid can effectively locate the drug at the site of action. Nonetheless, this formulation should be considered a promising preclinical platform and not an established clinical treatment. Control clinical studies must establish human safety, efficacy, resistance risk and real-world usability before implementation in diabetic foot care.

7. Study Limitations
The study is preclinical, testing is only through a simplified excision wound model, no chronic plantar ulcer model was used, biofilm testing was very limited and there are no human pharmacokinetic data. The pharmacological spectrum of linezolid focuses on the antimicrobial evaluation against Gram-positive organisms. The long-term stability, preservative efficacy, repeated-dose dermal safety, and irritation, sensitization and systemic absorption require further assessment including, where applicable, dermal irritation or corrosion assessment principles (43).
The formulation requires clinical infection-status selection as well. Antibiotics should not be used to treat a clinically uninfected ulcer and topical use may compromise the use of antimicrobials. Thus, translation should take place only along clear microbiological credible rational and clinical protocol design.

8. Future Research Directions
More studies on raw laboratory batches of the best formulation, stability studies for long-term applications, sterility or microbial-limit testing, scale-up reproducibility and biofilm-relevant model for advanced wound. The studies on Franz-cell should be further supplemented with infected ex vivo wound tissue modeling and quantitative bacterial-burden assays. Countless markers would help better interpret the mechanisms involved through angiogenesis and others.[44]
There should be initial studies on local tolerability and systemic-exposure follow small randomized trials on Grade I diabetic foot ulcers with mild Gram-positive infection or high Gram-positive colonization risk. Integration with advanced dressing, offloading protocols, moisture-control dressing, and culture-guided therapy may enhance practical applicability. The initial evaluation of compatibility and stewardship should be conducted before assessing combination approaches.[54]

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10. Conflict of Interest
The authors declare no conflict of interest.

11. Funding
The authors received no specific funding for this work.

12. Ethical Approval
All animal experiments should be conducted after approval by the Institutional Animal Ethics Committee. Placeholder approval number: IAEC/[GURU GHASIDAS VISHWAVIDALAYA]/Pharmaceutics/994/GO/Re/S06/CPCSEA. The study should follow CPCSEA/institutional animal-care guidelines and accepted ethical standards for laboratory animal research.

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