

Optimization and Assessment of Gallic Acid Proniosomal Gel for Transdermal Application

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Abstract

The current study investigates the development and assessment of a proniosomal gel incorporating gallic acid to treat *Staphylococcus aureus* infection. Gallic acid exhibits strong antimicrobial activity; its proniosomes were prepared to enhance its stability and transdermal delivery. Different formulations were developed by the coacervation phase separation approach in combination with a response surface design for optimization. Span 60 and soy lecithin served as the independent variables, with drug release and entrapment efficiency chosen as the dependent responses. The best formulation obtained was further analysed, incorporated into a Carbopol 940 gel base, and evaluated. The entrapment efficiency and drug release of different batches of proniosomes ranged between 76 ± 0.81 to $92.69 \pm 0.55\%$ and 44.06 ± 0.53 to $88.34 \pm 0.41\%$, respectively. The results demonstrated that the optimized batch of proniosomal gel exhibited a uniform vesicle size of 299.7nm with a polydispersity index of 0.232 and a zeta potential of -49.3 mV. pH and drug content of gel were 6.2 ± 0.23 and $99.51 \pm 0.65\%$, respectively. The zone of inhibition of proniosomal gel, marketed gel, and gallic acid was 33 ± 3.87 , 30 ± 2.89 , and 27 ± 3.19 mm, respectively; these results confirmed the superior antimicrobial effect of the formulation. To conclude, gallic acid-loaded proniosomal gel was found to be a promising formulation for the effective treatment of the infection caused by *Staphylococcus aureus*, with its superior drug entrapment and skin absorption properties, which enable the formulation to deliver the drug to the site of infection efficiently.

Keywords – Proniosomes, gallic acid, transdermal, coacervation phase separation method

1. Introduction

Gallic acid is a naturally occurring phenolic compound and is widely distributed among different families of higher plants. It is present throughout different parts of the plant, such as bark, wood, leaves, fruits, seeds, and roots, often occurring either in free form or as derivatives. It exists both in its free form and as components of more intricate structures, including ester derivatives and polymers (Daglia *et al.*, 2014). The therapeutic potential of gallic acid is quite comprehensive. It exhibits diverse biological traits such as antioxidant, anti-inflammatory, anticancer, antiviral, and anti-allergic. Furthermore, research has demonstrated its efficacy in addressing cardiovascular and metabolic disorders. Various investigations have highlighted the antibacterial properties of gallic acid. However, the precise mechanisms underlying its antimicrobial effects remain unclear in several cases. The antimicrobial mechanisms of polyphenols may involve various factors, which act independently or in synergy. These factors include alterations in the function of the cytoplasmic membrane, interference with intracellular processes, and induction of programmed cell death (Hadidi *et al.*, 2024). Despite numerous therapeutic benefits, applications of gallic acid

are restricted owing to fast metabolism, poor absorption, suboptimal distribution, low bioavailability, and quick elimination from the body (Bhuia *et al.*, 2023).

To date, gallic acid has been studied in various forms, including gels, microparticles, emulsions, microemulsions, and micelle formulations, to explore its therapeutic benefits. Stability aspects are a major concern about emulsion formulation (Dhandhi *et al.*, 2025). Vesicular drug delivery systems, such as liposomes, solid lipid nanoparticles, metal nanoparticles, and polymeric nanoparticles, were investigated by various researchers to overcome some drawbacks of gallic acid (Shukla *et al.*, 2022; Monteiro e Silva *et al.*, 2017; Consoli *et al.*, 2016; Patil *et al.*, 2021). Liposomes of gallic acid suffer from the drawbacks of poor encapsulation efficiency, burst release, and oxidative degradation (Enaru *et al.*, 2021). Nanoparticles in general undergo aggregation and are rapidly cleared, costly, and exhibit inconsistent drug release (Holghoomi *et al.*, 2024). The advancement of vesicular drug delivery systems has seen significant interest in the development of niosomes. Niosomes are advantageous owing to low cost, good stability, ease of formulation, and scaling-up (Ge *et al.*, 2019). These are preferred vesicles for transdermal drug delivery due to the modulation of drug transfer through the skin. The alterations in stratum corneum structures are among the

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primary factors contributing to increased permeability of drugs. The treatment with niosomes appears to significantly modify the intercellular lipid barrier within the stratum corneum, resulting in a more relaxed and permeable configuration (Barry, 2001). Niosomes, while beneficial, are not without their limitations. The formulations and compositions of niosomes can be physically unstable, leading to potential aggregation if the established preparation protocols are not strictly followed. Additionally, the process of preparing niosomes can be quite time-consuming, and there are infrequent instances of insufficient drug loading. Furthermore, variations in surface charges on vesicles can occur, which increases the risk of fusion, as vesicles with opposing charges are more likely to attract one another (Bashkeran *et al.*, 2023). These drawbacks can be overcome by proniosomes. These are liquid crystalline-compact niosomal hybrids, which are widely preferred for transdermal drug delivery (Tareen *et al.*, 2020). Upon skin application, these get hydrated with water from skin under occlusion and convert into niosomes (Keyvani-Ghamsari *et al.*, 2023). Some phospholipids can disrupt and fluidize the lipid bilayer of the stratum corneum, thereby improving diffusion across the skin layers. Proniosomes also provide practical benefits in terms of handling, storage, distribution, and dosing. Unlike conventional aqueous niosomal dispersions, they minimize common stability issues like aggregation, vesicle fusion, and drug leak. They also have the potential to improve the skin barrier's recovery rate (Ammar *et al.*, 2011).

Taking into account the above-mentioned factors, the aim of present study is to enhance penetration, stability, and modulate release of gallic acid by loading it into the proniosomal vesicular system. The formulation is further loaded into gel base for transdermal application to counteract microbial infection.

2. Materials and methods

2.1. Materials

Gallic acid, cholesterol, and soy lecithin were obtained from Research-Lab Fine Chem Industries, Mumbai, India. Additional ingredients, including Span 60, ethanol, Carbopol 940, triethanolamine, sodium benzoate, potassium dihydrogen orthophosphate, sodium hydrogen phosphate, and sodium hydroxide, were sourced from Thermosil Fine Chemical Industries Co., Ltd., India. All chemicals and reagents utilized in the formulation process were of analytical grade and highest purity.

2.2. Experimental design

A response surface optimal custom design was employed using Design Expert® software (Version 11, Stat-Ease, Minneapolis, USA) to generate different formulation batches (Table 1). Soya lecithin (X1) and Span 60 (X2) were chosen as independent variables; however, percent entrapment efficiency (Y1) and drug release (Y2) were taken as response factors. Statistical evaluation was done through analysis of variance (ANOVA) within software, and regression models were constructed to interpret the experimental domain. A significance level of $p \leq 0.05$ was considered for all analyses (Soliman *et al.*, 2016).

Table 1. Preparation of proniosomes by response surface optimal custom design

Independent variables	Symbols	Levels		
		Low	Medium	High
Amount of soya lecithin (mg)	X1	10	15	20
Amount of Span 60 (mg)	X2	160	180	200
Dependent variables				
Y1	Entrapment efficiency (%)			
Y2	Drug release (%)			

2.3. Preparation of gallic acid-loaded proniosomes

The preparation of gallic acid-loaded proniosomes was carried out using the coacervation phase separation method (Mehta *et al.*, 2016). Accurately measured quantities of gallic acid, soya lecithin, cholesterol, and Span 60 were placed into a 5 mL glass vial, subsequently by the addition of 0.3 mL of absolute ethanol. The mixture was blended with a glass rod, and the vial was immediately capped to avoid solvent loss through evaporation. The mixture was then subjected to heating in a water bath (Thermostate, India) at a temperature range of 60 to 65°C for approximately five min, ensuring that the surfactant blend was fully dissolved. After a clear solution was obtained, the aqueous phase (0.1 mL) was added at 60°C, and then the system was reheated. After achieving a transparent mixture, the vial was removed from the water bath and allowed to cool down at room temperature (Mohsen *et al.*, 2023). Different formulations are shown in Table 2.

Table 2. The composition of gallic acid proniosomes formulations

Batch no.	Drug (mg)	Cholesterol (mg)	Soya lecithin (mg)	Span 60 (mg)
P1	5	15	20	180
P2	5	15	15	200
P3	5	15	20	160
P4	5	15	10	180
P5	5	15	10	200
P6	5	15	15	160
P7	5	15	15	180
P8	5	15	20	200
P9	5	15	10	160

2.4. Characterization of proniosomes

2.4.1. Entrapment efficiency

0.2 g of the proniosomal gel was added to 10 mL of phosphate buffer (pH 7.4). The mixture was sonicated for 3 minutes to achieve uniform dispersion and subsequently centrifuged at 8000 rpm for 90 minutes at 4 °C (C24 Plus, Remi, India). The supernatant obtained was collected and analysed spectrophotometrically at 259 nm, using phosphate buffer (pH 7.4) as the reference blank. Entrapment efficiency was calculated in triplicate by equation (1) (Kandekar *et al.*, 2025).

$$EE\% = \frac{\text{Total amount of drug} - \text{Untrapped drug}}{\text{Total amount of drug}} \times 100 \quad (1)$$

2.4.2. Drug release

The in-vitro release profile of gallic acid from the proniosomal gel was evaluated using a Franz diffusion cell

(DBK Instruments, India), featuring a receptor compartment with a 25 mL volume and a diffusion area of 1 cm². A dialysis membrane (150 LA 401, HiMedia Laboratories, India) was positioned between the donor and receptor chambers to act as a permeation barrier. A sample containing 2 mg of gallic acid was introduced into the donor compartment, while the receptor side was filled with phosphate buffer at pH 7.4. The system was maintained at a temperature of 32 ± 0.5 °C and stirred continuously using a magnetic stirrer. At scheduled time points ranging from 1 to 8 hours, 1 mL samples were extracted from the receptor compartment. To preserve sink conditions, each withdrawn sample was immediately replaced with an equal volume of preheated phosphate buffer (pH 7.4). The samples were then analysed using UV spectrophotometry at 259 nm, with phosphate buffer (pH 7.4) serving as the blank.

2.4.3. Optimization of gallic acid proniosomes

Optimization was carried out to identify the levels of independent variables (X1 and X2) that would achieve the highest entrapment efficiency and sustain drug release over an 8h. The overlay plot was produced by Design Expert software, and the optimized formulation was selected. Then, it was evaluated and loaded into a gel base.

2.4.4. Characterization of optimized formulation

2.4.4.1. Particle size, polydispersity index (PDI), and zeta potential

0.2g of an optimized batch of proniosomal gel was added to 20 mL of phosphate buffer at pH 7.4, and then it was sonicated in a water bath for 10min. Following sonication, a 1 mL sample was extracted for analysis. Using dynamic light scattering (DLS) with a laser diffraction analyzer (SZ-100, Horiba, Osaka, Japan), vesicle size, polydispersity index, and zeta potential were assessed (Abu El-Enin *et al.*, 2019).

2.4.4.2. Scanning electron microscopy (SEM)

Vesicle morphology was studied by a scanning electron microscope operated at 20 kV. Precisely, 0.2g of proniosomes was dispersed in 10mL of pH 7.4 phosphate buffer. It was affixed to double-sided carbon tape and then positioned on an aluminium stub, where it underwent drying in a vacuum, following which the scanning process was conducted (Kandekar *et al.*, 2025).

2.4.4.3. Differential scanning calorimetry (DSC)

The thermal analysis of gallic acid, soya lecithin, Span 60, and the optimized formulation of proniosomes was performed using a differential scanning calorimeter (Mettler Toledo, SW STAR, United States). The scan was carried out at a heating rate of 10°C/min under a nitrogen atmosphere over a temperature range of 30°C-300°C (Kandekar *et al.*, 2021).

2.5. Preparation of proniosomal gel

Prepared proniosomes are incorporated into the gel base of Carbopol 940. Precisely, 1g of Carbopol 940 was soaked overnight. Optimized formulation of proniosomes was incorporated in a swollen base with constant stirring at a slow speed till a homogeneous mass was formed. Triethanolamine (0.1% v/w) was incorporated in the gel to adjust pH, while glycerine and sodium benzoate (each

0.1%) were added as humectant and preservative, respectively (Cheng *et al.*, 2024).

2.5.1. Determination of pH, viscosity, and drug content

The pH of the optimized proniosomal gel was measured using a pH meter in triplicate. The viscosity was assessed by Brookfield's viscometer (Brookfield Engineering Labs, USA) at 30, 50, and 100 rpm by using spindle number 64. To evaluate drug content, 1g of the prepared gel was mixed with 25mL of ethanol to produce the stock solution. The stock solution was then sonicated in a water bath for 10min, filtered, and analysed spectrophotometrically at 259nm. The content was estimated by equation (2).

$$\text{Drug Content (\%)} = (\text{Actual amount of gallic acid} / \text{Theoretical amount of gallic acid}) \times 100 \quad (2)$$

2.5.2. Texture analysis

The gel's texture was evaluated using a CT3-100 Texture Analyser (Brookfield Engineering Labs, USA) in triplicate. The formulation was placed into the female probe, and the test was conducted in compression mode. A conical male probe (35 mm diameter, 45° angle) was injected to the sample at a speed of 0.50 mm/s and trigger load of 2 g until reaching a depth of 10 mm. All measurements were performed in triplicate at 25 °C. (Dhakane *et al.*, 2023).

2.6. In-vitro diffusion study of gel

The *in-vitro* investigation of gallic acid-loaded carbopol gel, proniosomal gel, and marketed gel was carried out by the Franz diffusion cell apparatus by adopting the same procedure as mentioned under the *in-vitro* diffusion study of proniosomes and gallic acid (Pandit *et al.*, 2023).

2.7. Ex-vivo diffusion study of gel

A Franz diffusion cell with goat skin as the diffusion membrane was used for Ex-vivo diffusion studies of the drug-loaded Carbopol gel, proniosomes, proniosomal gel, and a marketed formulation. Fresh goat skin was collected from a local slaughterhouse. During transport, the skin was preserved in Ringer solution. The hair from the skin was shaved off, and care was taken to avoid transverse cuts. The skin was then attached between compartments, with the stratum corneum facing the donor chamber, and the diffusion study was carried out following the same process described for the *in-vitro* release study. Additionally, fresh goat skin was used with a placebo gel in the donor compartment and phosphate buffer (pH 7.4) in the receptor compartment to serve as a blank control (Shah *et al.*, 2021).

2.7.1. Ex-vivo skin permeation

Ex-vivo permeation of gallic acid through the skin membrane was determined by calculating steady-state flux ($J_{\max}$). The drug flux was calculated by the following equation (3).

$$J_{\max} = \frac{dQ}{dt} / A \quad (3)$$

Where dQ/dt is the skin permeation rate that indicates the cumulative amount of permeated gallic acid Vs time, and A is the effective diffusion area of the skin membrane (Dhakane; Pandit, 2023).

2.8. Antimicrobial activity against *Staphylococcus aureus*

The antimicrobial activity against *S. aureus* was evaluated using the agar well diffusion method against *Staphylococcus aureus* (*S. aureus*). Nutrient agar was prepared by dissolving in distilled water and sterilized in an autoclave (Space Lab Instruments, India) at 121 °C and 15 psi. The *S. aureus* culture was then spread uniformly over the surface of the agar plate. Sterilized borers were used to create wells in the agar plates, into which various samples were added separately. The plates were then transferred to an incubator and incubated at 37°C for 24 h. (Selvaraj *et al.*, 2022). The zone of inhibition (mm) was recorded in triplicate.

3. Result and discussion

3.1. Preparation of gallic acid-loaded proniosomes

Initial trials were conducted to select the level of Span 60 in the range of 5 to 25 mg and soy lecithin in the range of 150 to 250 mg based on literature survey. The proniosomes were not formed below 10 mg of soy lecithin. Similarly, for Span 60 proniosomes were not generated below 160 mg and above 200 mg. Hence, Span 60 and soy lecithin was selected in the range of 160 to 200 mg and 10 to 20 mg, respectively. Span 60 is a sorbitan sterate belonging to the category of non-ionic surfactants. It is a key structural component of proniosomes. The enhancement of the entrapment efficiency of the drug is mainly attributed to the presence of Span 60 (Barani *et al.*, 2018). Cholesterol is included in proniosomes at an optimal level to enhance formulation stability through its interaction with non-ionic surfactants. Cholesterol alone is not capable of forming the bilayer structure; however, it can integrate with the bilayer membrane, serving as a crucial regulator of both the membrane's structure and its flexibility (Ge;Wei;He;Yuan, 2019). Soya lecithin has the ability to form micelles in aqueous solution, significantly improving the drug entrapment characteristics. It not only facilitates the permeation of drugs but also plays a crucial role as a stabilizing agent (Govindarajan *et al.*, 2022).

3.2. Effect of soya lecithin and Span 60 on entrapment efficiency

Entrapment efficiency of vesicles depends on their stability, which is largely determined by the bilayer-forming components. The study focused on maintaining consistent levels of drug and cholesterol while systematically altering concentrations of soya lecithin and Span 60 to investigate their impact on entrapment efficiency. The different formulations showed entrapment efficiency between 76 ± 0.81 and $92.69 \pm 0.55\%$ (Figure 1a). Such high efficiency not only enhances the potential therapeutic efficacy of the formulation but also suggests its robustness as a carrier for targeted and sustained drug delivery applications. The results revealed that the higher entrapment was associated with a higher amount of Span 60. The increased entrapment can be attributed to the hydrophobic properties, solid characteristics, and higher phase transition temperature of Span 60. The results were found in agreement with Wen *et al.* (2014), where researchers reported that Span 60 and 80 showed better entrapment than Brij 52 (Wen *et al.*, 2014). The effect of soya lecithin on entrapment efficiency was found to vary,

but overall it contributed to an enhancement in entrapment efficiency. Soya lecithin, along with cholesterol, provides rigidity to the vesicle and prevents the leakage of the drug (Mehta *et al.*, 2016). These results were supported by the polynomial equation (4), contour (Figure 1b), and 3D graphs (Figure 1c).

$$\text{Entrapment efficiency (Y1)} = 91.24 + 4.95A + 2.14B - 3.14AB - 3.43A^2 - 0.9894B^2 \quad (4)$$

The positive sign for Span 60 and soya lecithin denotes the enhanced entrapment efficiency within the vesicles. The ANOVA test was used to analyse the effect of Span 60 and soy lecithin on entrapment efficiency. The test was found significant and confirmed by p-value of 0.0001 and F-value was 175.10. Adjusted and predicted R² values were 0.9831 and 0.9580, respectively. The difference between adjusted and predicted values was less than 0.2, indicating precise data. The lack of fit value is 4.99.b

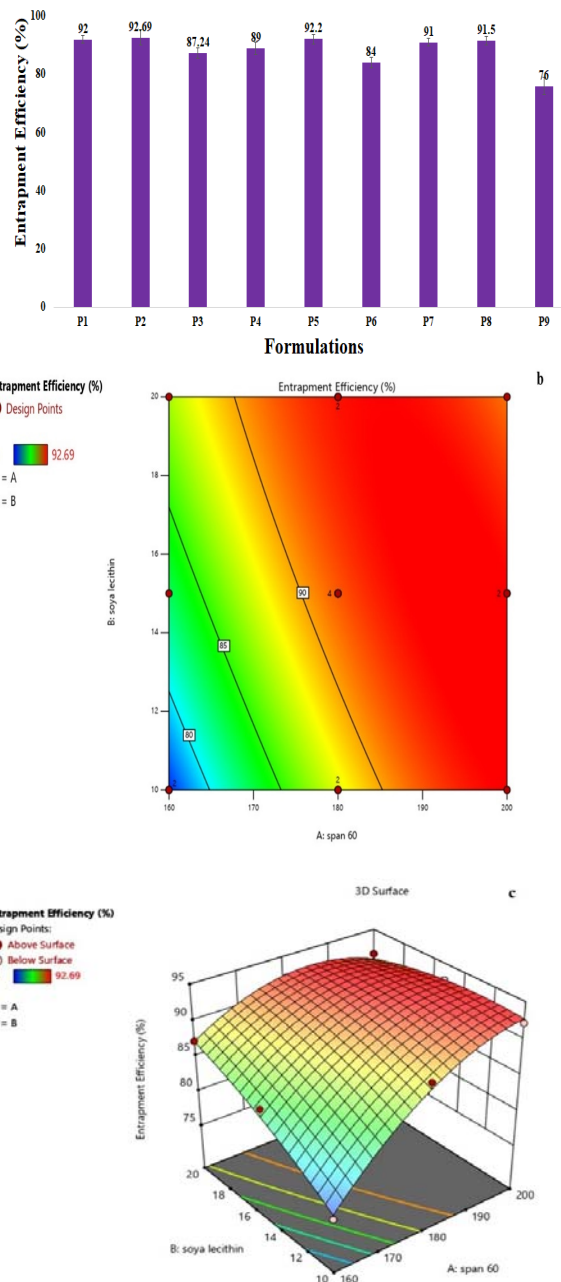


Figure 1. a) Entrapment efficiency, b) Contour plot, c) 3D graph

As evident in Figures 1b and c, the entrapment efficiency of proniosomes was directly proportional to the amounts of soya lecithin and Span 60. The change in colour from blue to red was indicative of enhanced entrapment.

3.3. Effect of soya lecithin and Span 60 on drug release

In-vitro release experiments revealed that Span 60 and soya lecithin had a notable impact on the drug release behaviour. The formulations exhibited drug release percentages ranging from $44.06 \pm 0.53\%$ to $88.34 \pm 0.41\%$. A higher concentration of soya lecithin was associated with enhanced drug release. Within vesicular delivery systems, phosphatidylcholine lecithin's primary constituent serves as an effective permeation enhancer, attributed to its elevated phase transition temperature.

Additionally, it acts to inhibit the leakage of the encapsulated drug (Wen;Farid;Kassem, 2014). The polynomial equation (5) generated during the ANOVA analysis is also supportive of the positive impact of Span 60 and the adverse effect of soya lecithin on drug release.

$$\text{Drug release (\%)} = 66.71 - 16.01A + 4.43B \quad (5)$$

The effect of Span 60 and soy lecithin was also analysed by ANOVA, where a p-value of 0.0001 indicates the test significance. F-value, adjusted and predicted R^2 values were 199.36, 0.9636 and 0.9448, respectively. The difference between the adjusted R^2 and the model predicted R^2 values was below 0.2, which further confirmed the adequacy of model. The lack of fit value is 7.22.

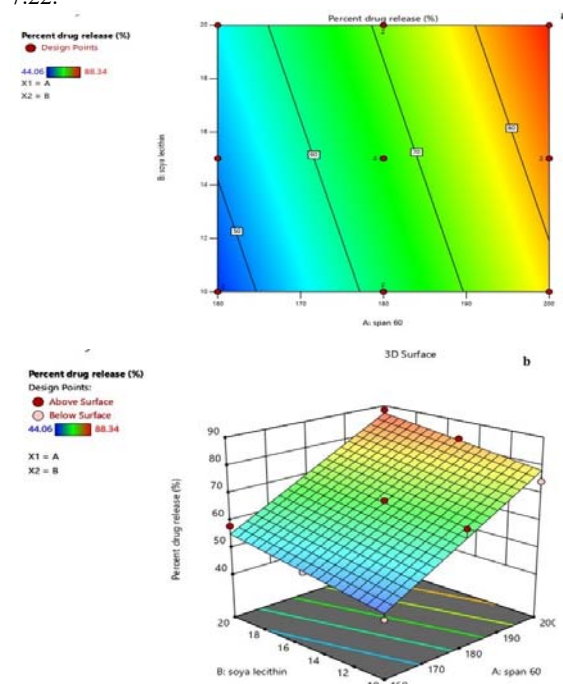


Figure 2. a) Contour plot, b) 3D graph of drug release from different formulations

Figures 2a and b were suggestive of drug release retardation with enhanced amounts of soya lecithin as indicated by blue colour. The opposite effect was observed

for Span 60, where the colour transitioned from blue to red.

3.4. Optimized formulation selection

Based on the results of drug release and entrapment efficiency, the optimized formulation was selected. According to the overlay plot (Figure 3), the predicted optimized batch demonstrates the entrapment efficiency and drug release of 91.82% and 79.79%, respectively. Practically, the value of entrapment efficiency and drug release was $92.51 \pm 0.62\%$ and $79.72 \pm 0.54\%$, respectively.

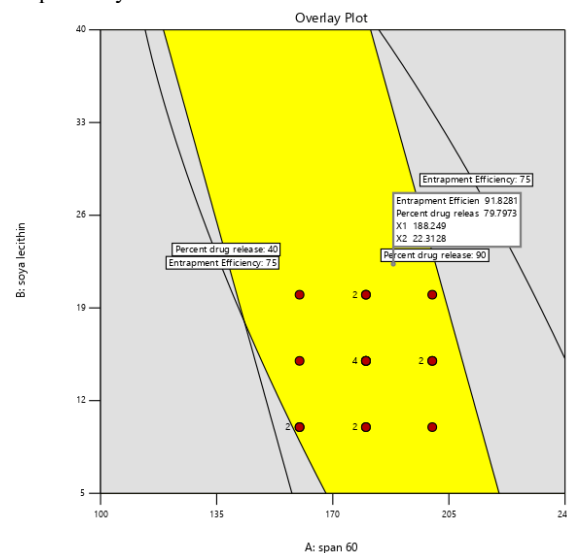


Figure 3 Overlay plot of entrapment efficiency and drug release

3.5. Characterization of optimized formulation

3.5.1. Vesicle size, PDI, and zeta potential

The vesicle size (Figure 2a) of proniosomes was 299.7 nm. This characteristic is especially beneficial for the transdermal administration of drugs. The polydispersity index was found to be 0.232. A poly-dispersity index (Figure 4a) of less than 0.3 is generally considered acceptable for drug delivery formulations. This index indicates the uniformity or homogeneity of particle sizes within the formulation. A lower polydispersity index suggests minimal variation in particle sizes, which is crucial for consistent drug delivery performance. The optimized formulation exhibited a zeta potential (Figure 4b) of -49.3 mV. This high negative zeta potential suggests strong repulsive forces between particles, which are beneficial for maintaining stability by preventing aggregation and flocculation. The presence of a high negative charge is often due to negatively charged excipients like soya lecithin, contributing to the formulation's stability (Danaei *et al.*, 2018).

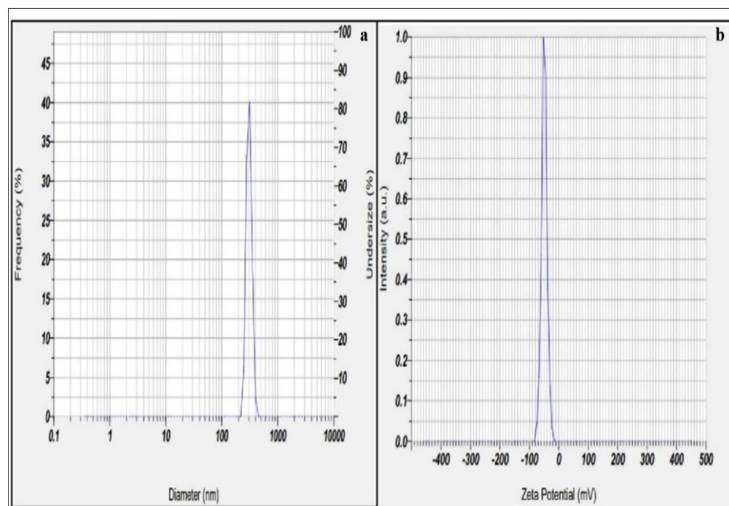


Figure 4. a) Particle size of optimized formulation, b) Zeta potential of optimized formulation

3.6. Scanning electron microscopy

Figure 4c presents the scanning electron microscopy (SEM) photomicrograph of the proniosomal formulation. According to the findings, the vesicles displayed a spherical shape, smooth texture, and were free from aggregation (Pinzaru *et al.*, 2021).

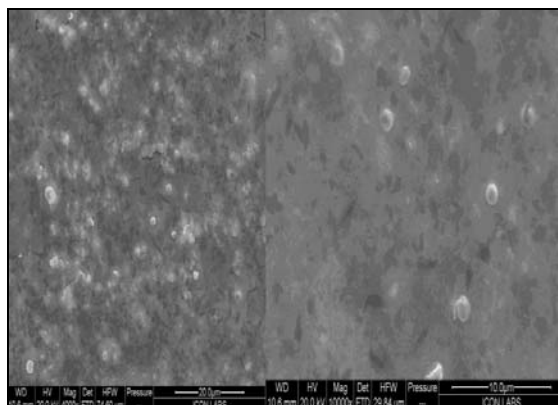


Figure 4c. SEM image of Proniosomes

3.7. Differential scanning calorimetry (DSC)

Thermal analysis (Figure 4d) studies were conducted for the drug, Span 60, soy lecithin, and proniosomes. The thermogram of gallic acid, Span 60, and soy lecithin exhibited an endothermic peak at 264.64°C, 57.42°C, and 262.60°C, respectively. In contrast, the broad endothermic peak of the formulation was displayed at 107.72°C. This broad peak suggests potential interactions between the drug, Span 60, and soya lecithin within the proniosomal structure. The disappearance of the endothermic peak of gallic acid in the formulation indicates that the drug has been encapsulated successfully within vesicles formed by Span 60 and soya lecithin (Shah;Nair;Shah;Jacob;Bharadia;Haroun, 2021).

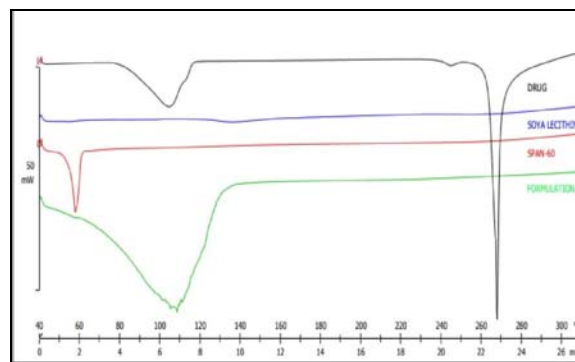


Figure 4d. DSC thermogram of gallic acid, soya lecithin, Span60 and proniosomal gel

The Composition and summary of various characteristics of the optimized formulation are listed in Table 3

Table 3 Composition and summary of key characteristics of optimized formulation

Soya lecithin (mg)	Span 60 (mg)	Vesicle size (nm)	Entrapment efficiency (%)	PDI	Zeta potential (mV)
22.31	188.24	299.7	92.51 ± 0.62	0.232	-49.3

3.8. Preparation of proniosomal gel

Carbopol 940 was employed as the gelling agent to convert the optimized proniosomal formulation into a gel-based system. Carbopol® 940 is a white, crosslinked polyacrylic acid polymer that acts as an efficient rheology modifier, providing high viscosity and forming clear gels.

3.9. Evaluation of proniosomal gel

3.9.1. Determination of pH, viscosity, and drug content

The gallic acid-loaded proniosomal gel had a pH of 6.2 ± 0.23 , which is matching to the pH of the skin. This

result was indicative of the appropriateness of the gel for topical application. The viscosity of proniosomal gel at speeds of 30 rpm, 50 rpm, and 100 rpm was 7900 ± 155 , 6389 ± 204 , and 3900 ± 139 cP. The viscosity of the marketed gel at speeds of 30 rpm, 50 rpm, and 100 rpm was 6440 ± 162 , 4820 ± 224 , and 2825 ± 192 cP, respectively. The formulation gel is more viscous than the marketed gel. As viscosity is directly proportional to adhesiveness, the results were suggestive of a prolonged contact time of the proniosomal gel with skin. The drug content of the optimized formulation was $99.51 \pm 0.65\%$, indicative of good entrapment of gallic acid in proniosomes.

The firmness of the proniosomal gel and the marketed gel (Figure 5a and 5b) was 750 g and 675 g, respectively. The tackiness of the proniosomal gel and the marketed gel was 200 g and 380 g, respectively (Figure 5a and 5b). The Work of adhesion, which is the ratio of area under the curve of tackiness and area under the curve of firmness of the proniosomal formulation and marketed gel, was 0.266 g/sec and 0.56 g/sec, respectively (Dhakane; Pandit, 2023). These findings may be due to the higher viscosity of the proniosomal gel compared to the marketed formulation.

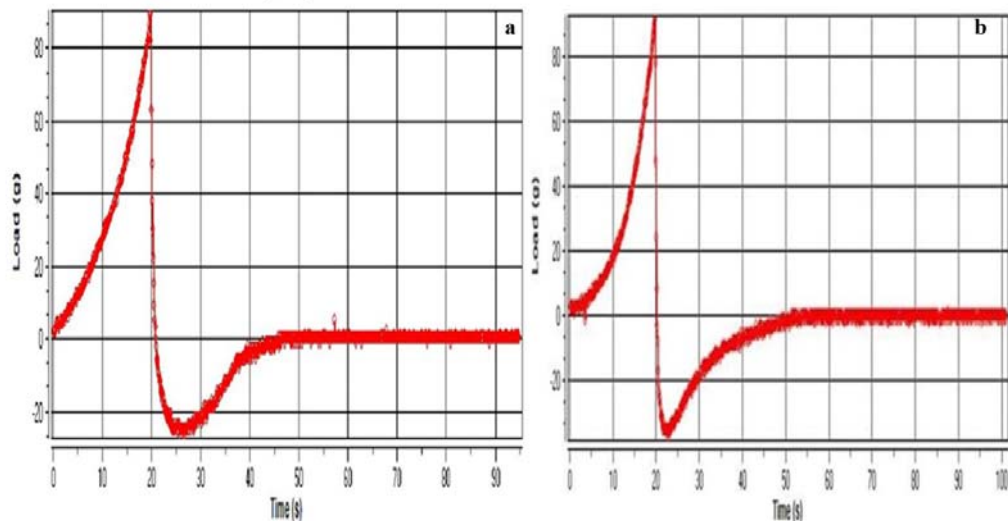


Figure 5. Texture analysis of a) Proniosomal gel, b) Marketed gel

3.9.2. In-vitro drug diffusion study

The diffusion of gallic acid from proniosomes and proniosomal gel was 86.55 ± 2.54 and $83.18 \pm 2.23\%$, respectively, whereas gallic acid without entrapment in vesicles exhibited $58.42 \pm 1.87\%$ drug diffusion within 8 h. The drug diffusion from gallic acid-loaded gel was 50.07 ± 2.87 (Figure 6a). A biphasic drug release pattern helps in the avoidance of saturation of the drug while diffusing through the skin. Initially, fast release leads to achieving transdermal delivery with a high concentration of the drug. Results are as per the given literature (Lather *et al.*, 2016). The release of proniosomes and proniosomal gel follows first-order diffusion kinetics; gallic acid and plain gel follow Korsmeyer-Peppas diffusion kinetics.

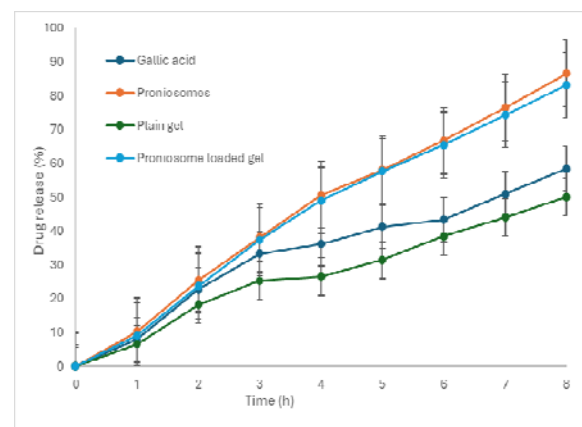


Figure 6a. In vitro drug diffusion study

3.9.3. Ex-vivo drug diffusion study

The ex-vivo drug diffusion (Figure 6b) for gallic acid-loaded proniosomes, proniosomal gel, marketed gel, and gallic acid-loaded gel were 82.5 ± 2.46 , 79.72 ± 2.89 , 75.99 ± 3.12 , and $47.47 \pm 2.43\%$, respectively. These results showed an increase in the drug diffusion of gallic acid by using the proniosomal vesicular system (Aldawsari *et al.*, 2023). The release of proniosomes and proniosomal gel follows first-order diffusion kinetics; gallic acid and plain gel follow Korsmeyer-Peppas diffusion kinetics.

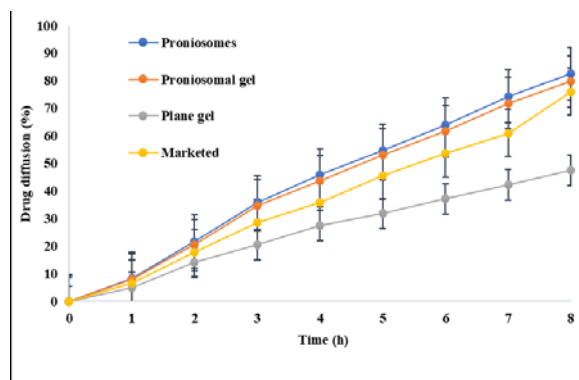


Figure 6b. Ex vivo drug diffusion study

3.9.4. Skin permeation study

The steady-state flux equation was used to determine the drug flux of the plain gel, marketed gel, proniosomes, and proniosomal gel. As per the results, the order of transdermal flux was proniosomes ($0.08610 \mu\text{g}/\text{cm}^2/\text{min}$) > proniosomal gel ($0.08237 \mu\text{g}/\text{cm}^2/\text{min}$) > marketed gel ($0.06032 \mu\text{g}/\text{cm}^2/\text{min}$) > plain gel ($0.03792 \mu\text{g}/\text{cm}^2/\text{min}$). Flux refers to the amount of drug permeated per unit time. In the case of the proniosome formulation, the drug was solubilized in the matrix of soya lecithin, and hence the amount of drug permeated was found more. In the gel formulation, the release of proniosomes was hindered due to entrapment in the gel matrix; hence, the flux was slightly low. In the marketed formulation, attempts were not made to enhance the solubility, and therefore the flux is lower. As gallic acid is insoluble in nature and entrapped in the gel base for plain gel, the flux is further lowered. These parameters illustrated that proniosomes containing surfactant Span 60 and soya lecithin improved the diffusion of gallic acid across the membrane (Dhakane; Pandit, 2023).

3.9.5. Antimicrobial activity against *Staphylococcus aureus*

The zone of inhibition (Figure 7) of proniosomal gel, marketed gel, and gallic acid was 33 ± 3.87 , 30 ± 2.89 , and 27 ± 3.19 mm. The value of the antibacterial study of gallic acid was consistent with the reported literature (Yu *et al.*, 2022). This study showed (Figure 9) that the proniosomal gel has a larger zone of inhibition than the pure drug and the marketed gel incorporated into the well.

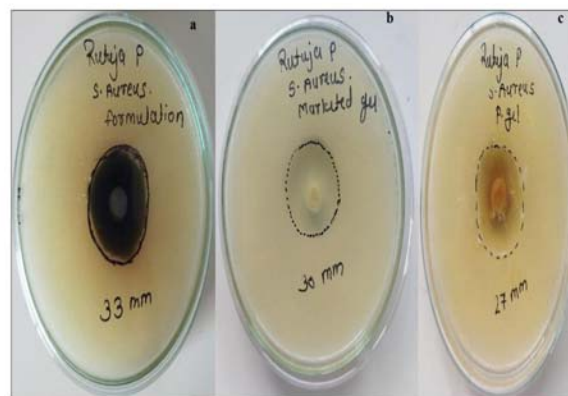


Figure 7 Zone of inhibition of a) Proniosomal gel, b) Marketed gel, c) Plain gel

The comparative summary of proniosomes, proniosomal gel, plain gel, and marketed gel is elaborated in Table 4.

Table 4: Comparative summary of proniosomes, proniosomal gel, plain gel, and marketed gel

Formulation	In-vitro diffusion (%)	Ex-vivo drug diffusion (%)	Flux ($\mu\text{g}/\text{cm}^2/\text{min}$)	Zone of inhibition (mm)
Proniosomes	86.55 ± 2.54	82.5 ± 2.46	0.08610	-
Proniosomal gel	83.18 ± 2.23	79.72 ± 2.89	0.08237	33 ± 3.87
Marketed gel	58.42 ± 1.87	75.99 ± 3.12	0.06032	30 ± 2.89
Plain gel	50.07 ± 2.87	47.47 ± 2.43	0.03792	27 ± 3.19

4. Conclusion

The findings of this study confirm the successful development of a gallic acid-loaded proniosomal gel. The optimized formulation exhibited high entrapment efficiency. SEM analysis revealed well-defined vesicles without signs of aggregation. The release of the drug in the formulation was extended. Antimicrobial activity test suggests that proniosomes hold strong potential as transdermal delivery systems for gallic acid to treat *S. aureus* infection. Further, preclinical studies must be carried out to evaluate the effectiveness of the formulation *in-vivo*.

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