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Fabrication and Optimization of Febuxostat-loaded Liposomal Gel Using the Box–Behnken Design for Gout Treatment

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Abstract

Monosodium urate crystals form and deposit in and around the joints due to hyperuricemia, a condition characterized by a prolonged increase in serum urate levels. This leads to tissue supersaturation with urate and results in gout, a prevalent form of inflammatory arthritis. Gout can be effectively managed with febuxostat (FBT), a nonpurine selective xanthine oxidase inhibitor. Despite the efficacy of FBT, its use is limited by poor oral bioavailability and aqueous solubility, which are influenced by its co-administration with food and enzymatic degradation. To address the formulation challenges, topical liposomal formulations can be utilized. These formulations, which leverage the affinity of liposomes for keratin and their ability to penetrate deeper into the skin, offer improved drug absorption. This study aimed to develop a liposomal formulation with soy lecithin and cholesterol using the thin-film hydration method, optimized via the Box–Behnken design. The optimized liposomal formulation was then incorporated into a transdermal gel prepared with Carbopol 934 using the dispersion method. The optimized liposomal formulation demonstrated favorable properties, including a vesicle size of 392.5 ± 11.02 nm, zeta potential of 28 ± 6.13 mV, polydispersity index of 0.127, and high entrapment efficiency as confirmed via transmission electron microscopy. The resulting transdermal gel exhibited the necessary antibacterial activity; excellent stability; *in vitro* release of 81.33% ± 1.19% over 7 h; and appropriate pH, viscosity, spreadability, and gel strength. Overall, the liposomal gel formulation represents a highly effective approach for transdermal drug delivery.

Keywords: febuxostat (FBT); liposome; transdermal gel; Box–Behnken design; antimicrobial study

Introduction

A vesicle is a small fluid-filled sac encased in a lipid bilayer for protection [1]. Vesicles naturally form during material secretion (exocytosis), uptake (endocytosis), and intracellular transport in both plants and animals. The term “Bingham body” was introduced by Bingham in 1965, who created a similar vesicle artificially. Weismann later termed these vesicles “liposomes” after evaluating their potential for drug delivery [2]. The phrase “vesicular drug delivery system” refers to structures formed by self-assembling amphiphilic molecules in water, including liposomes, niosomes, bilosomes, and other related systems [3].

In liposomes, an aqueous core is entirely enclosed

by a lipid bilayer, which is composed primarily of natural or synthetic phospholipids. These small, bilayered vesicles consist of phospholipids or other amphipathic lipids and are capable of encapsulating and transporting both hydrophilic and lipophilic substances effectively. Liposomes serve as a nontoxic delivery system for both these substances [4]. Drugs encapsulated in liposomes can maintain therapeutic levels for extended periods because the drug must first be released from the liposome before it can be metabolized and eliminated. The primary structural components of liposomes are phospholipids and cholesterol [5], which enhance the drug's efficacy and therapeutic index. Liposomal drug delivery offers protection from environmental degradation, provides site-specific and sustained release of medication into cells, and targets specific cellular compartments [6]. Incorporating liposomes into gelling agents improves their effectiveness and reduces toxicity compared with conventional formulations. Topical liposomal formulations have an affinity for keratin and can penetrate deeper into the skin, resulting in better drug absorption [7]. Liposomes can deliver sufficient amounts of poorly skin-permeable drugs to their target site. When a liposome reaches the stratum corneum and bursts, it releases its contents in an aqueous solution, effectively reaching deeper layers of the skin [8].

Febuxostat (FBT), a nonpurine selective xanthine oxidase inhibitor, is used to treat gout and can be formulated into liposomes. Food intake reduces the drug's maximum concentration (C_{max}) by 38%–39% and decreases its solubility in water due to oral bioavailability being compromised by enzymatic breakdown. Liposomal formulation addresses these issues, allowing for extended therapeutic activity. FBT is an FDA-approved medication of choice for managing chronic gout and its severe forms of arthritis.

The undesirable side effects associated with the oral administration of drugs can be effectively minimized using a liposome formulation via the transdermal route [9]. Liposomes serve as a targeted drug delivery system, allowing drugs to be delivered in a controlled manner. Liposomal gel formulations alter drug release dynamics, enhance bioavailability, and reduce the side effects of FBT [10].

This study aimed to develop an FBT-loaded liposomal gel using a quality-by-design approach for formulating and optimizing the liposomal dispersion. The developed formulation was characterized by evaluating vesicle size, zeta potential, entrapment efficiency (%EE), *in vitro* diffusion, and antimicrobial efficacy to assess its potential effectiveness.

Experimental

Materials

Micro-lab (Mumbai, India) provided FBT as a gift sample. Soya lecithin, cholesterol, Carbopol 934, propylene glycol, glycerol, triethanolamine, sodium hydroxide, potassium dihydrogen phosphate, methylparaben sodium, propylparaben sodium, methanol, chloroform, and dialysis membrane were purchased from Research-Lab Fine Chem Industries (Mumbai, India).

Methods

The infrared spectra of FBT were recorded using an Fourier-transform infrared (FTIR) spectrometer (Shimadzu IR Affinity-1S CE). The thin-film hydration method was used to form liposomes (Fig. 1), and the optimization process involved some trial and error. Various formulation batches were prepared by altering the drug and excipient ratios. The formulation process is as follows:

Determination of quality target product profiles (QTPPs) for liposomal formulation

The QTTP of the liposomal formulation was determined. QTPP refers to the intended product profile and associated quality standards. It typically covers the administration route, dosage form, dose, appearance, and pharmacokinetic data of the medicine, in addition to any specific safety or quality standards [11].

Determination of critical material attributes (CMAs), critical process parameters (CPPs), and critical quality attributes (CQAs) for liposomal formulation

Factors proposed as CQAs, CMAs, or CPPs were based on predetermined objectives, anticipated product quality, therapeutic requirements, and the chosen production process [11].

Preparation of liposomal dispersion containing FBT

Liposomal formulations containing FBT, soya lecithin, and cholesterol were prepared using the thin-

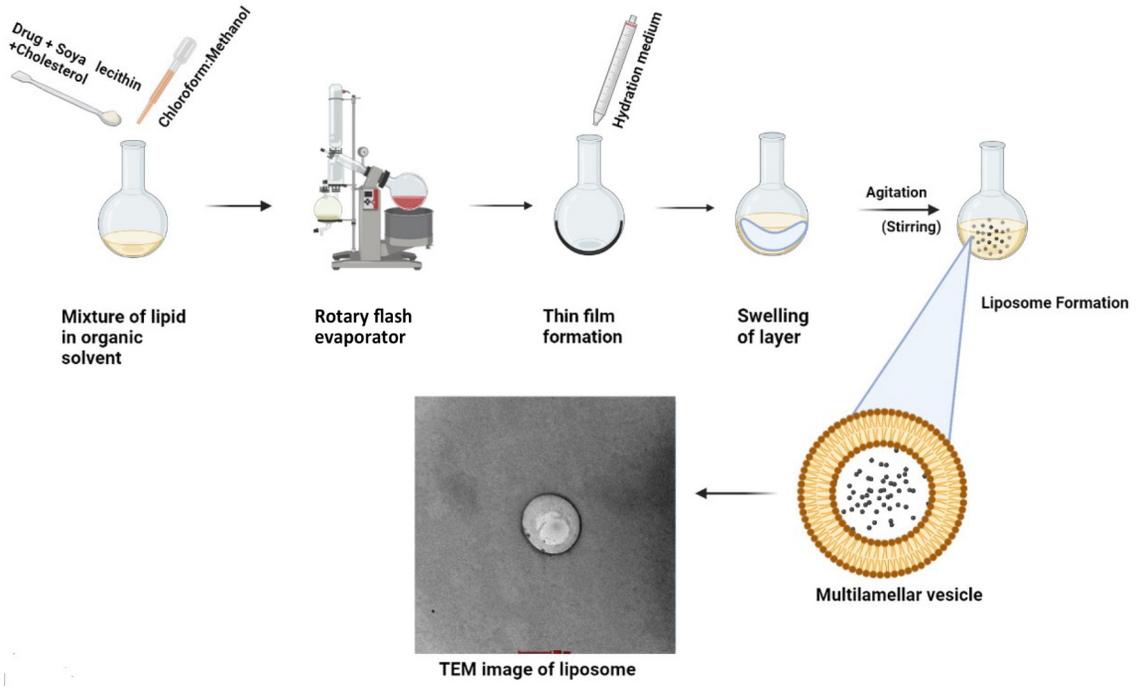


Fig. 1 Preparation of liposomal dispersion using the thin-film hydration method.

film hydration method (Fig. 1). The drug, phospholipid, and cholesterol were measured and dissolved in a mixture of chloroform and methanol at a 1:1 ratio before being added to a clean, dry round-bottomed flask. This solution was then transferred to the flask of a rotary flash evaporator. The solvent was evaporated at reduced pressure and 60 r/min over the lipid transition temperature for 30–40 min. An organic solvent thin coating formed on the inside surface of the flask's spherical bottom after evaporation. A thin lipid film was hydrated with the required phosphate buffer solution (pH 7.4) while rotating at 60 r/min for 1 h. The liposomal dispersion was then sonicated for 5 min to form homogeneous multilamellar vesicles and allowed to cool for 24 h [12].

Optimization of FBT-loaded liposomal gels using Box–Behnken design (BBD)

Design-Expert version 10.0.8.0 was used to apply the BBD for developing the experimental arrangement for FBT-loaded liposomal gel formation [13]. Batches were formulated using a 2^3 randomized BBD, which considered the effects of soya lecithin concentration, cholesterol concentration, and hydration volume as independent variables. The dependent variables measured were %EE, vesicle size, and zeta potential [14]. The independent variables were studied at three levels (–1, 0, and +1), as detailed in Table 1.

Preparation of liposomal gels

The dispersion process was used to prepare the gel. The best liposomal formulation was selected for inclusion in the Carbopol 934 gel base. A suitable amount of Carbopol 934 was dissolved in distilled water and continuously stirred using a magnetic stirrer (Table 2). This mixture was stored at room temperature for a 24-h hydration period to reduce swelling. Thereafter, the drug-containing liposomal suspension was added to the Carbopol 934 dispersion. The dispersion was then mixed with an appropriate

Table 1 Degree of independent variables for the formulation design.

Independent variables	Level		
	+1	0	−1
Soya lecithin (mg)	400	600	800
Cholesterol (mg)	80	100	120
Hydration volume (mL)	10	20	30

Table 2 Formulation for liposomal gel using different Carbopol 934 concentrations

Formulation code	Liposomal dispersion	Carbopol 934	Propylene glycol
LF13G1	1%	0.5%	10%
LF13G2	1%	1%	10%
LF13G3	1%	1.5%	10%
LF13G4	1%	2%	10%

amount of propylene glycol, glycerol, and other ingredients, and stirred continuously at room temperature. Triethanolamine was added to the dispersion to adjust its pH. Carbopol 934 was used in different batches of gel at varying concentrations (0.5%–2%) [15].

Evaluation of formulated liposomes

Factors affecting the shape and size of liposomes

The shape, micron-level size, and volume of the liposomes were examined using a compound microscope (Labline) at different magnifications. This assessment included evaluating the effects of sonication at various time intervals, the behavior of vesicle sizes with different hydration media, and other related factors [16, 17].

Determination of %EE

Centrifugation (Remi C-24 Plus) was used to measure the amount of FBT trapped in liposomes. Briefly, 10 mL of liposomal dispersion was placed in a centrifuge tube and centrifuged at 10 000 r/min for 10 min at 40 °C. After separating the nonentrapped FBT from the liposomes, the amount of FBT was determined. The vesicles were dissolved in methanol, and the drug concentration was measured via spectrophotometric analysis (Shimadzu UV-1800) with absorbance readings at 316 nm. The percentage of drug encapsulated in the vesicles, known as %EE, was calculated using the following formula [18, 19]:

$$\%\ \text{Entrapment efficiency} = \frac{\text{Drug entrapped}}{\text{Total drug added}} \times 100 \quad (1)$$

Size distribution and polydispersity index (PDI)

The size distribution and PDI of FBT-loaded liposomal gels were measured using a Zeta-sizer (Malvern Nano-ZS) [19, 20].

Transmission electron microscopy (TEM)

A TEM system (Tecnai G2 spirit Biotwin-FP 5018/41) was used to assess the morphological properties of the vesicles, providing detailed information about the sample based on the obtained images [21].

Zeta potential

The zeta potential of the synthesized liposomal gel was determined using Malvern Nano-ZS. To achieve optimal signal strength at 25 °C, the sample was diluted with water 6–8 times before analysis. The average zeta potential value was recorded and used for further data analysis [22–24].

Evaluation of liposomal gels

Homogeneity and grittiness

Homogeneity of the liposomal gels was visually assessed using a clear beaker. The presence of any aggregates and the overall appearance of the mixture were investigated [25].

pH

The pH of different gel formulations was measured using a digital pH meter (model EQ 610). To determine the pH, 1 g of gel was dissolved in 25 mL of distilled water. The measurements were performed in triplicate for accuracy, and the average pH value and standard deviation were calculated [26, 27].

Viscosity

The viscosity of the prepared gel was measured with a Brookfield viscometer (DV2T model) using a Helipath T spindle (D-94) [7, 28].

Spreadability

Spreadability was assessed using a wooden spreadability apparatus. To evaluate the spreadability of the gel, 1 g of the gel was placed on a glass slide mounted on a wooden block, and a second glass slide was placed on top of the first one. A pulley system, with one terminal attached to the top slide and the other to a weight, was used. The amount of time required for the top slide to slip across the bottom slide was recorded to determine spreadability [22]. The spreadability (S) was calculated using the following formula:

$$S = ML/T \quad (2)$$

where M indicates the weight tied to the upper slide, L indicates the length of the glass slide, and T indicates the time taken for the slides to separate.

Extrudability

The prepared gel was placed inside a collapsible aluminum tube with a standard cap, and the end was crimped shut to seal it. The weight of the tube was recorded. The tubes were then clamped between two glass slides, and a weight of 500 g was applied to the slides. After removing the cap, the volume of extruded gel was collected and weighed. This allowed for the calculation of the percentage of gel that was extruded [29].

Drug content

To determine the drug content, 1 g of the gel formulation was transferred into a 10-mL volumetric flask and diluted to the required volume with methanol. The solution was then filtered using Whatman filter paper. Another 10-mL volumetric flask was prepared by adding 1 mL of the filtered solution and diluting with methanol to the specified volume. The concentration of FBT was measured at 316 nm using an ultraviolet–visible spectrophotometer, with a blank used for calibration [25].

Gel strength

Gel strength was assessed by measuring the shear stress at a low shear rate after the gel had been quiescent for some time. Two reference points were marked on a cylinder. A 25-mL sample of the liposomal gel was placed into the cylinder. The time taken for the cylinder to move from the starting point to the ending point was measured and recorded. This measurement was used to determine the gel strength [30].

Drug deposition in the skin

To measure the amount of drug deposited in the skin, the Strat-M membrane was removed from the diffusion cell after 24 h of the permeation study. The membrane was carefully washed with distilled water for 10 s to remove any adhering gel formulation. The skin was then sonicated in 10 mL of methanol for 30 min using an ultrasonicator (Labline) to extract the drug. The FBT content was measured spectrophotometrically at 316 nm [31].

In vitro diffusion

To investigate the rate of drug release from the formulations, an *in vitro* diffusion study was conducted using a set of Franz diffusion cells. The receptor chamber was filled with receptor medium (phosphate-buffered saline (PBS), pH 7.4). The temperature of the receptor medium was maintained at (37 ± 1) °C throughout the experiment by circulating water through a jacket surrounding the cell body. The Strat-M membrane, with a pore size of 0.45 µm, was soaked in the receptor medium for 12 h before being clamped between the two chambers. Subsequently, 1 g of the formulation was applied to the donor cell. Samples (1 mL) were collected from the receptor cell at predetermined intervals. To maintain a constant volume, an equal amount of fresh receptor medium was added after each sample collection. Fresh receptor medium was used as the blank. The collected samples were analyzed spectrophotometrically, with necessary dilution, to determine the drug concentration at specific time intervals using a calibration curve [32].

Antimicrobial activity

FBT exhibits antibacterial activity; therefore, this study aimed to confirm the enhanced therapeutic response of FBT-loaded liposomal gels. The antibacterial activity of the formulations was evaluated using the cylinder cup method against *Pseudomonas aeruginosa*, *Escherichia coli* (Gram-negative), and *Staphylococcus aureus* (Gram-positive). The cylinder cup method is widely used to assess bacterial susceptibility or resistance to various antimicrobial agents by measuring the suppression of bacterial growth under controlled conditions. For the test, the bacterial cultures were uniformly and aseptically spread on Mueller–Hinton agar plates. Bores were created in the agar, and the test compounds were added to these wells. Each formulation was tested at a concentration of 100 mg/mL. The plates were incubated at 37 °C for 24 h. Antibacterial activity was indicated by a clear zone of inhibition around the bore. The inhibition zones were measured, and the results were compared to evaluate the effectiveness of the formulations [33, 34].

Stability

The stability of the formulation was assessed according to the guidelines of the International Council for Harmonisation (ICH). Freshly manufactured formulations were divided into groups and stored under ICH-regulated conditions. Samples were taken at regular intervals and evaluated for various parameters mentioned above. For a formulation to be considered stable, these evaluation parameters must remain consistent over time and under the specified storage conditions. Stability studies were conducted to assess *in vitro* pH, drug release, and drug content at (40 ± 2) °C and 75% ± 5% relative humidity over a period of 90 days [35].

Results and Discussion

FTIR studies

The FTIR spectrum of the pure FBT drug was similar

to the standard spectrum of the drug. The peak corresponding to the FBT drug was observed in the drug–excipient FTIR spectrum. C–H stretching of aldehyde was observed in both the drug and excipient at 2 893.22 cm^{-1}. N–H bending in FBT was found at 1 643.35 cm^{-1}. O–H bending of carboxylic acid was detected in both the drug and excipient at 1 419.21 cm^{-1}, as shown in Fig. 2.

QTPPs of the liposomal formulation

The QTPP was defined for FBT-loaded liposomal gels (Table 3).

Evaluation of liposomal dispersion

The formulation of liposomes was influenced by various factors, including hydration volume, hydration medium, and sonication time.

The highest %EE of FBT in liposomes, at 81.71%, was achieved with a 20 mL of PBS. The formulation was shaken or gently agitated for 1 h to create a liposomal dispersion with numerous lamellae. Insufficient PBS can hinder the hydrophilic head groups of phospholipids from extending into the aqueous solution, making liposome formation more difficult. The effect of PBS volume (pH 7.4) on %EE is shown in Fig. 3.

The %EE of FBT-loaded liposomal gels prepared with cholesterol and soya lecithin peaked at pH 7.4. An increase in pH results in enhanced %EE. When water is used as a hydration medium, crystals form because the thin film does not dissolve in water. Particle size increased in the following order: saline solution < PBS (pH 5.6) < PBS (pH 7.4), with particles being significantly larger in PBS (pH 7.4) than in the saline solution. The effect of the hydration medium on %EE is shown in Fig. 3.

The maximum %EE of the liposomal dispersion was 81.8% at 3 min of ultrasound time. Unilamellar liposomes were produced using ultrasound oscillation. However, excessive ultrasound can damage the liposomal structure, leading to leakage of the encapsulated FBT and a decrease in overall %EE. Initially, a multilamellar vesicle was formed (Fig. 3). As the sonication time increased, the vesicle size decreased and transformed into unilamellar vesicles (Fig. 3). The effect of sonication time on %EE is

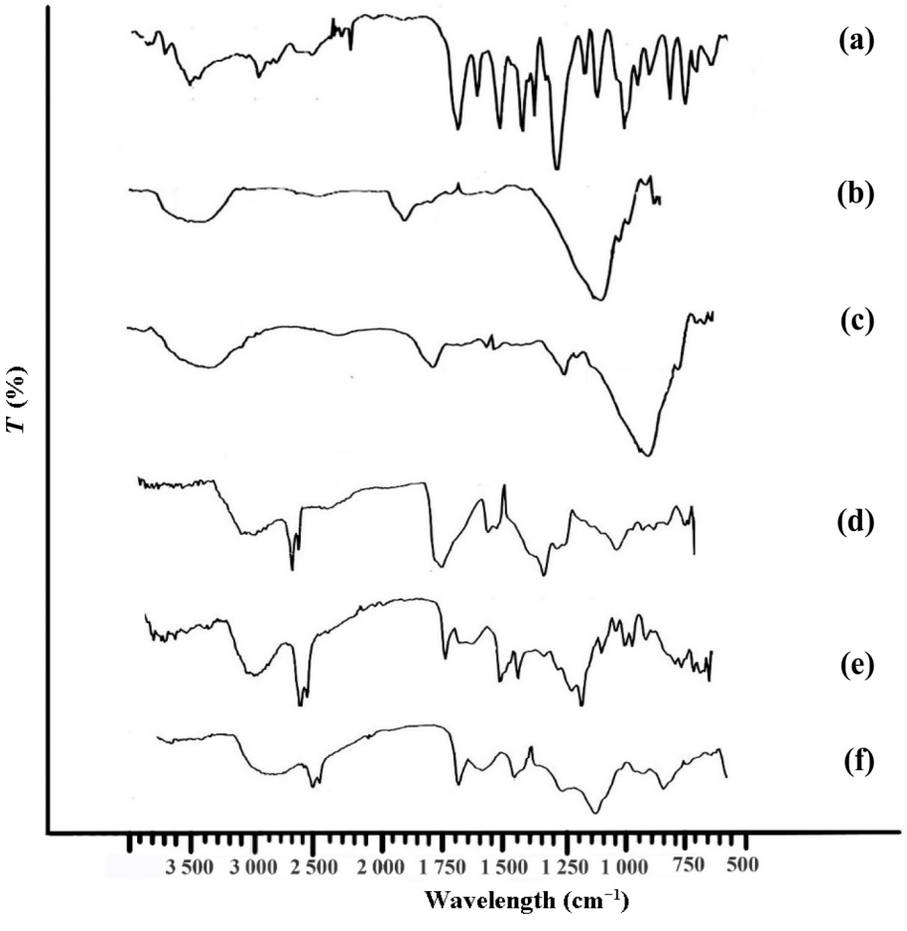


Fig. 2 FTIR results of **(a)** FBT, **(b)** liposome dispersion, **(c)** liposomal gel, **(d)** Carbopol 934, **(e)** cholesterol, and **(f)** soya lecithin.

Table 3 QTPP for FBT-loaded liposomal gels

Quality attributes	Target
Dosage form	Liposomal dispersion
Route of administration	Transdermal
Dose strength	200 mg
Physical attributes	Dispersion conforming to description: flocculation, sedimentation, and turbidity
%EE	> 70%
Zeta potential	± 25 mV
Vesical size	< 400 nm

illustrated in Fig. 3.

Optimization of FBT-loaded liposomal gels using BBD

Thirteen experiments were conducted using the BBD for the prepared three-component formulations. The three dependent variables were %EE (%), vesicle size (nm), and zeta potential (mV), with ranges of 55.89%–83.12%, 212–393 nm, and −11.43–−27.30 mV, respectively. The quadratic model obtained for the formulation was a good fit. The contour plots for particle size, %EE, and zeta potential of FBT-loaded liposomal gels are shown in Fig. 4.

Response 1 (Y_1): Effects of independent variables on %EE. The %EE ranged from 55.89% to 83.12% (Table 4).

$$\%EE = 67.69 + 10.43(X_1) - 2.04(X_2) - 1.34(X_3) \quad (3)$$

where X_1 is soya lecithin, X_2 is cholesterol, and X_3 is hydration medium. Table 5 shows the R^2 for each of the three responses.

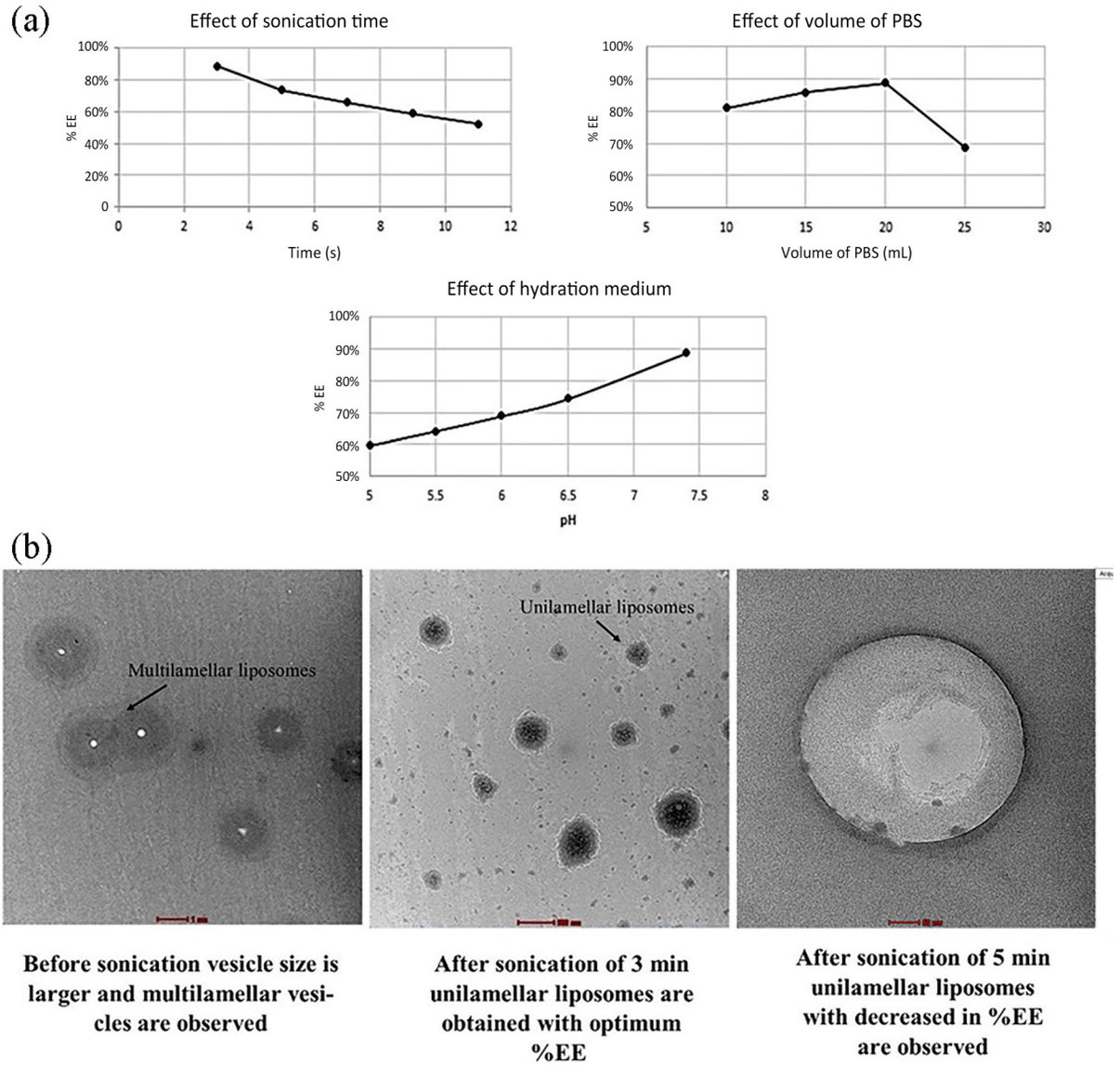


Fig. 3 (a) Effect of sonication time, hydration volume, and medium. **(b)** Effect of sonication at different time intervals.

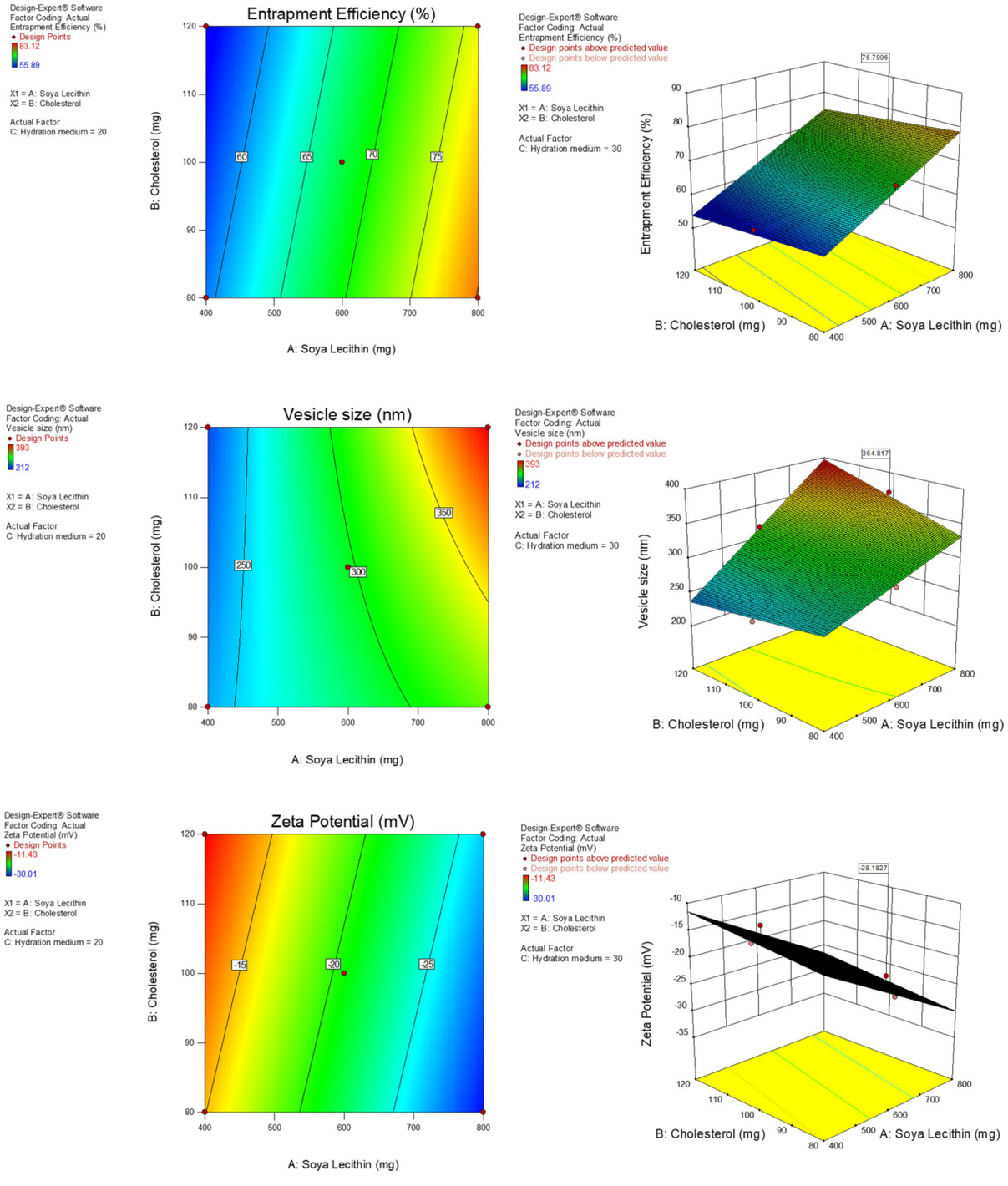


Fig. 4 Contour plots and response surface of %EE, vesicle size, zeta potential, and effect of variables.

%EE increases with the concentration of soya lecithin. Higher total lipid concentration enhances %EE by providing more space for drug accommodation and reducing drug leakage into the external phase. Increased lipid content helps entrap the drug within the lipid core, thus improving %EE.

Response 2 (Y_2): Effects of independent variables on vesicle size. Vesicle sizes for all 13 experiments ranged from 212 to 393 nm (Table 4).

$$\text{Vesicle size} = 296.69 + 62.50(X_1) + 14.37(X_2) + 10.88(X_3) + 22.75(X_1X_2) - 5.25(X_1X_3) - 5.00(X_2X_3) \quad (4)$$

where X_1 is soya lecithin, X_2 is cholesterol, and X_3 is hydration volume.

Based on this model, increasing the total lipid concentration also increases the vesicle size of liposomal gels. However, higher lipid concentrations decrease the viscosity and surface tension of the formulation, leading to a decrease in vesicle size.

Table 4 BBD for FBT-loaded liposomal gel formulation optimization based on practical responses

Runs	Factors (independent variable)			Response (dependent variable)		
	X_1: Soya lecithin (mg)	X_2: Cholesterol (mg)	X_3: Volume of hydration medium (mL)	Y_1: %EE	Y_2: Vesicle size (nm)	Y_3: Zeta potential (mV)
LF1	400	100	10	58.01%	212	−12.09
LF2	600	100	20	66.63%	285	−21.25
LF3	800	100	30	73.11%	370	−27.33
LF4	600	80	10	71.03%	262	−23.04
LF5	600	120	30	61.49%	318	−17.39
LF6	600	80	30	69.83%	293	−22.89
LF7	800	100	10	78.63%	358	−25.76
LF8	400	120	20	55.89%	233	−11.43
LF9	400	80	20	60.44%	256	−15.07
LF10	600	120	10	64.59%	307	−19.41
LF11	800	80	20	80.09%	325	−30.01
LF12	400	100	30	57.15%	245	−13.66
LF13	800	120	20	83.12%	393	−28.7

Table 5 R^2 results for the response surface plot

	Quadratic model	Predicted R^2	Adjusted R^2
Response	%EE	0.887 9	0.815 7
	Vesicle size	0.970 0	0.932 5
	Zeta potential	0.925 7	0.954 6

Response 3 (Y_3): Effects of independent variables on zeta potential. The zeta potential for all formulations ranged from −11.43 to −27.30 mV (Table 4).

$$\text{Zeta potential} = -20.62 - 7.44(X_1) + 1.76(X_2) - 0.12(X_3) \quad (5)$$

where X_1 is soya lecithin, X_2 is cholesterol, and X_3 is a hydration medium.

Cholesterol concentration affects the negative charge on the vesicles. The negative zeta potential observed is attributed to cholesterol concentration. Increased cholesterol concentration enhances zeta potential, indicating more stable vesicles.

Lipid concentrations significantly influenced the drug release patterns from liposomal gels. Increasing lipid concentration accelerated drug release while maintaining sustained release. The optimized liposomal gel formulation was determined using the BBD point prediction method. After evaluation, the desirability function, which reached a value of 1, identified the formulation containing 800 mg of soya lecithin and 120 mg of cholesterol as optimal. This optimized formulation of FBT-loaded liposomal gels achieved a particle size of 393 nm, %EE of 83.12%, and zeta potential of −28.7 mV.

%EE

The LF13 formulation showed the highest %EE (83.12%) compared with other prepared formulations (Table 4).

Vesicle size distribution and PDI

The average size of the LF13 liposome was 392.5 ± 71 nm, which is considered promising for delivery applications. Additionally, the PDI value of 0.127 indicates a homogeneous dispersion, contrary to other formulations that exhibited greater variability in vesicle size and higher PDI values (Fig. 5).

TEM study

TEM imaging of LF13 liposomal vesicles confirmed that the prepared liposomes closely matched the size distribution observed in studies. The TEM images also indicated that the formulation was homogenously dispersed (Fig. 6).

Zeta potential

Zeta potential studies of LF13 indicated that the prepared liposomes were stable, with an observed value of −28 ± 6.13 mV. This value, which exceeds −20 mV (Fig. 7), suggests a highly stabilized dispersion compared with other formulations. Consequently, the robust stability implies a longer shelf life.

Evaluation of formulated liposomal gels

Homogeneity and grittiness

The LF13G2 formulation exhibited excellent

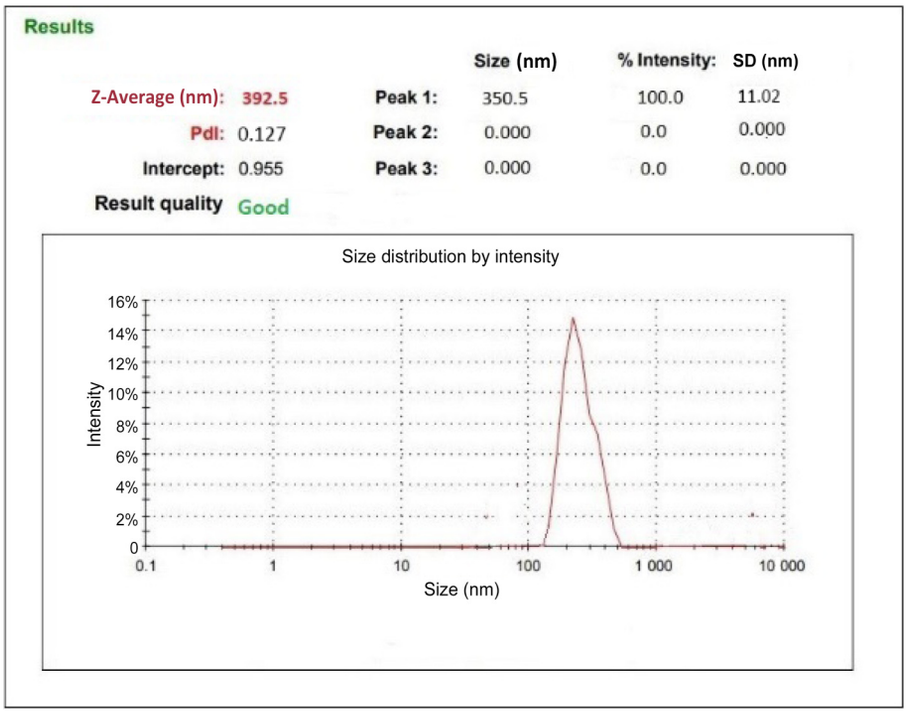


Fig. 5 Vesicle size distribution and PDI of optimized liposomal vesicles.

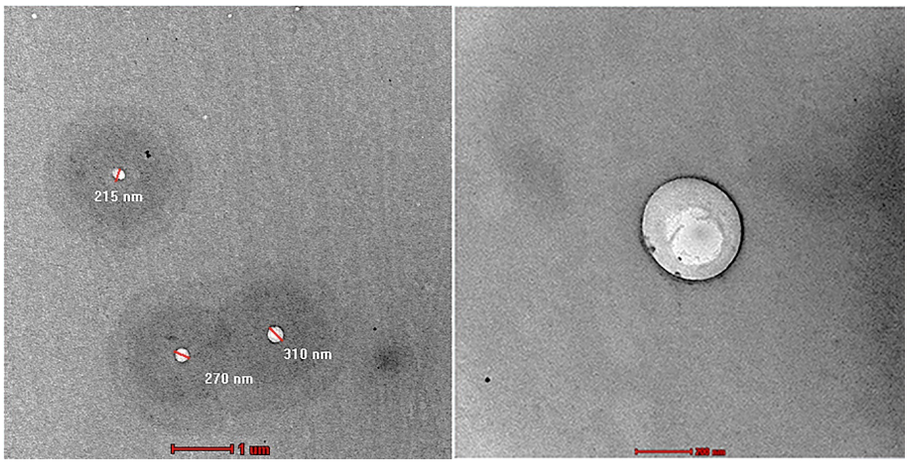


Fig. 6 TEM images of liposomal vesicles.

homogeneity and minimal grittiness compared with other liposomal gel formulations. It also displayed no visible aggregates during visualization (Table 6).

pH

The pH values of the liposomal gels (Table 7) ranged from 7.12 to 7.36, indicating that the gels are suitable for topical use and are unlikely to cause irritation during application.

Viscosity

The viscosity of the LF13G2 formulation was optimal for topical delivery. Conversely, other formulations displayed either too low or too high viscosity due to variations in Carbopol 934 concentrations (Table 7).

Spreadability

Spreadability, which measures how easily the gel can be spread with minimal pressure, was best for LF13G4 and LF13G2 compared with LF13G1 and LF13G3. LF13G1 and LF13G4 had significantly lower spreadability (Table 7).

Extrudability

Extrudability, assessed by the amount of gel extruded from the tube under pressure, was good for all formulations, ranging from 68% to 94.6% (Table 7). LF13G2 exhibited the highest extrudability among

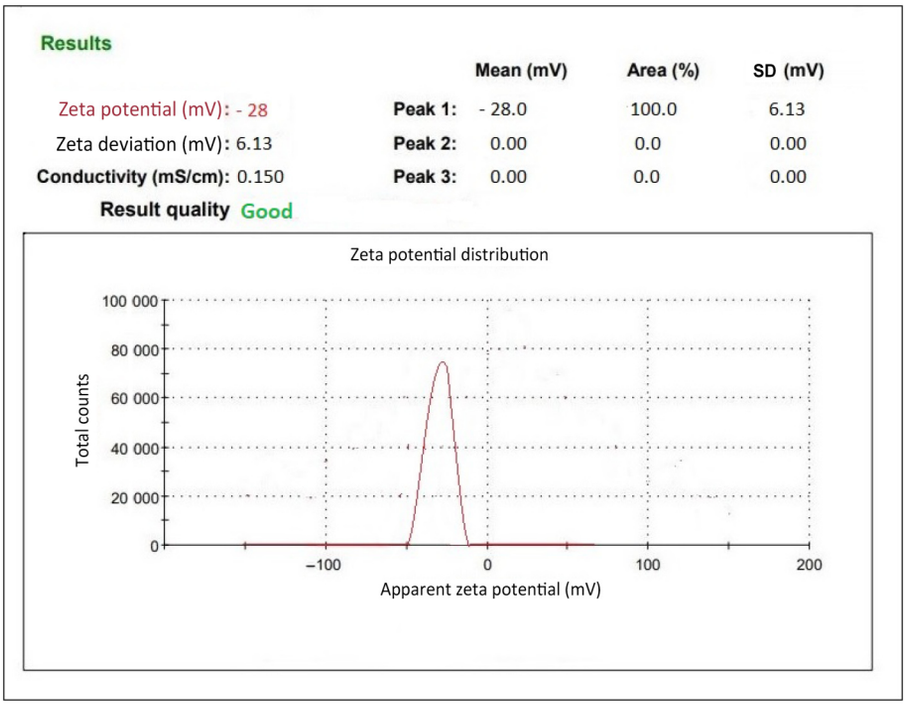


Fig. 7 Zeta potential of optimized liposomal vesicles.

Table 6 Homogeneity and grittiness of liposomal gel formulations

Formulation	Grittiness	Homogeneity
LF13G1	+ +	+ + +
LF13G2	+ + +	+ + +
LF13G3	++	+ +
LF13G4	+ +	+ +

+ + +, excellent; + +, good.

Table 7 Evaluation of liposomal gel formulations

Formulation	pH	Viscosity (cP)	Spreadability (g·cm·s^{-1})	Extrudability	Drug content	Drug deposition	Gel strength (s)
LF13G1	7.12 ± 0.17	29 209 ± 754	51.42 ± 0.42	68% ± 3.85%	71.54% ± 0.55%	19.67%	32
LF13G2	7.36 ± 0.24	32 160 ± 571	72 ± 0.01	94.6% ± 2.37%	80.66% ± 0.67%	11.24%	35
LF13G3	7.14 ± 0.31	35 516 ± 831	42.3 ± 0.33	93.8% ± 3.12%	74.58% ± 0.59%	21.45%	40
LF13G4	7.20 ± 0.22	42 313 ± 734	25.61 ± 0.61	93.06% ± 4.05%	69.07% ± 0.79%	23.09%	42

the preparations.

Drug content

The LF13G2 formulation had the highest drug content at 80.66% (Table 7), indicating a homogeneous distribution within the liposomal gel. The drug content across all formulations ranged from 69.07% to 80.66%.

Gel strength

The gel strength of the formulations ranged from 32 to 45 s. LF13G2 demonstrated superior gel strength compared with the other formulations (Table 7).

Drug deposition in the skin

LF13G2 had the highest drug deposition in the skin, with only 11.24% of the drug remaining in other compartments after 24 h of diffusion (Table 7). This indicates that 81.63% of the drug was effectively released during diffusion, demonstrating LF13G2's superior ability to penetrate skin layers and retain the drug within them.

In vitro diffusion

The drug diffusion through the membrane at various time intervals is shown in Fig. 8. LF13G2

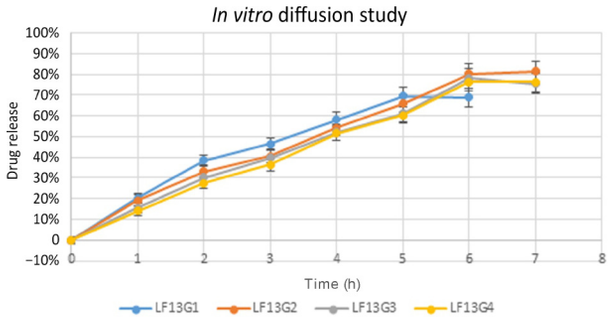


Fig. 8 *In vitro* diffusion study of liposomal gels.

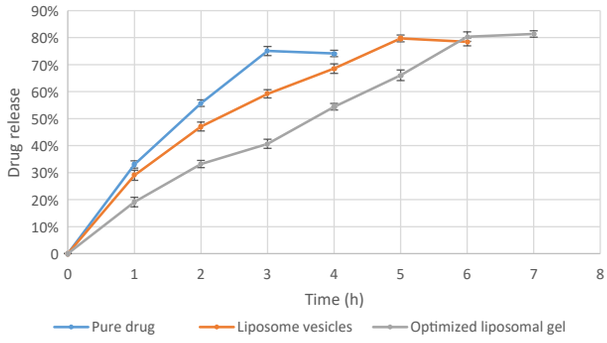


Fig. 9 Comparative *in vitro* diffusion study.

demonstrated superior diffusion compared with other formulations, achieving approximately 81.33% ± 1.19% drug release. This is attributed to its high drug content and favorable parameters affecting diffusion. Kinetic studies revealed that the Higuchi model had the highest R^2 value of 0.978, indicating that the LF13G2 gel follows a diffusion-controlled release mechanism. All evaluation parameters for LF13G2, prepared with 1% Carbopol 934, were optimal for topical delivery. Owing to its superior performance, LF13G2 was selected as the optimal formulation and was subsequently subjected to antimicrobial and stability studies.

The *in vitro* diffusion of the optimized liposomal gel (LF13G2) was compared with that of a liposomal vesicle and a pure drug dispersion. The LF13G2 liposomal gel demonstrated superior diffusion, with an 81.33% ± 1.19% release, compared with both the liposomal vesicle and the pure drug dispersion (Fig. 9).

Antimicrobial activity

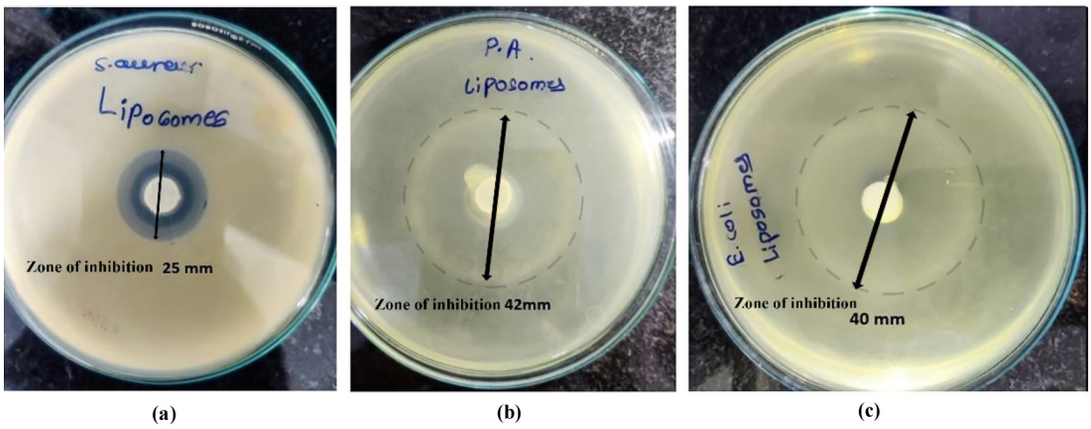
The antibacterial efficacy of various liposomal gel formulations was assessed by evaluating the zone of inhibition against the pathogens *P. aeruginosa*, *E. coli*, and *S. aureus*, as summarized in Table 8. The antibacterial activity of these formulations is illustrated in Fig. 10. The results indicated that Gram-positive bacteria (*S. aureus*) were less susceptible to the inhibitory effects of the FBT-loaded liposomal gel compared with Gram-negative bacteria (*P. aeruginosa* and *E. coli*). All formulations demonstrated enhanced inhibitory efficacy compared with the pure drug dispersion (FBT). Among them, the LF13G2 formulation exhibited the highest antimicrobial activity.

Table 8 Antibacterial activity of liposomal gel formulations

		Zone of inhibition (mm)		
No.	**Formulation**	***P. aeruginosa***	***E. coli***	***S. aureus***
1	LF13G1	39	38	23
2	LF13G2	42	40	25
3	LF13G3	40	39	23
4	LF13G4	37	36	22
5	Drug dispersion (FBT)	35	34	22

Stability

A stability of the liposomal gel was evaluated according to ICH guidelines. The results



(a) **(b)** **(c)**

Fig. 10 Antimicrobial activity of LF13G2. Zones of inhibition of **(a)** *S. aureus*, **(b)** *P. aeruginosa*, and **(c)** *E. coli*.

Table 9 Stability study of the optimized liposomal gel

Optimized formulation	**LF13G2**			
Storage conditions	40 °C, 75% relative humidity			
Time interval (day)	0	30	60	90
pH	7.36 ± 0.24	7.34 ± 1.12	7.33 ± 0.86	7.32 ± 1.12
Drug content	80.66% ± 0.67%	80.53% ± 1.32%	80.43% ± 0.98%	79.67% ± 1.21%
In vitro release	81.33% ± 1.19%	80.98% ± 0.63%	80.21% ± 1.13%	80.67% ± 1.56%

demonstrated that the optimized formulation remained stable across all evaluated parameters, with no significant changes observed at various intervals over a 3-month period (Table 9).

Conclusion

In this study, an FBT-loaded liposomal gel was successfully formulated using various concentrations of Carbopol 934. The liposomes were prepared using the thin-film hydration method with cholesterol as a stabilizer and soya lecithin as the phospholipid. The average liposome size was 392.5 ± 11.02 nm, with a PDI of 0.127 and zeta potential of −28 ± 6.13 mV, indicating strong stability. The maximum %EE achieved was 83.12% ± 4.88%. When incorporated into the gelling agent, a 1% Carbopol 934 concentration proved optimal in terms of pH, drug content, extrudability, spreadability, and viscosity. The gel also demonstrated 81.33% ± 1.19% drug release over 7 h. Compared with the drug solution, the optimized formulation exhibited effective antimicrobial activity against *S. aureus*, *E. coli*, and *P. aeruginosa*. Based on these findings, this vesicular liposomal delivery system has the potential to enhance both the therapeutic efficacy and safety of medications.

CRediT Author Statement

Conceptualization, **Punam Gadekar** and **Bhushan Rane**; writing-original draft preparation, **Punam Gadekar**, **Akash Amkar**, **Vaibhav Patil**, and **Bhushan Rane**; writing-review and editing, **Akash Amkar**, **Bhushan Rane**, and **Ashish Jain**. **Punam Gadekar** and **Vaibhav Patil** created all the figures presented. The graphical abstract created by **Punam Gadekar** and **Bhushan Rane** using BioRender.com. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interests

The authors declare that no competing interest exists.

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