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Original Article

FORMULATION, OPTIMIZATION AND CHARACTERIZATION OF BACLOFEN-LOADED LIPOSOMES FOR TOPICAL APPLICATION USING A QUALITY BY DESIGN APPROACH

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ABSTRACT

Objective: The current study aimed to develop and optimize a liposomal gel for topical baclofen delivery to enhance skin permeation and therapeutic efficacy in muscle spasticity.

Methods: A thin film hydration approach was used to create liposomes loaded with Baclofen. To optimize the baclofen liposomes, the effects of lipid concentration and hydration volume on vesicle size and entrapment efficiency were examined using a 3^2full factorial design. A topical liposomal gel was created by incorporating an optimized baclofen liposome batch into a 1% Carbopol gel, and it was assessed for many parameters.

Results: The optimized formulation demonstrated a mean vesicle size of 244.6±3.34 with a PDI of 0.145 and an entrapment efficiency of 63.78±2.66. The values from the experiments were very close to the predicted values given by the software, with a result error of ±5%, confirming the reliability of the statistical model at 95% confidence interval. A zeta potential of-28.8 mV for optimized formulation reflected good physical stability. The formulated liposomes were found to have a smooth surface texture with discrete particles in spherical shape as demonstrated in TEM analysis. The release profile of the drug from the liposomal gel formulation has shown a sustained release pattern up to 8 h with maximum release of 96.95%, following the Higuchi drug release kinetics model with non-fickian diffusion as predicted by Kores-Peppas model. The *in vivo* skin irritation test conducted on *wistar rats* revealed no signs of irritation. Furthermore, the histopathological evaluation established the safety of the formulation for topical application.

Conclusion: It was concluded that the baclofen-loaded topical liposomal gel represents a promising drug delivery approach for the treatment of muscle spasticity.

Keywords: Liposomes, Baclofen, Vesicle size, Zeta potential, Carbopol

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INTRODUCTION

The drug baclofen belongs to BCS class III category with high solubility and low permeability. It is a GABA agonist used as a skeletal muscle relaxant, aimed at treating severe muscle spasms resulting from cerebral or spinal cord disorders, like multiple sclerosis [1, 2]. Baclofen stimulates the presynaptic GABA receptors. It reduces the entry of calcium ions into the neuron terminals and consequently reduces the excitatory neurotransmitter glutamate. The result of this mechanism eventually leads to reduced excitability of the neuron [3]. Baclofen is exclusively indicated for multiple sclerosis to reduce muscle spasticity. The flexor spasms in addition to pain and muscle stiffness, can also be managed with Baclofen. baclofen is also employed to treat spinal cord injuries and various spinal cord disorders [4].

In case of low skin permeability drugs like Baclofen, vesicular formulation with encapsulation increases the interaction of drugs with biological membranes, leading to improved drug permeation across the skin barrier. While several nanocarrier based vesicular formulation approaches, such as nanoemulsions, niosomes, ethosomes, and polymeric nanoparticles have been explored for topical delivery, liposomes offer distinct advantages. Due to their phospholipid bilayer structure, liposomes closely resemble biological membranes, enhancing biocompatibility, skin affinity, and reduced irritation [5].

Liposomal preparations employ lipid vesicles for the encapsulation and targeting of drugs. It is spherical in shape and consists of a single or multiple layers of lipids enveloping an aqueous core, identical to lipids found in cell membranes. The liposomes can encapsulate both hydrophilic and lipophilic drugs, making their potential broad for application in a drug delivery system [6]. The other applications of liposomes are enhancing biological-barrier transport, improving the bioavailability, reducing the toxicity, and providing a controlled and targeted drug delivery approach. Encapsulation of drugs by the liposomal vesicular system can protect drug degradation and enhance their stability [7].

Besides the systemic delivery approaches, liposomal formulations offer a promising topical delivery system for Baclofen, allowing for localised therapeutic action and reducing systemic exposure. In the case of musculoskeletal pain and spasticity, liposomal gel formulations can be used to encapsulate Baclofen, enhancing skin permeation with the aid of vesicle-skin lipid interactions that work to modulate the skin barrier and facilitate deeper drug penetration to the underlying muscle tissues. This drug delivery approach also avoids the first pass hepatic effect, providing improved bioavailability and reducing the side effects of baclofen oral dosage forms [8].

Design of experiment (DoE) is an important tool used by researchers to effectively optimize variables and identify their interactions in influencing key responses efficiently. The 3^2full factorial design is a statistical tool used to analyze the two independent variables at three levels, to study individual as well as combined effects in the formulation. For optimizing the formulation of Baclofen-loaded liposomes, DoE has been utilized to test the effect of the lipid concentration and hydration volume on key quality attributes of the formulation, namely vesicle size and entrapment efficiency. With this technique, it is possible to get a formulation with improved performance features with a reduced number of experimental trials [9, 10].

In the preparation of baclofen liposomes, the choice of soya phosphatidylcholine (SPC), cholesterol and stearic acid is critical in influencing membrane properties, drug release and skin interactions, thereby influencing overall topical activities. SPC is the primary lipid component responsible for bilayer formation and providing structural integrity to the formulated liposomes. Cholesterol is an important component in influencing membrane rigidity

and permeability. It intercalates between phospholipid acyl chains SPC formulated liposomes, thus decreasing the molecular mobility. This process also suppresses the rapid elimination of baclofen and ensures sustained drug release by increasing the rigidity of the membrane [11].

The stearic acid also plays pivotal role in the modification of surface charge and the stabilization of membrane. By improving the occlusivity and skin hydration, stearic acid indirectly enhances the permeability of baclofen through the hydrated stratum corneum. The synergistic action of these lipids tends to improve the skin-lipid interactions, thus making the formulation suitable for topical delivery of Baclofen. The topical baclofen liposomal gel formulation encloses the drug within the lipid vesicles. In this formulation, efforts are made to circumvent the skin barriers using highly diffusible liposomes [12].

MATERIALS AND METHODS

Materials

Baclofen was obtained from Bal Pharma Limited, Bangalore, India. Soya phosphatidylcholine (Lecithin) was procured from Yarrow Chem, Mumbai, India. Cholesterol, ethanol, triethanolamine, carbopol 940, methyl paraben, and propyl paraben were purchased from Loba Chemie, Mumbai, India. Stearic acid was procured from SRL Chemical, Mumbai, India.

Fourier transform infrared spectroscopy (FTIR) study

The FTIR spectra of baclofen pure drug, Soya phosphatidylcholine (Lecithin), cholesterol, and a physical mixture of baclofen with lecithin and cholesterol were obtained using the ATR Bruker spectrophotometer (Alpha II, Massachusetts, USA). The FTIR interpretation study was done to investigate comprehensive information about the molecular vibrations of various bands in the samples to comprehend their chemical behaviour and compatibility with other excipients. The major peaks with functional peaks were confirmed from the respective spectra obtained [13].

Optimization of baclofen liposomes

The optimization of Baclofen-loaded liposomes was performed by 3^2 full factorial design. This statistical design was selected as it enables efficient assessment of main and interaction effects of two independent variables at three levels with a minimum number of experimental runs. In contrast to Box-Behnken or central composite designs, which are well suited for optimization involving multiple variables and complex response surfaces, the 3^2 full factorial design provides a simpler framework with sufficient analysis to detect curvature and facilitate clear interpretation of factor-response relationships when only two key formulation variables are used. The independent variables selected at three distinct levels: low (-1), medium (0), and high (+1), were namely the lipid concentration and hydration volume. Ten formulation trials with varying levels of independent variables were created, which included nine factorial runs with one centre point using the DoE approach, which was achieved through the Design Expert 11.0 software. In all the trials, the amount of cholesterol and stearic acid was kept constant. The dependent variables chosen were vesicle size (nm) and entrapment efficiency (%) [14, 15].

The proposed model can be represented by the following quadratic equation that incorporates the effect of individual coefficients, their interactions, and polynomial terms.

$$Y = b0 + b1X1 + b2X2 + b12\,X1X2 + b11\,X12 + b22X22 \quad \text{(Eq. 1)}$$

In this model, Y stands for the observed response based on different level of the components.

X_1 and X_2 are the standard independent variables, b_0 is the intercept and b_1,b_2 are regression coefficients obtained from experimental runs. b_{12} is the interaction coefficient representing the combined effects of X_1 and X_2 and b_{11} and b_{22} are the quadratic coefficients representing the curvature effects of $X_1^2$and X_2^2.

The independent variables selected at different levels and their influence on the dependent factors are represented in table 1.

Table 1: 3^2full factorial design variables for baclofen liposomes: coded levels and actual values for each factor

Factors	Levels, actual(coded)		
	-1 (Low)	0 (Medium)	+1 (High)
Independent variables			
A=Lipid Concentration (mg)	20	60	100
B=Hydration volume (ml)	10	20	30
Dependent variables		Constrains	
R1=Vesicle size (nm) (R1)		Minimize	
R2=Entrapment Efficiency (%) (R2)		Maximize	

Formulation of baclofen liposomes

Baclofen liposomes were formulated by the thin film hydration method. Variable concentration of lecithin was used with 20 mg of cholesterol and 10 mg of stearic acid as stabilizing lipids. The lipids were dissolved in 5 ml of ethanol in a beaker. The required quantity of baclofen was added to this solution and dissolved by stirring continuously. A 100 ml round-bottom flask was filled with the drug lipid mixture, which was then fixed to a rotary evaporator set to 50 C and rotated at 100 rpm for 20 min. Following the formation of dry film, it was rinsed and hydrated with a variable quantity of phosphate buffer pH 7.4 to produce liposomal dispersion using a rotary evaporator for 60 min for complete hydration [16].

Formulation of baclofen liposomes loaded topical gel

The optimized liposome batch was formulated into 1% Carbopol gel by dissolving 1g of Carbopol 934 in distilled water, adjusting the total volume to 100 ml, followed by mechanical stirring and allowing the mixture to soak overnight. Methylparaben and propylparaben were added as preservatives. Triethanolamine was used to achieve the required consistency of gel, along with pH adjustment of the formulated gel to yield the pH in the human skin range [17].

Vesicle size and polydispersity index (PDI)

Using a Malvern zeta sizer utilizing the electrophoretic light scattering (ELS) method, the vesicle size and PDI of baclofen liposomes and the optimized formulation were determined at 25 °C. Before analysis dilution of 1:10 ratio for each sample was done using distilled water [18].

Zeta potential

The Electrophoretic Light Scattering Method with a Malvern zeta sizer (Malvern Instruments, UK) was used to measure zeta potential of the optimized formulation at 25 °C [19].

Percentage entrapment efficiency (% EE)

The entrapment efficiency (%) of all the liposomal formulations, including the optimized formulation, was determined using the centrifugation technique. To gather the liquid supernatant, the formulations were centrifuged in a cold centrifuge at 4 °C [R-8C Remi, Vasai, India] for 40 min at 10,000 rpm. The concentration of the free drug was measured using the recovered liquid after it had been diluted with a phosphate buffer pH 7.4. The % EE was estimated with a UV-visible spectrophotometer at 220 nm [20].

The following formula was used to determine the drug entrapment efficiency of all formulations.

$$\text{Entrapment efficiency } (\%) = [(\text{Ct} - \text{Cf}) \div \text{Ct}] \times 100$$

Where, C_t – Total amount of drug

C_f-Concentration of unentrapped drug

Transmission electron microscopy (TEM) analysis

The surface structure analysis of the optimized liposomal formulation was done using TEM analysis (JEM-1230, Joel, Tokyo, Japan). On the copper grid, a drop of liposomal dispersion was placed. A 60-W incandescent bulb was used for drying the grid with the sample for two days. Then the sample was analyzed [21].

pH

The digital pH meter was used to measure the pH of the optimized formulation. Three trials were conducted by submersing the electrode into a beaker containing 1g of liposomal gel diluted with 10 ml of distilled water [22].

Spreadability

Spreadability was performed using a parallel plate approach. 1 g of gel was placed between the transparent glass slides, and a 100g weight was placed over it. The time it took for the gel to spread completely was observed by measuring the spread length [22].

The following equation was used to calculate spreadability.

$$S = (M \times L)/T$$

Where, M – Mass of gel,

L – Length of spread gel on the glass slide,

T – Time taken in seconds for complete spreading of gel.

Drug content (%)

UV spectroscopy was used to estimate the drug content (%) of the optimized formulation. A 100 µg/ml concentration was prepared by diluting the optimized formulation in 100 ml of phosphate buffer pH 7.4. The UV spectrophotometer was used to scan the dilutions at 220 nm, and the absorbance value was recorded [23].

Viscosity

The viscosity of the baclofen liposomal gel was measured by employing a Brookfield viscometer, using spindle no. 96 at varying shear rates of 3, 6, 12, 30, 50 and 100 rpm. The flow nature of the optimized formulation was assessed by plotting a graph of rpm versus viscosity [24, 25].

Drug release study

The drug release study of the optimized formulation was investigated by employing a Franz diffusion cell apparatus. A dialysis membrane is positioned in between the donor and receptor chambers of the assembly as a diffusion barrier. 1g of the baclofen liposomal gel was placed onto the donor compartment, and the receptor chamber contained 100 ml of phosphate buffer pH 7.4, as dissolution medium. The medium was continuously stirred, with the aid of a magnetic bead at 100 rpm, with temperature maintained at 37±0.5˚C during the experiment. At specified intervals (0.5, 1, 2, 3, 4, 5, 6, 7 and 8 h), 1 ml sample was withdrawn from the receptor compartment and replaced with an equal volume of fresh buffer pH 7.4 in a volumetric flask and was assayed at 220 nm by using a UV spectrophotometer to measure the drug concentration. The percentage cumulative drug release (%CDR) was then calculated based on these measurements. The data collected in the study of drug release were further analyzed through curve fitting analysis [26, 27].

Drug release kinetics study

To examine the drug release mechanism of the optimized formulation, drug release data were analyzed using various kinetic models, such as zero-order, first-order, Higuchi, and Korsmeyer-Peppas. The best model was predicted based on a higher regression (R^2) value obtained [28].

***Ex vivo* permeation studies**

The *ex vivo* permeation study was performed using a modified Franz diffusion chamber with excised rat abdominal skin. The abdominal hair was removed using an electric clipper followed by a depilatory cream, ensuring no damage to the skin. After excision, adhering subcutaneous fat was carefully removed, and the skin was washed with normal saline. Skin integrity was confirmed by visual inspection to ensure the absence of cuts or damage before mounting on the diffusion cell. The excised skin was stored in 10% formalin and soaked overnight in phosphate buffer (pH 7.4) prior to the study. All procedures involving animals were approved by the Institutional Animal Ethics Committee (IAEC) with approval number-NGSM/IAEC/APR-2024/444. The release study was carried out by the same procedure as described in the drug release study for a time period of 8 h

[29].

***In vivo* animal skin irritation studies**

An *in vivo* irritation test was conducted on *wistar rats* to assess skin safety, following ethical clearance from IAEC at NGSMIPS, under reference number NGSM/IAEC/APR-2024/444. The research was conducted on 9 male wistar rats representing three groups: (each group containing 3 animals) control, standard and test. The animals were allowed to acclimatize to the stimulated condition for seven days. 24 h before the study, a 2 cm. sq area of hair on the dorsal side of rats was shaved. The test group was treated with baclofen liposomal gel; plain carbopol gel was used in the standard group, while the control group remained untreated. Immediately after treatment, the animals were put back in their assigned cages. All the animals were systematically observed for any skin reactions like edema and erythema at 24, 48 and 72 h, and grading scores were documented according to standard scores as indicated in table 2 [30].

Table 2: Standard score grades for skin irritation study

Erythema formation grading score	
No erythema	0
Very slight erythema	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema	4
Edema formation grading score	
No edema	0
Very slight edema	1
Well-defined edema	2
Moderate to severe edema	3
Severe edema	4
Total possible score formation	8

(Skin irritation scoring was performed according to the Draize dermal irritation scale), (A total irritation score ≤ 1 was considered indicative of negligible skin irritation.)

Histopathology study

To evaluate the topical irritation potential and dermal toxicity, a skin histopathology study was conducted using the wistar rats that were experimented in the skin irritation study. The animals were divided into different treatment groups, including an control group with untreated skin, standard group treated with plain Carbopol gel (1% w/v), and the test group receiving the optimized formulation. After the treatment period, the animals were euthanized and the treated skin area was carefully excised. To keep the structure of tissue intact, the sliced skin tissues were quickly fixed with 10% formalin solution. After fixation, the samples were processed using standard histological procedures, embedded and sectioned into thin slices using a microtome. All the obtained sections were stained with hematoxylin and eosin to enhance the visualization of skin structures. The histopathology analysis was performed using an high resolution microscope, and photographs were taken at high magnification to evaluate any structural changes in the animal skin caused by the treatment [31].

Stability studies

The optimized formulation was assessed for short term stability studies for a period of 30 d. The study was conducted by storing the formulation in a stability chamber at 4 °C and 25 °C temperature conditions. The formulation was assessed for parameters such as vesicle size, PDI, entrapment efficiency and pH [32].

RESULTS AND DISCUSSION

FTIR study

The IR spectrum of pure drug baclofen exhibited characteristic absorption bands at 3289, 1625, 732 and 3447 cm^{-1}, corresponding to O-H (alcohol) stretching, C=O (carbonyl) stretching, C-Cl stretching, and N-H (amino) stretching, respectively. In case of pure Lecithin, notable peaks were observed at 736, 292, and 1286 cm^{-1}, indicating the presence of C=O (carbonyl) stretching, C-H (aliphatic) stretching, and C-O-C (ether) stretching functional groups. The IR spectrum for pure cholesterol showed absorption peaks at 3384, 2926, and 1540 cm^{-1}, which are attributed to O-H (alcohol) stretching, C-H (aliphatic) stretching, and C=C (alkene) stretching. In the IR spectrum of the physical mixture containing Baclofen, Lecithin, and Cholesterol, peaks appeared at 3300, 1626, 1240, 2922, and 3441 cm^{-1}, which corresponds to O-H (alcohol) stretching, C=O (carbonyl) stretching, C-O-C (ether) stretching, C-H (aliphatic) stretching, and N-H (amino) stretching, pointing the presence of these functional groups in the mixture. These peaks closely matched those of individual components without any significant shift, disappearance or formation of any new peaks. The absence of additional absorption bands confirmed that no chemical interaction was found between baclofen and lipids used, indicating good compatibility and the physical nature of mixing. The FTIR spectra of Baclofen, lecithin, cholesterol and their physical mixture were depicted in fig. 1 (A, B, C, D).

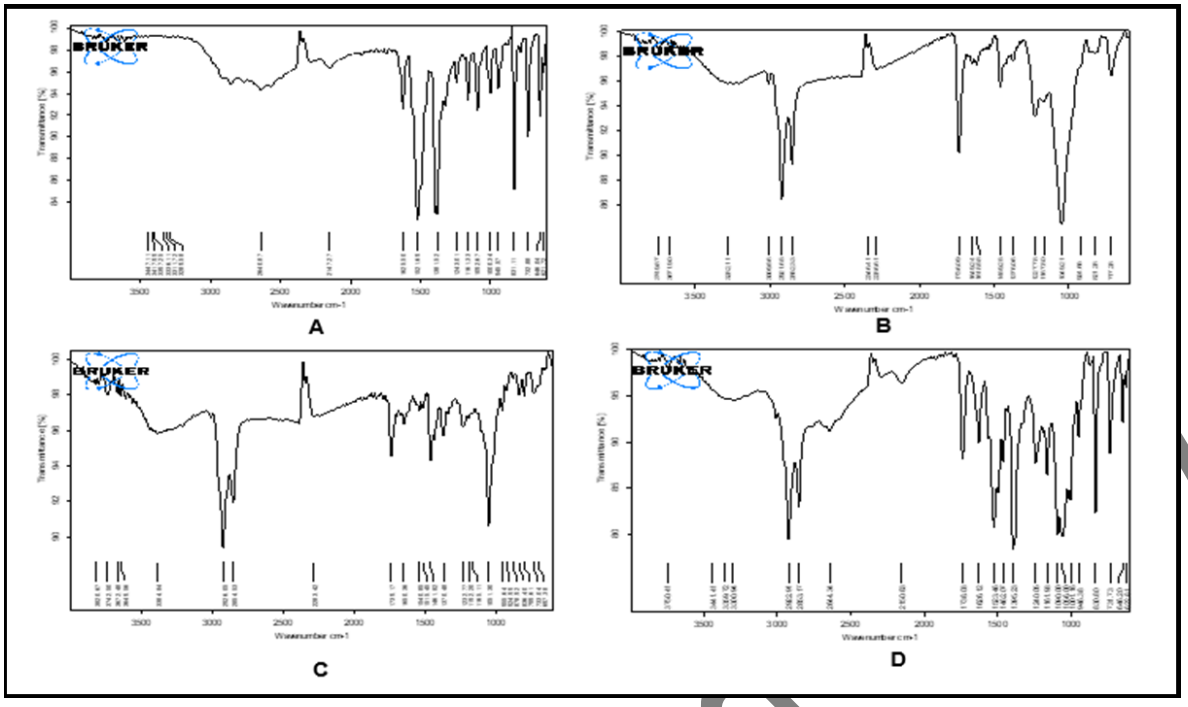


Fig. 1: (A) FTIR of pure Baclofen, (B) FTIR of Lecithin, (C) FTIR of Cholesterol, (D) FTIR of physical mixture of drug, lecithin, and cholesterol (Spectra were recorded using an ATR-Bruker Alpha II spectrophotometer over the range of 4000–400 cm^{-1}.)

Optimization of baclofen liposomes

The design of experiments (DoE) was conducted to study the impact of various factors on the response variables, vesicle size (R1) and entrapment efficiency (R2). The 3^2full factorial design created all 10 liposomal formulations at the recommended concentrations, and all the formulations were done using the thin film hydration method. Vesicle size and % entrapment efficiency of each liposome formulation were assessed. The vesicle size of all the liposomes ranged from 209.5 to 310 nm, and the % EE was in the range of 54.5 to 73.34%.

Effect of vesicle size

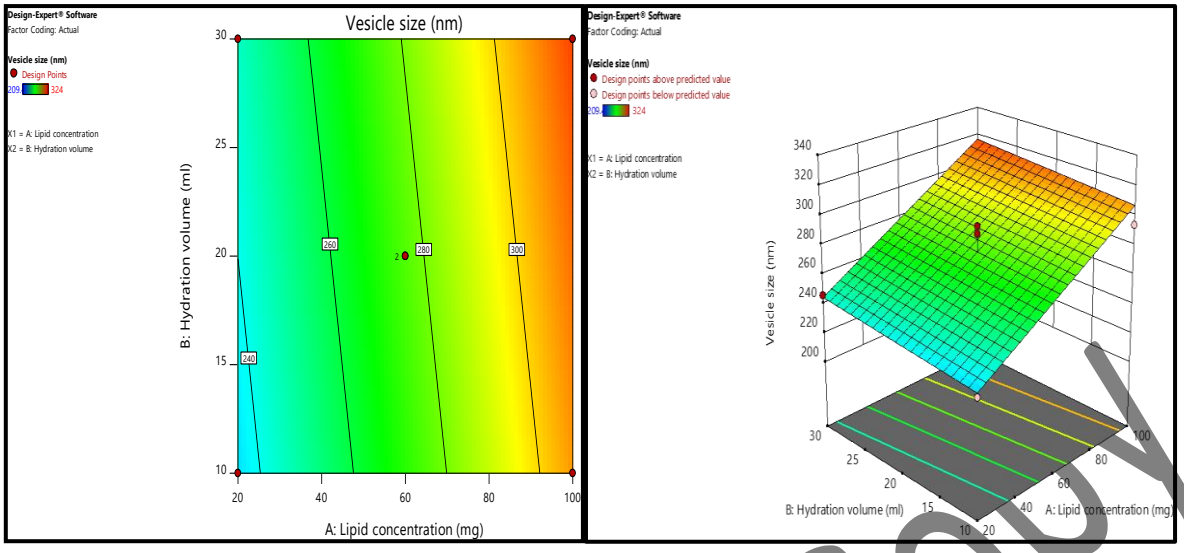
The vesicle size of liposomes significantly affects the efficacy, stability and permeation. The smaller size of vesicles enhances the ability to navigate through the stratum corneum and reach deeper skin layers. They are generally more stable in formulations, resisting aggregation and fusion. Smaller vesicle size provides faster drug release due to their higher surface area to volume ratio. The quadratic model was determined to be significant based on its F and P values, which were 36.57 and 0.02, respectively. The predicted R^2 of 0.8358 was found to be in reasonable accordance with the Adjusted R^2 of 0.8877.

The ANOVA results for baclofen liposomes were used to create the following quadratic equation for vesicle size:

Vesicle size =+276.00+34.94 A+4.78 B (coded terms)

Where; A-Lipid concentration (mg), B-Hydration volume (ml)

Fig. 2(A) and (B) illustrate the contour and 3D surface plots of vesicle size versus lipid concentration and hydration volume factors. It was noticed that vesicle size had increased, with increasing hydration volume and lipid concentration. This may be due to the formation of larger or multilamellar vesicles at higher concentrations. The hydration volume can indirectly affect liposome size due to its influence on the self-assembly process. Larger hydration volumes can give rise to slower dispersion of lipids during hydration and hence increased vesicle size. The hydration volume had only minor indirect influence, likely affecting the rate of lipid dispersion during hydration but this did not translated into a meaningful change in vesicle size. As the primary objective was to obtain smaller vesicles both lipid concentration and hydration volume there were considered non-significant contributors to vesicle size reduction in the present study.



A **B**

Fig. 2: Contour (A) and 3D surface plots (B) of vesicle size on lipid concentration and hydration volume factors

Effect of entrapment efficiency (%)

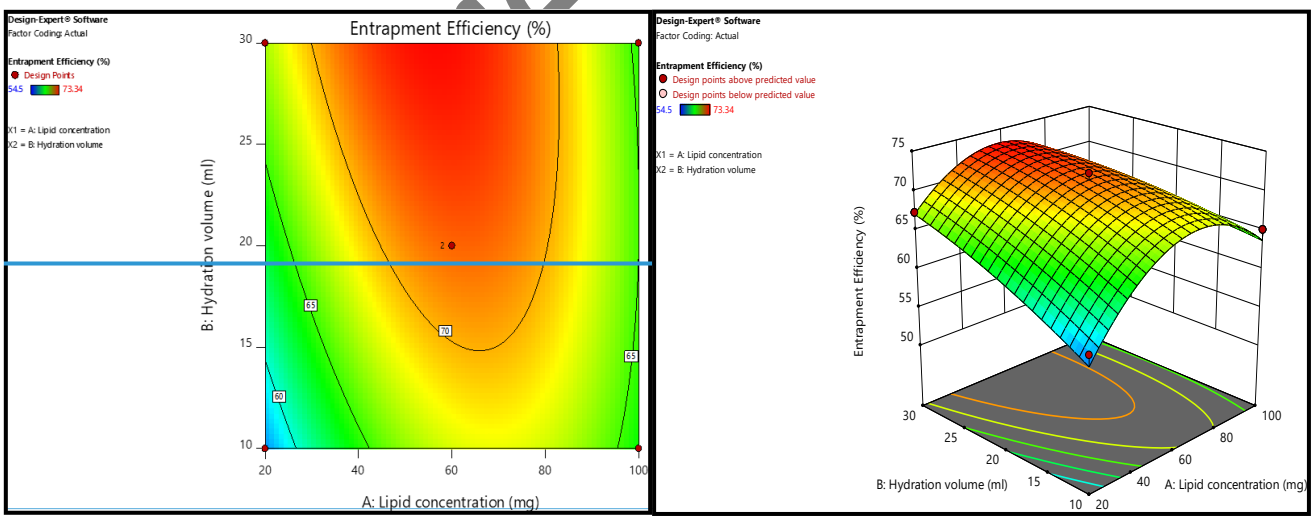
The quadratic model describing the entrapment efficiency (%) showed statistical significance as indicated by the F-value of 31.93 and the P-value of 0.02. However, the predicted R^2 (0.8372) and adjusted R^2 (0.9450) differed by more than 0.2, suggesting a larger than expected discrepancy. This discrepancy indicates a possible constraint of the model, perhaps due to overfitting, wherein the model accurately aligns with the experimental data yet exhibits reduced reliability in predicting new observations.

Based on ANOVA results for baclofen liposomes, a quadratic equation was generated to describe the percentage of entrapment efficiency (%).

% Entrapment efficiency =+71.45+0.9478 A+2.63 B – 2.34 AB – 7.40 A^2-0.9306 B^2 (coded factors)

Where, A – Lipid concentration (mg), B – Hydration volume (ml)

The contour and 3D surface plots for entrapment efficiency (%) are represented in fig. 3 (C and D).



C **D**

Fig. 3: Contour (C) and 3D surface plots (D) of entrapment efficiency (%) on lipid concentration and hydration volume factors

From the obtained 2D and 3D surface plots of the entrapment efficiency factor, it was noted that as there was an increase in lipid concentration, entrapment efficiency was increased up to a certain optimal level and beyond that it declined. This might be due to the fact that increasing lipid concentration to a larger extent will lead to the formation of multilamellar lipid vesicles that reduce the encapsulated volume per unit lipid. Hence, it was noted that the effect of lipid concentration on entrapment efficiency was not significant. In the other case, as there was an increase in the hydration volume, the entrapment efficiency also increased substantially, demonstrating that hydration volume has a strong influence on enhancing entrapment efficiency. The possible reason for this effect might be because of more availability of phospholipids, which generally increases vesicle formation

essential for the complete drug encapsulation. Additionally, increased hydration volume enables uniform distribution of lipids, leading to the bilayer formation, and provides an adequate area for better drug encapsulation. Therefore, it was revealed that hydration volume had a significant role in enhancing the entrapment efficiency of baclofen liposomes.

Vesicle size and polydispersity index (PDI)

The vesicle size is an important parameter in preparing liposomal topical formulations, balancing penetration, stability, release kinetics, and achieving optimal therapeutic performance. Liposomes with vesicle sizes particularly in the range of 100-300 nm are considered optimal for topical delivery. These smaller vesicles can navigate the intercellular lipid pathways of the stratum corneum more effectively than larger vesicles**.** The optimized formulation exhibited a vesicle size of 244.6±3.34 nm with a PDI of 0.145 confirming its nanoscale size range and indicating the uniform monodisperse vesicle distribution without any evidence of segregation. The results of the vesicle size and PDI of the optimized formulation are represented in fig. 4.

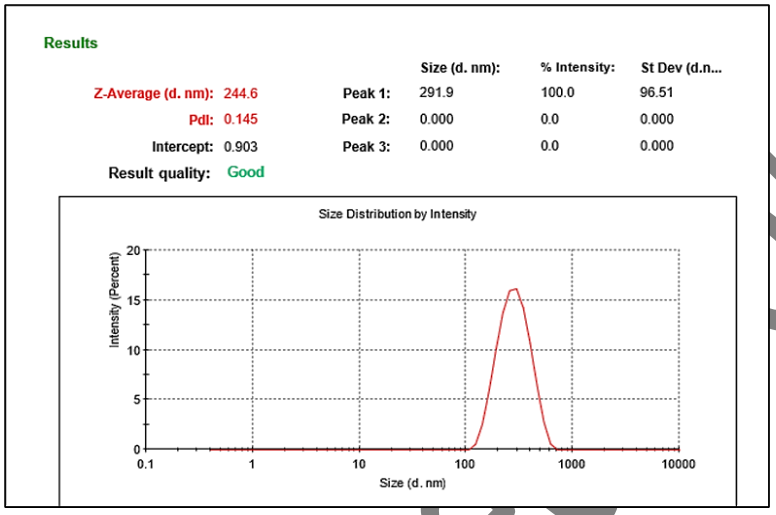


Fig. 4: Vesicle size and PDI of optimized formulation

Zeta potential

The optimized formulation had a zeta potential of-28.8 mV, as depicted in fig. 5. The obtained value was within the acceptable stability range of-25 to-40 mV. This value shows that there is enough surface charge to keep the liposomal dispersion based formulation during storage and use. The negative charge is attributed to the phosphate group of phosphatidylcholine. This gives the vesicles an anionic nature and reduces the chances of aggregation and fusion. DLVO theory states that colloidal dispersion stability depends on the balance between vander walls attractive and electrostatic repulsive forces from the electric double layer. When the zeta potential is greater than±25 mV, repulsive forces are stronger than attractive forces, which prevents aggregation. Hence, the measured value of zeta potential indicates that electrostatic repulsion is dominant, ensuring better physical stability of the formulation.

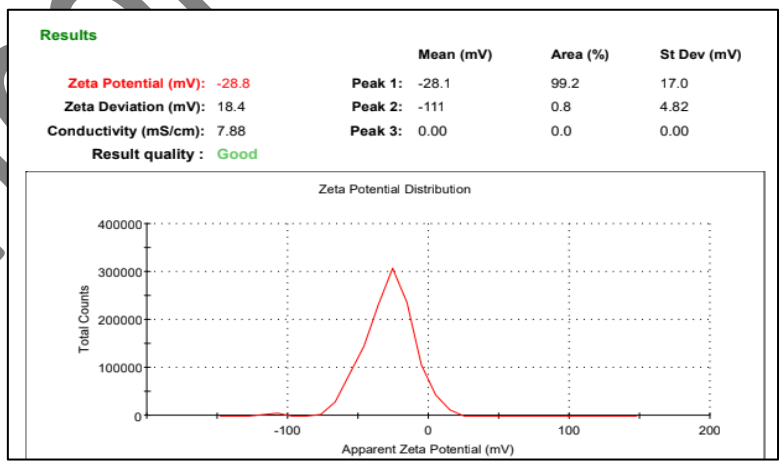


Fig. 5: Zeta potential of optimized formulation

Transmission electron microscopy (TEM)

The TEM analysis is a crucial parameter that provides complete information regarding surface morphology characteristics of the liposomal formulations. As evident from fig. 6, the optimized formulation exhibited spherical shaped vesicles with smooth surface and the measured sizes of the

vesicles were in close proximity to the values obtained from the Malvern Zetasizer. The obtained results suggest that the drug was successfully encapsulated within the lipid vesicular system, signifying successful encapsulation within the bilayer structure.

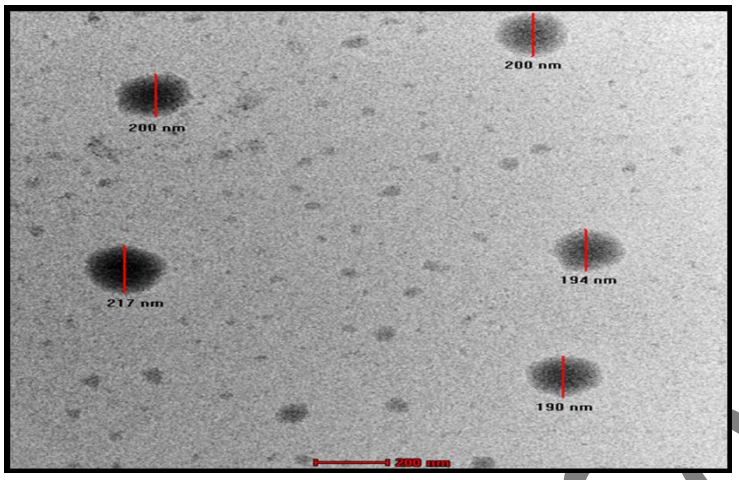


Fig. 6: TEM image of optimized formulation

pH

The optimized formulation was found to have a skin-compatible pH of 6.7±0.12, which was in between the skin pH range of 5.5 – 7.4.

Spreadability

The spreadability influences the ease of application of a formulation on skin, along with patient compliance and therapeutic efficacy. Spreadability value for this optimized formulation was found to be 12.68±1.26 g. cm/sec. The formulation's rheological behaviour and its suitability for topical delivery were clearly depicted by the results.

Drug content (%)

The optimized formulation showed good content uniformity with a drug content of 98.9±2.66. Results revealed a uniform distribution of the drug throughout the formulation.

Viscosity

Viscosity is an important parameter for predicting the nature of flow of topical formulations. Viscosity affects many factors, such as skin penetration and release rate. The viscosity of the optimized formulation was determine using a Brookfield viscometer. The viscosity ranged from 365 – 5895 cps, which was well within the 50 – 50,000 cps acceptable range for semisolid topical formulations. A rheogram was plotted as shown in fig. 7. The optimized formulation's viscosity profile showed shear-thinning effects with pseudoplastic flow, where viscosity drops as shear rate increases, enabling the gel to flow readily on the skin on application in accordance with previously mentioned spreadability. This makes it easier for liposomes to disperse uniformly across the skin surface, improving drug delivery and contact [33].

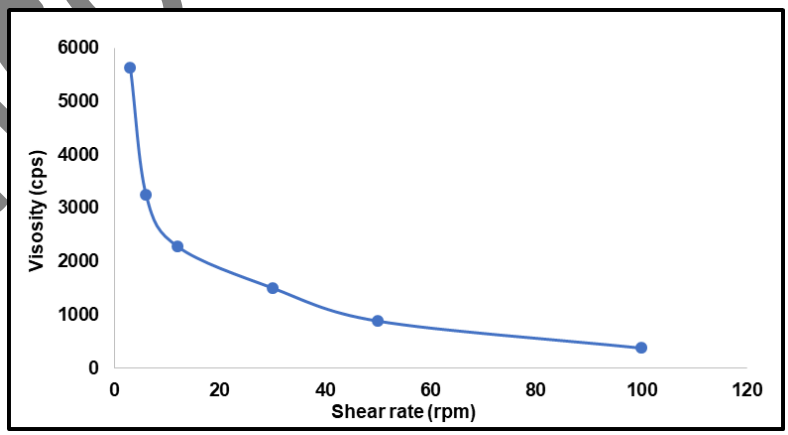


Fig. 7: Rheogram of baclofen liposomal gel (n=3)

Drug release study

The drug release profile of the baclofen liposomal gel is depicted in fig. 8. The release study lasted for 8 h, achieving a maximum drug release of 96.95±3.23 %. The formulation demonstrated a sustained release pattern. Initially, there was a burst release of 20% within 1.5 h, which corresponds

to adsorption of baclofen on the liposome surface, which is released rapidly upon contact with the release medium, and also might be due to the high surface area of the liposomes. After the initial burst effect, the drug release was sustained till 8 h, and the possible reason for this may be due to the gradual diffusion of baclofen from the liposomal bilayer, controlled by the lipid membrane integrity and the viscosity of gel matrix.

In comparison, a previously reported baclofen loaded nanovesicular system demonstrated nearly 85% cumulative drug release over a similar duration of hrs with sustained release ascribed primarily to the vesicle nanosize and the presence of non-ionic surfactants. In relation to this study report, the present liposomal formulation achieved better cumulative drug release while retaining sustained release characteristics, suggesting that phospholipid based liposomal systems may offer improved drug retention and controlled diffusion compared to surfactant based nanovesicles [34].

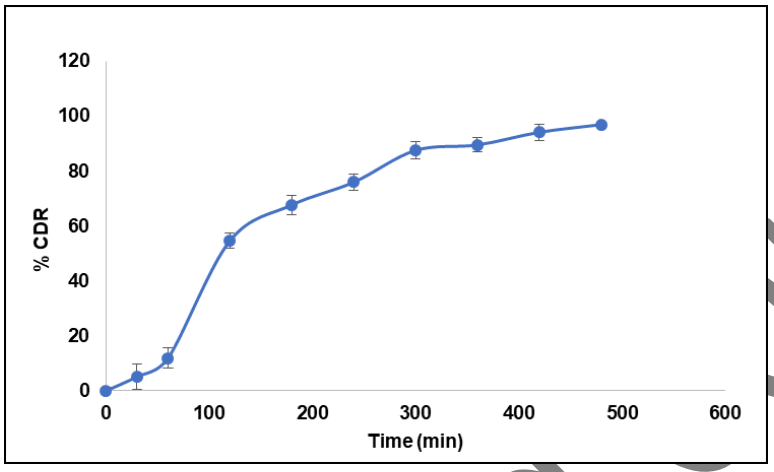


Fig. 8: Drug release profile of baclofen liposomal gel (n=3)

Drug release kinetics

Different kinetic models were employed to understand the drug release mechanism of the optimized formulation, emphasizing the importance of regression coefficients. The regression coefficient values of zero order, first order, higuchi and korsmeyer-peppas release kinetic models were represented in table 3. Among all the models evaluated, the Higuchi release model demonstrated the highest regression value, as shown in fig. 9, and the optimized formulation was found to follow the Higuchi release kinetics mechanism for the drug release following swelling and erosion of the lipid membrane. In addition to this, korsmeyer-peppas model indicated n-value of 1.0477 that helps to understand drug release from the formulation was diffusion or non-diffusion. Since the obtained n value was higher than 0.89, the release mechanism can be attributed to supercase II transport. This suggests that the drug release mechanism follows non-Fickian diffusion, which involves a combination of mechanisms.

Table 3: Results of release kinetic models of optimized formulation

	Kinetic models								
	Zero-order		**First order**		**Higuchi**		**Korsmeyer-peppas**		
	R^2	**K**	**R^2**	**K**	**R^2**	**K**	**R^2**	**K**	**n**
Optimized Baclofen liposomal gel	0.8936	0.2023	0.9297	-0.0028	0.9806	4.777	0.912	1.0477	1.047

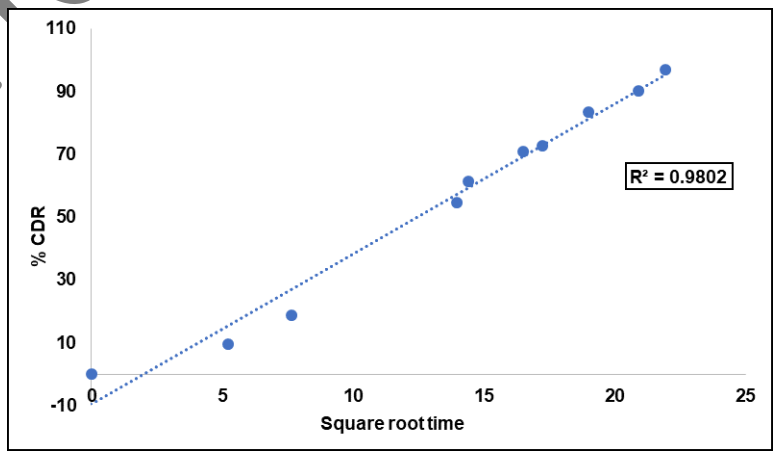


Fig. 9: Higuchi release kinetics model of baclofen liposomal gel

Ex vivo permeation studies

The *ex vivo* drug permeation studies are commonly employed to investigate the drug penetration across different skin layers, providing insight into the barrier function of the skin and the role of the physiological properties of the drug and formulation vehicle in governing the drug release. In the present study, the *ex vivo* study was conducted for a duration of 8 h using excised rat skin as a diffusion membrane. The optimized formulation exhibited a sustained release pattern with a cumulative release of 85.1±2.78% as depicted in fig. 10.

The intricate, multilayered structure of the rat skin is responsible for the delayed release as observed in *ex vivo* permeation study as compared to the *in vitro* drug release profile. The main rate limiting barrier which greatly restricts the drug transport is stratum corneum which is highly organized lipid matrix composed of compact corneocytes. In addition, the apparent permeation of baclofen may be further diminished by skin thickness, skin hydration, and potential metabolism by skin associated enzymes. Although appendageal pathways such as hair follicles provide auxiliary routes, their contribution is minimal. Additionally, the interaction between the lipid components and skin lipids may enhance drug retention in the layers of skin. Collectively, all these factors contribute for the slower and more extended *ex vivo* drug permeation in comparison to *in vitro* drug release [35].

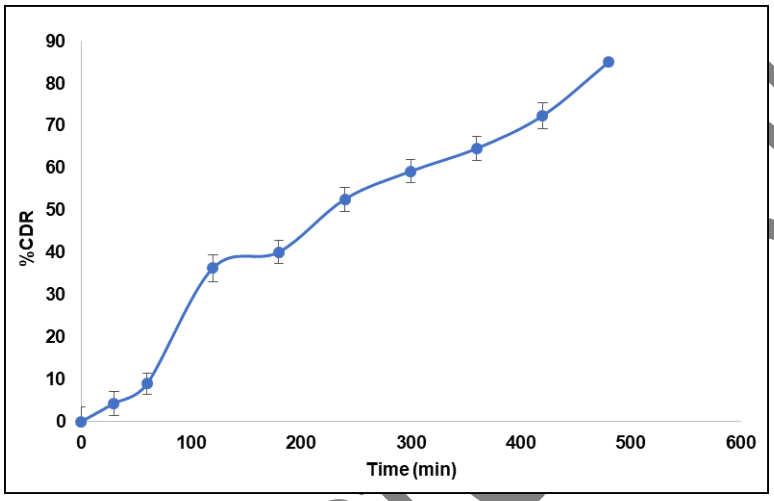


Fig. 10: ***Ex vivo*** **drug permeation profile for optimized formulation (n=3)**

Skin irritation study

An *in vivo* study on skin irritation was carried out using *wistar rats*. The goal of the study was to predict the optimized formulation's potential for irritation by comparing it with standard and control groups. No signs of irritation were noted in any groups after 72 h. The test images were depicted in fig. 11.

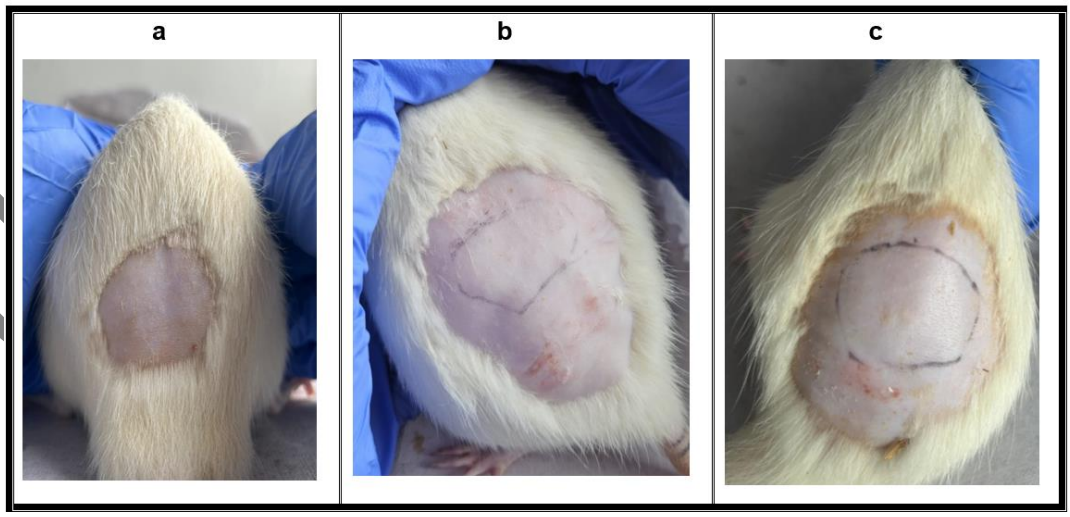


Fig. 11: Skin irritation test images (a-control, b-standard, and c-test)

Histopathological study

A histopathological study was conducted on the excised skin of the tested wistar rats. Dyes like haematoxylin and eosin were used to fix the skin samples that were stored in 10% formalin solution. The tissue sections of the standard and test showed no changes in the epidermis and dermis layers of the skin as compared to the control section. Histological changes like inflammatory infiltration, edema, ulcer, and abscess are not seen.

The epidermis in the control and standard groups had a thin stratified squamous keratinized epithelium with nearly 3-5 cell layers. There were no signs of inflammatory cell infiltration, edema, swelling or abscess formation in the dermis with normal collagen fiber structure, fibroblast distribution and vascular structures. Skin structures such as sebaceous glands and hair follicles were evenly distributed and intact. The test group's epidermis composed of a thin stratified squamous keratinized epithelium with approximately 2-4 cell layers retained its normal structure. There was a slight reduction in epidermal thickness as compared to the standard group, but this variation remained within normal physiological limits and did not correspond to any inflammatory alterations. The dermal layer displayed normal vascularity, intact connective tissue structure with no signs of necrosis. Additionally, there were no signs of structural damage and epidermal rupture. The histopathology study results were depicted in fig. 12.

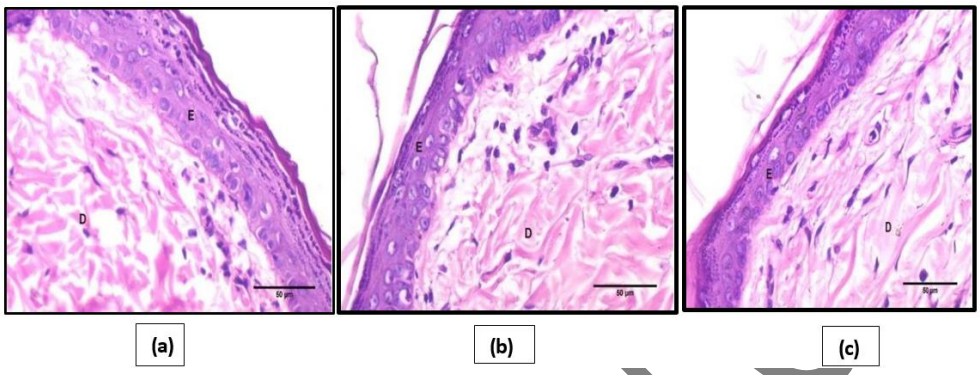


Fig. 12: Histopathology study images (a-control, b-standard, c-test)

Stability studies

The stability study results of the optimized formulation are summarised in table 4. Evaluation of stability parameters indicated that the formulation remained stable at 4 °C and 25 °C for up to 15 d with no significant differences in the measured values. Although minor changes were observed at the end of 30 day study period, these variations did not significantly affect the formulation stability. Importantly, no visible changes in the physical appearance were noted throughout the study, including the absence of precipitation and particle segregation. Overall, the formulation retained its stability under the tested storage conditions.

Table 4: Stability study results of optimized formulation

Temperature	Days	Vesicle Size	PDI	% EE	pH
4 °C	0	248.9±3.18	0.156±1.98	67.04±2.57	6.5±2.66
	15	250.2±2.25	0.207±0.56	62.33±2.05	6.3±3.38
	30	243.5±1.76	0.185±2.74	65.65±1.77	7.0±3.18
25 °C	0	238.6±1.43	0.178±2.20	69.55±1.52	6.8±2.68
	15	242.3±2.33	0.221±1.83	71.45±2.63	7.2±2.18
	30	240.7±2.06	0.196±1.48	67.82±2.31	6.5±2.76

(Results are given in mean ±SD, n=3)

CONCLUSION

In the present study, an attempt was made to formulate the baclofen loaded liposomal gel for topical drug delivery baclofen liposomes were prepared by the thin film hydration technique with the aid of soya phosphatidylcholine, cholesterol, and stearic acid as lipids. Optimization of the formulated liposomes was carried out utilizing 3^2 full factorial statistical design by assessing the effect of lipid concentration and hydration volume on particle size and entrapment efficiency factors. The results of vesicle size and entrapment efficiency of the optimized formulation were in compliance with the predicted values of the software. The zeta potential analysis revealed that the formulation exhibited good physical stability. TEM study results confirmed that the drug was well entrapped within the lipid vesicles, which had a smooth, spherical surface with no segregation. The optimized batch of baclofen liposomes was incorporated into carbopol gel to get a liposomal gel, and the formulated gel was investigated for evaluation studies such as pH, viscosity, spreadability and drug content. The obtained results of these parameters were found comply with the acceptance. Studies on *in vitro* and ex vivo release showed that the optimized formulation had a sustained drug release for 8 to10 h. *In vivo* skin irritation study conducted on *wistar rats* confirmed that the formulation was non-irritant and non-toxic to the animal skin. Furthermore, the histopathology study revealed that the formulation was safe for topical application, as it did not affect the animal skin structure. The formulated gel was found to be stable for a period of 30 d with no significant variations in the results of the evaluated parameters. By improving sustained release and localized drug delivery, the liposomal system could reduce systemic exposure and the other associated adverse effects of the oral dosage forms. In chronic disorders, such as muscle stiffness

and spasticity topical administration may also enable lower dose frequency, enhancing patient compliance. Overall, the study findings suggests that baclofen loaded liposomal topical gel represents a promising and patient friendly drug delivery approach for the effective management of muscle spasticity and stiffness.

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Nil

ABBREVIATIONS

BCS: Biopharmaceutical classification system; DoE: Design of experiment; % EE: Percentage entrapment efficiency; ELS: Electrophoretic light scattering; GABA: Gamma-Aminobutyric Acid; mg: Milligram; ºC: Degree Celsius; g: Grams; %: Percentage; nm: Nanometre; ml: Millilitre; µg: Microgram; hrs: Hours; min: Minute: FTIR: Fourier transform infrared spectroscopy; PDI: Polydispersity Index; Rpm: Revolutions per minute; Cps: Centipoise; TEM: Transmission electron microscopy; mV: Millivolts; UV: Ultra violet; %CDR: Percentage cumulative drug release.

AUTHORS CONTRIBUTIONS

All the authors contributed significantly to this work; Riya Gurudas Kalsekar designed the work, prepared the manuscript, and took part in the execution of the data. Ritiksha Poojari drafted and revised the manuscript, Sandeep DS reviewed the final version, and approved the manuscript.

CONFLICT OF INTERESTS

Declared none

REFERENCES

1. Ertzgaard P, Campo C, Calabrese A. Efficacy and safety of oral baclofen in the management of spasticity: a rationale for intrathecal baclofen. J Rehabil Med. 2017;49(3):193-203. doi: 10.2340/16501977-2211, PMID 28233010.
2. Yomiya K, Matsuo N, Tomiyasu S, Yoshimoto T, Tamaki T, Suzuki T et al. Baclofen as an adjuvant analgesic for cancer pain. Am J Hosp Palliat Care. 2009;26(2):112-8. doi: 10.1177/1049909108327968 [ePub]. PMID 19114602.
3. Davidoff RA. Antispasticity drugs: mechanisms of action. Ann Neurol. 1985;17(2):107-16. doi: 10.1002/ana.410170202, PMID 2858176.
4. Mohamed A, Bendas ER, Mohamed S, Abdel-Jaleel GA, Nasr-Alla SM. Formulation and evaluation of topical niosomal gel of baclofen. J Chem Pharm Res. 2015;7(1):277-88.
5. Witika BA, Mweetwa LL, Tshiamo KO, Edler K, Matafwali SK, Ntemi PV et al. Vesicular drug delivery for the treatment of topical disorders: current and future perspectives. J Pharm Pharmacol. 2021;73(11):1427-41. doi: 10.1093/jpp/rgab082, PMID 34132342.
6. Antimisiaris SG, Marazioti A, Kannavou M, Natsaridis E, Gkartziou F, Kogkos G, et al. Overcoming barriers by local drug delivery with liposomes. Adv Drug Deliv Rev. 2021;174:53-86. doi: 10.1016/j.addr.2021.01.019 [ePub]. PMID 33539852.
7. Liu P, Chen G, Zhang J. A review of liposomes as a drug delivery system: current status of approved products, regulatory environments, and future perspectives. Molecules. 2022;27(4):1372. doi: 10.3390/molecules27041372, PMID 35209162, PMCID PMC8879473.
8. Kamra M, Diwan A, Sardana S. Topical liposomal gel: a review. Int J Pharm Sci Res. 2017;8(6):2408-14.
9. Honmane SM, Chimane SM, Bandgar SA, Patil SS. Development and optimization of capecitabine loaded nanoliposomal system for cancer delivery. Ind J Pharm Educ Res. 2020;54(2):376-84. doi: 10.5530/ijper.54.2.43.
10. Asif AH, Desu PK, Alavala RR, Rao GS, Sreeharsha N, Meravanige G. Development, statistical optimization and characterization of fluvastatin loaded solid lipid nanoparticles: A 3^2 Factorial Design Approach. Pharmaceutics. 2022;14(3):584. doi: 10.3390/pharmaceutics14030584, PMID 35335960.
11. Jiang Y, Li W, Wang Z, Lu J. Lipid-based nanotechnology: liposome. Pharmaceutics. 2023;16(1):34. doi: 10.3390/pharmaceutics16010034, PMID 38258045, PMCID PMC10820119.
12. Nsairat H, Khater D, Sayed U, Odeh F, Al Bawab A, Alshaer W. Liposomes: structure, composition, types, and clinical applications. Heliyon. 2022;8(5):e09394. doi: 10.1016/j.heliyon.2022.e09394, PMID 35600452, PMCID PMC9118483.
13. Patel A, Dayaramani R. Novel formulation for facial acne using liposomal gel containing lipid soluble naphthoic acid derivative. Ind J Pharm Educ Res. 2022;56(2s):s245-52. doi: 10.5530/ijper.56.2s.95.
14. Sailor G, Seth AK, Parmar G, Chauhan S, Javia A. Formulation and *in vitro* evaluation of berberine containing liposome optimized by 32 full factorial designs. J App Pharm Sci. 2015;5(07):23-8. doi: 10.7324/JAPS.2015.50704.
15. El-Hashemy HA. Design, formulation and optimization of topical ethosomes using full factorial design: in-vitro and ex-vivo characterization. J Liposome Res. 2022;32(1):74-82. doi: 10.1080/08982104.2021.1955925, PMID 34697998.
16. Tiwari G, Tiwari R, Pranay W, Ankita W. Development and optimization of liposomes containing 5 fluorouracil and tretinoin for skin warts: 3^2 Experimental design. Fabad J Pharm Sci. 2019;44(1):17-26.
17. Rahamathulla M, Pokale R, Al-Ebini Y, Osmani RA, Thajudeen KY, Gundawar R, et al. Simvastatin-encapsulated topical liposomal gel for augmented wound healing: optimization using the box-Behnken model, evaluations, and *in vivo* studies. Pharmaceuticals (Basel). 2024;17(6):697. doi: 10.3390/ph17060697, PMID 38931364, PMCID PMC11206487.
18. Şahin Bektay H, Sağıroğlu AA, Bozali K, Güler EM, Güngör S. The design and optimization of ceramide NP-loaded liposomes to restore the skin barrier. Pharmaceutics. 2023;15(12):2685. doi: 10.3390/pharmaceutics15122685, PMID 38140026, PMCID PMC10747297.
19. Bhattacharyya S, Sudheer P, Das K, Ray S. Experimental design supported liposomal aztreonam delivery: *in vitro* studies. Adv Pharm Bull. 2021;11(4):651-62. doi: 10.34172/apb.2021.074 [ePub]. PMID 34888212, PMCID PMC8642795.
20. Song J, Shi F, Zhang Z, Zhu F, Xue J, Tan X et al. Formulation and evaluation of celastrol-loaded liposomes. Molecules. 2011;16(9):7880-92. doi: 10.3390/molecules16097880, PMID 22143548, PMCID PMC6264578.
21. Yalçin TE, Yetgin C. Influence of formulation composition on the characteristic properties of 5-fluorouracil-loaded liposomes. Turk J Pharm Sci. 2025;21(6):551-6. doi: 10.4274/tjps.galenos.2024.11278. PMID 39801089.
22. Fatima N, Ilyas N, Babu S. Formulation and in-vitro Evaluation of Baclofen loaded Transferosomal Gel. J Drug Delivery Ther. 2023;13(12):4-14. doi: 10.22270/jddt.v13i12.6015.
23. Yussef A, Fayez S, Sakran W. Formulation and evaluation of baclofen polymeric nanoparticles for transdermal delivery in-vitro and ex-vivo optimization. J Adv Pharm Res. 2021;5(2):285-96. doi: 10.21608/aprh.2021.68835.1125.

24. Tripathi N, Butani S, Shah R. Development and optimization of Cuscuta reflexa-based hydrogel formulation for management of eczema: *in vitro* and *in vivo* approach. Futur J Pharm Sci. 2025;11(1):1-17. doi: 10.1186/s43094-025-00900-9.
25. Baig RP, Wais M. Formulation and development of proniosomal gel for topical delivery of amphotericin B. Int J Pharm Pharm Sci. 2022;14(1):38-49.
26. Mane T, Mohite M. Design, formulation, and characterization of apremilast-saccharin cocrystals loaded with topical gel. Asian J Pharm Clin Res. 2020;13(4):60-7. doi: 10.22159/ajpcr.2020.v13i7.37693.
27. Wang Y, Wang M, Lin F, Zhang X, Zhao Y, Guo C, et al. Preparation, characterization, and evaluation of liposomes containing oridonin from *Rabdosia rubescens*. Molecules. 2022;27(3):860. doi: 10.3390/molecules27030860, PMID 35164121.
28. Mourtas S, Fotopoulou S, Duraj S, Sfika V, Tsakiroglou C, Antimisiaris SG. Liposomal drugs dispersed in hydrogels. Effect of liposome, drug and gel properties on drug release kinetics. Colloids Surf B Biointerfaces. 2007;55(2):212-21. doi: 10.1016/j.colsurfb.2006.12.005. PMID 17223020.
29. Shah H, Madni A, Rahim MA, Jan N, Khan A, Khan S, et al. Fabrication, *in vitro* and *ex vivo* evaluation of proliposomes and liposomal derived gel for enhanced solubility and permeability of diacerein. PLOS One. 2021;16(10):e0258141. doi: 10.1371/journal.pone.0258141. PMID 34665836, PMCID PMC8525764.
30. Jain S, Kale DP, Swami R, Katiyar SS. Codelivery of benzoyl peroxide and adapalene using modified liposomal gel for improved acne therapy. Nanomedicine. 2018;13(12):1481-93. doi: 10.2217/nnm-2018-0002.
31. Niu J, Yuan M, Zhang Z, Wang L, Fan Y, Liu X, et al. Hyaluronic acid micelles for promoting the skin permeation and deposition of curcumin. Int J Nanomedicine. 2022;17:4009-22. doi: 10.2147/IJN.S372711. PMID 36105622, PMCID PMC9464638.
32. Lalit KV, Kiran KT, Rajesh N, Ashish KP. Development and characterization of topical liposomal gel formulation for anti-cellulite activity. Int J Pharm Pharm Sci. 2013;5(3):512-16.
33. Ortan A, Parvu CD, Ghica MV, Popescu LM, Ionita L. Rheological study of a liposomal hydrogel based on Carbopol. Rom Biotechnol Lett. 2011;16(1):47-54.
34. M Sheta N, A El-Gazar A, M Ragab G, A Essa M, M Abdel-Haleem K, El-Dahmy RM. Transcending traditional treatment: the therapeutical potential of nanovesicles for transdermal baclofen delivery in repeated traumatic brain injury. Adv Pharm Bull. 2024;14(2):346-63. doi: 10.34172/apb.2024.031, PMID 39206406.
35. Puşcaşu C, Zanfirescu A, Negreş S. Recent progress in gels for neuropathic pain. Gels. 2023;9(5):417. doi: 10.3390/gels9050417, PMID 37233008, PMCID PMC10217.