RESEARCH PAPER

QbD-optimized oxaprozin-eucalyptus oil using Glycerosome-enabled nanogel as targeting inflammation in rheumatoid osteoarthritis

Chauhan Rachna[1] Ms Shabana Zaffar[2] Dr Shankar Lal Soni[3] Dr Vandana Sharma[4] Ms Deepika Biswas[5]

1. Research Scholar, Department of Pharmaceutics, Arya College of Pharmacy, Kukas, Jaipur, Rajasthan, India.
2. Assistant Professor, Arya College of Pharmacy, Kukas, Jaipur, Rajasthan, India.
3. Professor, Arya College of Pharmacy, Kukas, Jaipur, Rajasthan, India.
4. Principal, Arya College of Pharmacy, Kukas, Jaipur, Rajasthan, India.
5. Assistant Professor, Arya College of Pharmacy, Kukas, Jaipur, Rajasthan, India.

Corresponding author: Ms Shabana Zaffar (Shabanazafar2350@gmail.com)

Abstract

Osteoarthritis is a chronic degenerative joint disorder associated with cartilage degradation, pain, stiffness, and reduced mobility. Oral non-steroidal anti-inflammatory drugs (NSAIDs) may cause gastrointestinal adverse effects, highlighting the need for alternative topical delivery systems. This study aimed to develop and optimize an oxaprozin-loaded glycerosomal nanogel containing Gaultheria oil for potential topical management of osteoarthritis-related pain and inflammation. Preliminary studies evaluated the physicochemical properties, solubility, and UV absorption characteristics of oxaprozin. The maximum absorption wavelength was identified at 285 nm, and calibration studies demonstrated a linear relationship between drug concentration and absorbance. Oil screening identified Gaultheria oil as a suitable vehicle based on drug solubilization capacity. A Quality by Design (QbD) approach was applied to identify critical formulation variables and quality attributes. Oxaprozin-loaded glycerosome were prepared using thin-film hydration, followed by sonication and centrifugation. Optimization was performed using a three-factor, three-level Box–Behnken design comprising 17 experimental runs. Phospholipon 90G, cholesterol, and glycerol were selected as independent variables, while vesicle size, polydispersity index (PDI), and entrapment efficiency were evaluated as responses. The optimized formulation, F10, exhibited a predicted vesicle size of approximately 128 nm, a PDI of 0.06, and an entrapment efficiency of approximately 79.44%. The optimized dispersion was incorporated into a Carbopol-based gel and evaluated for physicochemical properties. The gel exhibited a smooth, uniform texture, a pH of 5.4 ± 0.53, and a reported drug content of 93.23 ± 2.97%. Accelerated stability testing for four weeks at 40 ± 5°C and 75 ± 5% relative humidity revealed changes in vesicle size and PDI during storage. Overall, QbD-assisted optimization supported the development of an oxaprozin-loaded glycerosomal nanogel as a promising topical drug delivery system.

Keywords: Oxaprozin, Osteoarthritis, Glycerosome, Nanogel, Quality by Design (QbD)

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Introduction

Osteoarthritis stands out as one of the most common degenerative joint disorders that progressively worsen over time, frequently triggering chronic pain. This condition exhibits a remarkably high prevalence among adults globally, positioning itself as the second most frequent rheumatologic ailment. This condition stands as the most prevalent joint disorder in India, impacting approximately 22% to 39% of the population. Furthermore, osteoarthritis affects women at higher rates than men, with its incidence scaling markedly alongside advancing age. Specifically, roughly 45% of women over the age of 65 report clinical symptoms, while radiological evidence of the disease is observable in 70% of individuals within this demographic age group.(1) Osteoarthritis is a musculoskeletal disorder characterized by the gradual breakdown of articular cartilage, localized inflammatory changes, and persistent joint pain that can significantly impair mobility and daily activities.(2) Clinical signs and symptoms of osteoarthritis include disability with typical clinical symptoms of pain, stiffness, and decreased function. These three symptoms are used in diagnosis by the EULAR (The European Alliance of Associations for Rheumatology). (3) Given the absence of disease-modifying pharmacotherapies capable of reversing or halting

osteoarthritis progression, clinical management relies heavily on palliative and symptomatic interventions, including non-steroidal anti-inflammatory drugs (NSAIDs), analgesics, monoclonal antibodies, and glucocorticoids.(4) Non-steroidal Anti-inflammatory drugs (NSAIDs) are frequently prescribed to manage osteoarthritis. While oral formulations are widely used by patients, they carry a high risk of severe gastrointestinal complications, such as bleeding, ulcers, and irritation. Consequently, extensive research is dedicated to developing effective topical NSAID delivery methods to minimize systemic side effects while maximizing local drug concentration.(5)

Topical administration offers notable benefits over oral delivery, such as providing steady, continuous medication straight to the target site while requiring lower doses and avoiding systemic side effects. Oxaprozin, a newer non-steroidal anti-inflammatory drug belonging to the propionic acid family, is utilized to manage the painful inflammation linked to osteoarthritis.(6) Despite presenting minor adverse effects, the medication is classified as a BCS Class II compound, meaning its poor oral absorption stems primarily from low solubility throughout the gastrointestinal tract. Consequently, topical delivery offers a superior alternative to enhance therapeutic efficiency by lowering required doses and minimizing associated side effects. Furthermore, transdermal administration extends the drug residence time by bypassing first-pass metabolism.(7)

Nanoparticles, ultra-small carrier systems typically measuring 1 to 400 nm, encapsulate, shield, and transport therapeutic drugs. Glycerosome nanoparticle topical delivery represents an advanced film thin hydrate technology designed to enhance transdermal and percutaneous administration. As an evolution of traditional liposomal systems, glycerosome are engineered to penetrate the stratum corneum, the skin's primary protective barrier.(8) Studies on plants belonging to the Gaultheria genus have primarily focused on their analgesic and anti-inflammatory properties. Gaultheria oil, whose major constituent is methyl salicylate, is widely used to relieve pain associated with rheumatoid arthritis.(9) Building on these properties, the present study aimed to develop a novel glycerosomal gel combining oxaprozin and Gaultheria oil for targeted topical management of pain and inflammation associated with osteoarthritis.(10)

Glycerosome are specialized bilayer vesicles designed for dermal and transdermal drug delivery. Distinguished from standard liposomes by the inclusion of phospholipids and substantial amounts of glycerol (5-15% v/v), they derive their properties from this high glycerol content. These vesicles efficiently transport active compounds into the skin, offering greater stability and fluidity than traditional liposomes, which makes them ideal for topical applications. Furthermore, glycerol enhances the deformability of the vesicular bilayers, thereby boosting skin penetration.(11)

An innovative drug delivery technique called nano-glycerosomal gels has been developed as a promising drug delivery system to improve the therapeutic potential of poorly water-soluble drugs. These formulations combine glycerosome-based nanocarriers with a gel matrix to enhance their pharmaceutical performance. The relatively low viscosity of glycerosomal dispersions may lead to insufficient skin retention and inadequate spreadability; however, these limitations can be addressed by incorporating a suitable gelling agent to form a glycerosomal gel. The gel matrix may also improve formulation stability, prolong residence time at the application site, and modulate drug-release characteristics. Compared with several conventional nanocarrier systems, glycerosome may offer advantages such as high drug-loading capacity, enhanced skin penetration and diffusion, and reduced skin irritation, depending on the formulation composition and drug characteristics.(12)

MATERIALS AND METHODS

Materials

Oxaprozin was obtained from Prince Scientific (Hyderabad, India). Various essential and carrier oils—including Gaultheria, sunflower, apricot, clove, and kalonji—were sourced locally. Carbopol 974® was provided by SD Fine Chemicals India Pvt. Ltd. (Mumbai, India), and Phospholipid 90G was acquired from Lipoid (Ludwigshafen, Germany). Cholesterol was purchased from Sisco Research Laboratories Pvt. Ltd. (Mumbai, India). Analytical-grade solvents, such as methanol, ethanol, and chloroform, were supplied by CDH (Delhi, India), while triethanolamine was sourced from Loba Chemie Pvt. Ltd. (Mumbai, India). All additional chemical reagents used in the study were of analytical grade.

Methods

Pre-formulation and Formulation studies

Evaluation of Organoleptic Properties

The organoleptic characteristics of the drug—encompassing colour, Odor, taste, and physical appearance were assessed through direct observation. Specifically, the visual appearance was evaluated to determine whether the substance existed in a crystalline or amorphous state.(13)

Melting Point Determination

The melting behaviour of the sample was evaluated using a differential scanning calorimeter (DSC; PerkinElmer). An accurately weighed sample was placed in an aluminium pan and crimped non-hermetically. The sample was heated from 40°C to 250°C at a constant heating rate of 10°C/min under a continuous nitrogen purge. The resulting thermogram was analyzed to determine the characteristic melting transition of the sample.(14)

Solubility Assessment

Determining drug solubility is a vital component of Preformulation studies. For oral drugs, solubility directly dictates the release rate and bioavailability, thereby influencing therapeutic efficacy. The equilibrium solubility method was conducted as follows: 2 grams of the drug were added to separate vials or ampules containing 30 mL of each test solvent. Initial mixing was performed using a vortex mixer, after which the sealed containers were agitated in a shaker incubator at a controlled temperature between 25°C and 37°C. Sampling occurred at regular 24-hour and 48-hour intervals until equilibrium was achieved. Finally, the samples were centrifuged, the supernatant was collected, and absorbance was measured using a UV spectrophotometer.(15)

UV Spectrophotometric Analysis in Ethanol

Given that the drug exhibits good solubility in ethanol, a standard calibration curve was established to quantify the drug in subsequent formulations and bioanalytical samples. A primary stock solution was prepared in ethanol and serially diluted to create standard working solutions. These dilutions were then analyzed using a UV spectrophotometer at an absorption wavelength of 285 nm.(16)

Spectrophotometric Analysis in Aqueous Solution

Since the drug is soluble in water, a calibration curve was constructed to quantify drug concentrations in subsequent formulations and bioanalytical samples. A primary stock solution was prepared using water and serially diluted to generate standard working solutions. The absorbance of these dilutions was then measured via UV spectrophotometry at a wavelength of 285 nm.(16) **Oil Screening**

Oils were selected based on their accessibility and ability to dissolve the drug. To evaluate drug solubility, 1 mg of the drug was added to 1 mL of each test oil in 5 mL glass vials. The mixtures were vortexed to assist dissolution and agitated in an incubator shaker at 37°C for 60 hours. Following this incubation period, the upper layer was sampled, appropriately diluted with ethanol, and analyzed via a UV spectrophotometer to quantify the dissolved drug concentration. Ultimately, the oil exhibiting the highest drug-solubilization capacity was selected for the formulation.

Quality by Design (QbD) Element Identification

A Quality Target Product Profile (QTPP) was established to guide the Quality by Design (QbD) framework, ensuring the final formulation maintains high standards of safety, efficacy, and consistency. The QTPP outlines the product's intended performance goals and serves as a foundation for development. Based on these targets, Critical Quality Attributes (CQAs) were determined to set acceptance criteria and evaluate quality. Potential variations in CQAs stem from Critical Material Attributes (CMAs) and Critical Process Parameters (CPPs). Following ICH Q9 recommendations, risk assessment tools such as fault tree analysis, Failure Mode and Effects Analysis (FMEA), and Ishikawa fishbone diagrams are utilized to pinpoint sources of variability. In this study, Minitab 17 (Minitab Inc., Philadelphia, USA) was used to construct an Ishikawa diagram to analyze potential cause-and-effect relationships.(17)

Glycerosome Formulation Overview

Glycerosome are vesicular drug delivery systems structurally comparable to traditional liposomes, consisting mainly of phospholipids and cholesterol. When hydrated and dispersed in an aqueous medium, phospholipids naturally assemble into bilayer vesicles. In contrast to standard liposomes, glycerosome incorporate glycerol—typically at 5%, 10%, or 15% concentrations—alongside conventional lipids. Because of their beneficial characteristics, glycerosome are recognized as promising vehicles for topical and transdermal drug delivery. Furthermore, their biocompatible nature and high skin tolerability offer a safe, non-toxic strategy for localized therapeutic administration.(18)

Preparation of Oxaprozin-Loaded Glycerosome via Rotary Evaporation

Oxaprozin-encapsulated glycerosome were synthesized using a modified thin-film hydration method. Precise quantities of oxaprozin,

Phospholipid 90G, and cholesterol were dissolved in a chloroform solution containing 1% methanol and stirred mechanically at 40°C for one hour to achieve homogeneity. The organic solvents were then evaporated under reduced pressure using a rotary evaporator (Hahnshin Scientific Co., HS-2005V-N), forming a thin, transparent lipid film inside the round-bottom flask. To remove residual solvent, the film was maintained under a vacuum overnight. Subsequently, the dried lipid film underwent a two-step hydration process using a predetermined glycerol-water mixture, followed by mechanical stirring at 40°C for one hour to promote vesicle formation. To decrease vesicle size and ensure uniformity, the dispersion was sonicated for 60 seconds (using 5-second pulses separated by 3-second intervals). Finally, the formulation was centrifuged at 7500 rpm and 4°C for 10 minutes to separate unentrapped drug, after which the resulting glycerosome were lyophilized and stored for future characterization.

Optimization of Drug-Loaded Formulation by Design of Expert (DOE)

The optimization of oxaprozin-loaded glycerosome was carried out using a three-factor, three-level Box–Behnken design (BBD) to evaluate the effects of selected formulation variables on the physicochemical characteristics of the developed system. A total of 17 experimental formulations were generated using statistical software and subsequently prepared and evaluated. The independent variables were the concentrations of Phospholipid 90G (X1), cholesterol (X2), and glycerol (X3). The dependent variables included vesicle size (Y1), polydispersity index (PDI) (Y2), and entrapment efficiency (Y3). The experimental design enabled systematic assessment of the individual and combined effects of the formulation variables on the selected responses, thereby facilitating the identification of an optimized oxaprozin-loaded glycerosomal formulation.(19)

Characterization of optimized Oxaprozin-loaded Glycerosomal formulation.

Particle Size and Polydispersity Index (PDI) Characterization

The particle size of the oxaprozin-loaded glycerosome was evaluated to understand their physical behavior. Measurements were performed using a Malvern Nano-ZS Zetasizer (Malvern, UK) equipped with DTS software, operating on the principle of dynamic light scattering (DLS). For the analysis, the formulation was diluted with distilled water, transferred into a quartz cuvette, and assessed. To ensure statistical reliability and consistency, all measurements were conducted in triplicate.(18,20)

Zeta Potential Analysis

The zeta potential of the formulation was determined using a Malvern Nano-ZS Zetasizer (Malvern, UK) equipped with DLS software, operating on the principle of dynamic light scattering. Following the same sample preparation protocol used for particle size analysis, the diluted formulation was placed in a quartz cuvette for measurement. To ensure reproducibility and reliability, all evaluations were performed in triplicate.(18,20)

Entrapment Efficiency (% EE) Determination

The entrapment efficiency of the oxaprozin-loaded glycerosome was evaluated using an ultracentrifugation method. The sample was filtered and subjected to ultracentrifugation at 25,000×g and 4°C for 45 minutes using a Beckman Coulter LE 80 ultracentrifuge. Following separation, the concentration of unentrapped drug present in the supernatant was determined by diluting the sample with methanol and analyzing it with a Shimadzu UV-1600 UV spectrophotometer at a wavelength of 285.(18)

The percentage of entrapped drug (% EE) was calculated using the following equation:

$$\text{Entrapment Efficiency (\%)} = \frac{D_T - D_S}{D_T} \times 100$$

Where,

DS = the amount of drug in the Glycerosomal sediment

DT = the theoretical amount of drug used to prepare the formulation

Preparation of Oxaprozin-loaded Glycerosomal gel

The optimized oxaprozin-loaded glycerosomal formulation was incorporated into a Carbopol gel base. Initially, Carbopol 934 (1% w/w) was gradually added to distilled water under continuous stirring until a uniform dispersion was formed. The dispersion was then left undisturbed overnight to ensure complete hydration and swelling of the polymer. Subsequently, the hydrated gel was sonicated for 15 minutes to eliminate entrapped air bubbles. Triethanolamine was added as a neutralizing agent to adjust the pH and promote gel formation, while benzalkonium chloride was incorporated as a preservative. Finally, the optimized oxaprozin-loaded glycerosomal dispersion was gradually mixed into the prepared Carbopol gel base with gentle, continuous stirring to obtain a uniform and homogeneous glycerosomal gel formulation.(18,21,22)

Characterization and evaluation of oxaprozin-loaded glycerosomal gel

Spreadability and Extrudability Studies

Spreadability

The spreadability of the prepared gel was evaluated using the apparatus described by Multimer. Approximately 1 g of gel was placed on a ground glass slide fixed to a wooden block. A second glass slide of identical dimensions was placed over the gel, and a weight of 500 g was applied to the slides for 5 minutes. After the specified period, the extent of gel spreading was measured by recording the increase in diameter. This test was performed to assess the ease with which the gel spreads over the application surface.(23)

Extrudability

The extrudability study was conducted to determine the force required to expel the gel from its container or tube. This parameter was used to evaluate the ease of dispensing the formulation and its suitability for convenient application.

The extrudability of the optimized formulation was determined using the following equation:

$$E = \frac{M}{A}$$

Where,

E represents the extrudability value

M denotes the applied weight (mass) required to expel the gel

A corresponds to the cross-sectional area through which the gel is extruded.

pH and Homogeneity Assessment

The pH of the nano-formulation was measured at 25°C using a calibrated pH meter, while visual inspection was performed to verify homogeneity and check for the presence of any aggregates. To ensure data reliability and consistency, all evaluations were conducted in triplicate.(24)

Drug Content Quantification

The drug concentration within the final gel formulation was determined using UV spectrophotometry. Specifically, 1 g of the gel was dissolved in 100 mL of methanol within a volumetric flask. To ensure reliability and consistency, the sampling and analysis were performed in triplicate, and the percentage of drug content was subsequently calculated.(25,26)

Accelerated predictive stability study

A stability study was conducted to assess the physical and chemical stability of the optimized Oxaprozin-loaded glycerosomal formulation under accelerated storage conditions. The formulation was stored in an amber-coloured borosilicate glass container at 40±5°C/75±5% relative humidity for four weeks. Samples were collected at predetermined intervals of 0, 1, 2, 3, and 4 weeks for evaluation. The stability of the formulation was assessed by monitoring changes in vesicle size, polydispersity index (PDI), and drug entrapment efficiency throughout the storage period. These parameters were used to evaluate the formulation's ability to maintain its physicochemical characteristics under the specified conditions.(27)

Results and discussion

7.1.1. Authentication of drug sample

Organoleptic Evaluation: Oxaprozin powder was inspected visually to check color, odor, nature, and other physical characteristics.

Table. 1: Organoleptic Properties of Oxaprozin

S. No.	Parameter	Observation
1	Nature	Solid crystalline powder
2	Colour	White to pale yellow
3	Odour	Odourless
4	Solubility	Freely soluble in water, ethanol and methanol
5	Melting point	260.7°C

Solubility Studies

The solubility profile of the drug was investigated using different solvents, including water, methanol, ethanol, and phosphate-buffered saline (PBS, pH 7.4)**.** The drug exhibited appreciable solubility in water, ethanol, and PBS**,** whereas its solubility in methanol was comparatively lower.

Table. 2: Solubility Profile of the Drug in Different Solvents

S. No.	Solvent System	Observation
1	Water	Freely soluble
2	Methanol	Highly soluble
3	Ethanol	Highly soluble
4	Phosphate buffer (pH 7.4)	Sparingly soluble

Melting Point Determination

Capillary Method

The melting point of oxaprozin was determined using the capillary tube method. The drug was observed to melt within the temperature range of 280–285°C. This result was recorded as part of the preliminary characterization of oxaprozin to

assess its thermal properties and verify its identity.

Determination of Maximum Absorption Wavelength (λ_{max})

A standard oxaprozin solution (20 μg/mL) was prepared in ethanol and scanned across a wavelength range of 200–400 nm using a UV-Visible spectrophotometer to identify the drug's wavelength of maximum absorption (λ_{max}).

Table. 3: Showing λ_{max} of oxaprozin in Ethanol

Drug	Media	λ_{max}
Oxaprozin	Ethanol	285

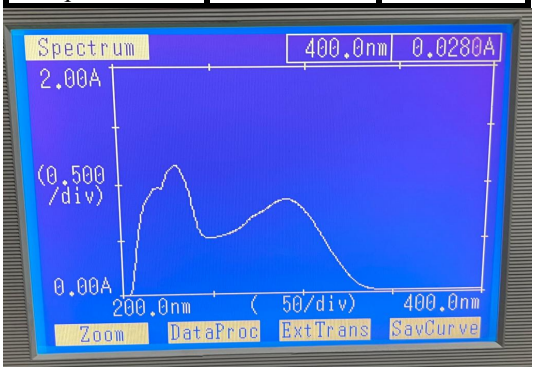


Fig. 1: UV Spectrum

By UV Spectrophotometric Method

Preparation of the Standard Calibration Curve in Ethanol

A stock solution of oxaprozin (100 µg/mL) was prepared by dissolving an accurately weighed 2 mg quantity of the drug in 20 mL of ethanol. Working standard solutions ranging from 2 to 20 µg/mL (in 2 µg/mL increments) were subsequently prepared via serial dilution of the stock with ethanol. The absorbance of each concentration was recorded at the characteristic λ_{max} of 285 nm using a UV-Visible spectrophotometer, and the resulting calibration curve is illustrated in Figure 3.

Table. 4: Absorbance of Oxaprozin in ethanol

Concentration (µg/ml)	Mean Absorbance ± SD (n=3)
0	0.000
2	0.103 ± 0.009
4	0.162 ± 0.003
6	0.239 ± 0.005
8	0.348 ± 0.005
10	0.438 ± 0.005
12	0.539 ± 0.004
14	0.649 ± 0.005
16	0.729 ± 0.007
18	0.812 ± 0.005
20	0.890 ± 0.011

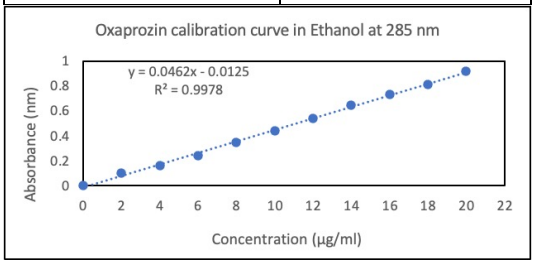


Fig. 2: Calibration Curve of Oxaprozin in ethanol

Screening of Oil

Gaultheria oil was chosen as the lipid vehicle for this investigation because preliminary screening demonstrated that the drug exhibited maximum solubility in this medium. The solubility values (mg/mL) of the drug across various tested oils are summarized in Table 5 and shown in fig 3.



Fig. 3: Screening of Oil

Table. 5: Solubility of Oxaprozin in different oils

S.No.	OIL	Drug Solubility (mg/ml)
1	Gaultheria Oil	2±0.9
2	Apricot Oil	0.5±0.117
3	Kalonji Oil	2± 0.226
4	Lemongrass Oil	0.4±0.442

5	Rosemary Oil	1±0.3

Identification of Elements for Quality by Design (QbD)

QbD Approach for Robust Formulation Development

Quality by Design (QbD) offers a structured framework to engineer robust and reliable pharmaceutical formulations. In this investigation, a Quality Target Product Profile (QTPP) was defined to pinpoint essential Critical Quality Attributes (CQAs). Potential formulation and process variables impacting these attributes were systematically examined through risk assessment using an Ishikawa fishbone diagram, with the resulting cause-and-effect relationships. Overall, the QbD strategy proved highly effective in establishing correlations between process parameters, material attributes, and product quality. The fishbone diagram enabled efficient risk identification and prioritized critical factors for subsequent optimization. While this risk-driven methodology lays the groundwork for consistent and reproducible manufacturing, empirical studies remain essential to validate the precise impact of these variables on the target CQAs.

Formulation Development

Optimization of Oxaprozin-Loaded Glycerosome Using Design of Experiments (DoE)

Design-Expert software (Version 13; Minneapolis, Minnesota, USA) was used to optimize the oxaprozin-loaded glycerosomal formulation through a Box–Behnken design (BBD). A three-factor, three-level experimental design comprising 17 runs was developed to investigate the effects of formulation variables on the physicochemical properties of the glycerosome. The independent variables were the concentrations of Phospholipon 90G (X1), cholesterol (X2), and glycerol (X3), while the dependent responses included particle size (Y1), polydispersity index (PDI) (Y2), and entrapment efficiency (Y3). The experimental plan consisted of 12 runs with different combinations of factor levels and five replicate runs at the central point to estimate experimental variability and improve the reliability of the analysis. Polynomial regression equations were developed to describe the relationships between the independent variables and the measured responses. These equations were used to assess the individual effects and interactions of the formulation components. Linear and quadratic statistical models were evaluated, and response surface plots were generated to visualize the influence of the selected variables on the responses. The quadratic model was employed to examine potential nonlinear relationships, as well as the individual and interactive effects of the formulation factors. Finally, the optimization process was used to identify the formulation conditions that could provide the desired physicochemical characteristics of oxaprozin-loaded glycerosome.

Table. 6: Oxaprozin-loaded Glycerosome: The Box-Behnken design experimental dependent and independent variables with levels (low, medium, and high).

Independent variable	Low (-1)	High (+1)
X1: Phospholipid 90G (mg)	70	90
X2: Cholesterol (mg)	5	15
X3: Glycerol (%)	5	15
Dependent variable	**Constraints**	
Y1: Vesicle Size (nm)	Minimize	
Y2: Polydispersity Index (PDI)	Minimize	
Y3: Entrapment efficiency (%)	Maximize	

Optimization of drug-loaded formulation using Box-Behnken design

Optimization of Oxaprozin-loaded Glycerosome by Statistical design

The Box-Behnken design (BBD) is a robust statistical strategy frequently utilized in pharmaceutical development to optimize formulations by establishing mathematical relationships between independent variables and experimental responses while minimizing the total number of required trials. In this investigation, BBD was applied to optimize oxaprozin-loaded glycerosome. Glycerol concentration significantly impacts vesicular properties; lower levels (5% or less) yield rigid structures prone to rapid deformation, thereby limiting dermal penetration. Conversely, elevating the glycerol content enhances membrane flexibility and mechanical resilience—with the 15% formulation exhibiting optimal flexibility and stress resistance—attributable to its interaction with the phospholipid bilayer. Additionally, incorporated glycerol mitigates skin irritation typical of alcohol-based vesicular systems. Concurrently, cholesterol reinforces vesicular stability by regulating membrane fluidity, preserving structural integrity, and

preventing aggregation. The experimental outcomes for the 17 BBD runs, generated via Design-Expert software, are summarized in Table 6 and were utilized to evaluate factor influences and identify the optimal formulation.

Table. 7: X_1: Phospholipid 90G (mg), X_2: Cholesterol (mg), X_3 Glycerol (%w/v), Y_1: Vesicle Size (nm), Y_2: Polydispersity Index, Y_3: Entrapment Efficiency (%).

Formulation Code				Independent Variables			Dependent Variables		
Actual Variables				Predicted Variables					
S. No	X1	X2	X3	Y1	Y2	Y3	Y1	Y2	Y3
F1	80	15	5	167.30	0.30	76.44	167.41	0.29	76.64
F2	80	5	5	138.70	0.33	94.03	138.20	0.33	94.29
F3	80	10	10	127.46	0.08	82.79	127.67	0.09	84.01
F4	90	5	10	168.81	0.01	71.05	168.91	0.02	72.26
F5	70	5	10	67.61	0.16	80.16	68.12	0.16	80.28
F6	80	15	15	147.40	0.44	73.44	147.90	0.44	74.76
F7	80	10	10	128.40	0.09	80.82	128.00	0.11	81.55
F8	70	10	15	75.93	0.28	84.28	75.54	0.28	84.85
F9	90	10	15	177.40	0.46	81.76	177.41	0.46	82.84
F10	**80**	**10**	**10**	**128.19**	**0.07**	**80.13**	**128**	**0.06**	**79.44**
F11	70	15	10	107.72	0.27	83.27	107.62	0.27	82.84
F12	70	10	5	96.43	0.62	95.62	96.42	0.62	95.09
F13	80	5	15	106.77	0.27	87.27	106.66	0.27	88.23
F14	90	10	5	197.20	0.04	90.04	197.59	0.04	91.61
F15	80	10	10	126.90	0.10	81.12	128.97	0.11	82.84
F16	90	15	10	210.37	0.03	82.03	209.86	0.03	82.84
F17	80	10	10	129.90	0.09	81.12	127.47	0.10	82.84

Influence of Independent Factors on Vesicle Size

Smaller vesicular dimensions facilitate deeper dermal penetration compared to larger particles. Across all prepared glycerosomal batches, vesicle diameters varied from 67.61 nm to 210.37 nm, as summarized in Table 7. As demonstrated by the polynomial equation, each independent variable exerted a distinct effect on particle size. Cholesterol concentration exhibited a direct, positive correlation with vesicle size; elevated cholesterol levels disrupt the tight packing of the lipid bilayer, allowing a greater volume of the aqueous phase to be entrapped within the core, thereby expanding the vesicle dimensions. Additionally, increased cholesterol enhanced drug encapsulation, contributing to the observed size enlargement. Conversely, surfactants reduced interfacial tension between water and cholesterol components, shrinking the molecular spacing and promoting smaller vesicular diameters. Overall, particle size increased proportionately with rising concentrations of Phospholipon 90G (X_1) (ranging from 70 mg to 90 mg) and cholesterol (X_2), whereas extended sonication times reduced vesicle size. Optimal size parameters were achieved at higher Phospholipon 90G levels paired with appropriate sonication protocols.

Influence of Independent Factors on Entrapment Efficiency (% EE)

The entrapment efficiency (% EE) of the optimized oxaprozin-loaded glycerosome ranged from 71.05% to 94.03%, as detailed in Table 7. As indicated by the generated polynomial equations, each independent formulation variable exerted a measurable impact on % EE. Specifically, cholesterol concentration demonstrated a direct, positive correlation with % EE; elevating cholesterol levels enhanced the hydrophobicity and rigidity of the lipid bilayer membrane, thereby improving structural stability and accommodating greater drug encapsulation. Furthermore, increasing the surfactant concentration contributed to a higher % EE, which can be attributed to the formation of more uniform and homogeneous glycerosomal vesicles.

Influence of Independent Factors on Polydispersity Index (PDI)

The polydispersity index (PDI) of all fabricated oxaprozin-loaded glycerosome ranged from 0.001 to 0.062 (Table 7), reflecting exceptional homogeneity and a narrow particle size distribution. As indicated by the polynomial equations, every independent variable significantly influenced the PDI values. Specifically, increasing concentrations of cholesterol and Phospholipon 90G exerted a positive, direct effect on PDI, which can be attributed to increased vesicular dimensions and higher drug-loading capacities contributing to broader size variation. In contrast, surfactant content demonstrated an inverse effect compared to cholesterol and phospholipid levels; escalating surfactant concentrations led to a marked reduction in PDI. This improvement is explained by the ability of sufficient surfactant quantities to lower interfacial tension between the aqueous and lipid phases during preparation, thereby stabilizing the primary emulsion and preventing vesicle aggregation.

Optimized Formulation Selection via Point Prediction

The optimal oxaprozin-loaded glycerosomal formulation was identified using the point prediction numerical optimization tool within Design-Expert software, targeting minimized vesicle size, maximized entrapment efficiency, and an acceptable polydispersity index (PDI). Among the evaluated solutions, formulation F10 emerged as the optimal batch, yielding predicted values of 128 nm for particle size, 79.44% for entrapment efficiency, and 0.06 for PDI. These findings confirm that formulation F10 successfully satisfied all pre-determined optimization constraints, exhibiting exceptional

physicochemical properties suitable for further study.

Characterization of Oxaprozin-loaded glycerosome

Characterization and Evaluation of Optimized Formulation F10. The vesicle size of the optimized formulation F10 was determined to be 128 nm. Vesicle dimensions are critical for topical administration, as nanometric sizing provides an expanded surface area and enhanced skin permeability (Figure 4), ultimately leading to increased drug bioavailability and therapeutic efficacy. Furthermore, the polydispersity index (PDI) of batch F10 was 0.06, demonstrating a narrow, monodisperse size distribution and a highly homogeneous colloidal dispersion (Figure 4). The entrapment efficiency of the oxaprozin-loaded glycerosome was also evaluated for formulation F10. Drug encapsulation is heavily influenced by the physicochemical properties of oxaprozin; elevating phospholipid concentrations enhanced partitioning into the lipid bilayer, while increasing cholesterol reinforced bilayer rigidity, preventing premature leakage and securing high drug-loading capacity.

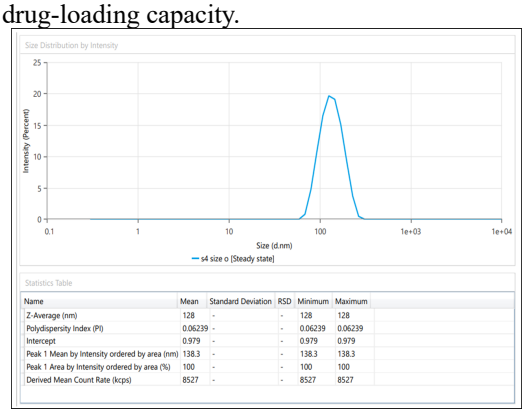


Fig. 4: Vesicle size and PDI of Opt-Oxaprozin-loaded glycerosome.

Zeta Potential

Zeta Potential Analysis of Optimized Formulation F10. The zeta potential of the optimized oxaprozin-loaded glycerosome formulation was determined to be -33.64 mV. A magnitude of ± 30 mV is widely recognized as the threshold for achieving optimum stabilization in colloidal nano-dispersions (Figure 5). Zeta potential measures the electrical charge at the surface of the nanoparticle and across the lipid bilayer, serving as a standard metric to characterize surface charge characteristics (cationic, anionic, or neutral). High absolute zeta potential values—whether positive or negative—generate strong electrostatic repulsion between particles, thereby preventing aggregation and ensuring long-term shelf stability. Consequently, these findings confirm the robust physical stability of the developed glycerosomal system.

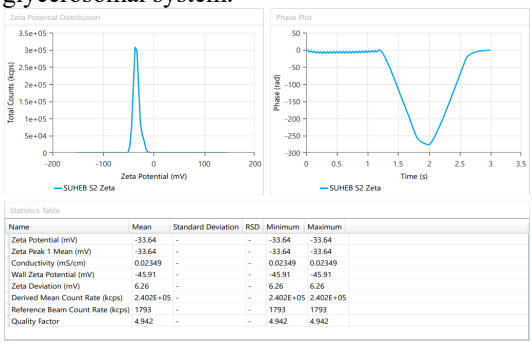


Fig. 5: Zeta potential of Opt-Oxaprozin-loaded glycerosome

Characterization and evaluation of oxaprozin-loaded glycerosomal gel

pH and homogeneity

pH and Organoleptic Evaluation of Formulation F10 Gel. The pH of the optimized oxaprozin-loaded glycerosomal gel formulation (F10) was recorded as 5.4 ± 0.53 using a calibrated pH meter (Table 8), demonstrating excellent compatibility with normal physiological skin pH and minimizing the risk of dermal irritation. Furthermore, organoleptic evaluation revealed that the gel possessed a smooth, uniform texture with desirable consistency and a complete absence of grittiness, confirming its suitability for topical administration.

Spreadability and Extrudability Evaluation

The spreadability assessment revealed that the optimized oxaprozin-loaded glycerosomal gel (F10) could be effortlessly distributed across a surface under minimal applied force, yielding a favorable spreadability value of 13.82 ± 0.38 cm. Comprehensive physicochemical and performance parameters for formulation F10 are detailed in Table 8. Furthermore, the gel exhibited an extrudability value of 48.73 ± 2.50 g/cm^2, confirming its excellent dispensing behavior and suitability for smooth extrusion from a container tube under standard conditions.

Table. 8: Characterization Parameters of Oxaprozin-Loaded Glycerosomal Gel

Rutin hydrate glycerosomal gel		
S. No	**Parameters (Mean±SD) (n=3)**	**Value**
1	Homogeneity	The formulation exhibited a smooth, uniform texture with no visible aggregates

2	Drug content (%)	93.23±2.97
3	pH	5.4 ± 0.53
4	Extrudability	48.73 ± 2.50
5	Spreadability	13.82 ± 0.38

STABILITY STUDIES

Short-Term Accelerated Stability Evaluation of Formulation F10 The physical stability of the optimized oxaprozin-loaded glycerosomal formulation (F10) was assessed over a four-week period under accelerated conditions of 40 ± 5°C and 75 ± 5% relative humidity (RH). As detailed in Table 9, formulation F10 exhibited high stability, displaying only minor, negligible shifts in vesicle size and polydispersity index (PDI), alongside a complete absence of drug degradation or loss. These minimal variations indicate robust resistance to particle aggregation and negligible drug leakage over the duration of the study. Overall, while these findings confirm the short-term physicochemical integrity and drug-retention capabilities of the system, long-term stability assessments under extended storage conditions are recommended to fully validate its shelf-life profile.

Table. 9: Accelerated predictive stability study parameters

40 ± 5°C/75±5% RH					
Parameter	**0 Week**	**1Week**	**2 Week**	**3 Week**	**4 Week**
Vesicle size	128.01±0.7	131.36±1.33	139.4±0.79	146.67±1.86	151.8±1.11
PDI	0.06±0.004	0.04±0.003	0.14±0.08	0.26±0.009	0.365±0.01
Entrapment efficiency	79.49±0.05	81.17±0.95	82.87±1.53	83.91±1.48	87.84±1.40

Conclusion

The present study successfully demonstrated the development and statistical optimization of an oxaprozin-loaded glycerosomal nanogel incorporating Gaultheria oil for potential topical application in the management of inflammation and pain associated with osteoarthritis. Preliminary characterization confirmed the suitability of oxaprozin for formulation development, while oil screening identified Gaultheria oil as a promising vehicle for drug solubilization. The application of the Quality by Design (QbD) approach and Box–Behnken experimental design enabled systematic evaluation of the effects of Phospholipon 90G, cholesterol, and glycerol on the critical physicochemical characteristics of the glycerosomal system. Among the 17 experimental formulations, formulation F10 was selected as the optimized batch, exhibiting a predicted vesicle size of approximately 128 nm, a polydispersity index of 0.06, and an entrapment efficiency of approximately 79.44%. Incorporation of the optimized glycerosomal dispersion into a Carbopol-based gel produced a formulation with a smooth and homogeneous appearance, a pH of 5.4 ± 0.53, and a reported drug content of 93.23 ± 2.97%. These findings indicate the potential of glycerosomal nanogel technology to provide a suitable platform for topical delivery of oxaprozin. However, the accelerated stability study showed an increase in vesicle size and PDI over four weeks, indicating the need for further formulation refinement and comprehensive stability evaluation. Additional investigations, including in vitro drug-release studies, ex vivo skin-permeation experiments, skin-irritation assessments, and in vivo anti-inflammatory studies, are necessary to establish the formulation's safety, efficacy, and therapeutic potential. Overall, the developed formulation represents a promising research approach for topical oxaprozin delivery, although its clinical effectiveness requires further experimental validation.

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