Niosomal Marvel: Advancements in Ketoprofen Delivery via Gel Formulation

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Abstract:
NSAIDs possess significant anti-inflammatory, analgesic, and antipyretic qualities, making them valuable for treating osteoarthritis and rheumatoid arthritis. While oral NSAID therapy is highly effective, its clinical use is often limited due to potential adverse effects like gastrointestinal mucosa irritation and ulceration. Niosomes are expected to interact favorably with human skin when used in topical preparations, particularly by improving the characteristics of the skin's outer layer. This enhancement includes reduced trans-epidermal water loss and increased smoothness through replenishing lost skin lipids. Gel formulations typically facilitate faster drug release compared to ointments and creams. Therefore, the project aimed to formulate, characterize, and evaluate niosomes containing nonsteroidal anti-inflammatory drugs embedded in a gel matrix. The objective was to develop niosome-based gel formulations for in vitro permeation studies, followed by evaluation in in vivo studies on animals.

Keywords: Niosomes, NSAIDs, Ketoprofen, Gel
DOI Number: 10.48047/nq.2022.20.9.nq44873 **NeuroQuantology 2022;20(9):7475-7495**

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are extensively used medications due to their effectiveness in reducing pain and inflammation, leading to their inclusion in the WHO's Model List of Essential Medicines. NSAIDs demonstrate significant anti-inflammatory, analgesic, and antipyretic properties and are frequently utilized in the management of osteoarthritis and rheumatoid arthritis(Bindu et al., 2020). Although oral NSAID treatment is quite effective, its clinical application is frequently limited due to potential adverse effects, including irritation and ulceration of the gastrointestinal mucosa. Patient noncompliance adds to the complexity of managing chronic inflammatory conditions since many NSAIDs necessitate multiple daily doses to maintain therapeutic blood levels(Bindu et al., 2020; McGettigan and Henry, 2013).

The administration of these agents through the skin may address the limitations associated with oral intake, maintaining relatively stable plasma levels for long-term therapy(Janssen and Genta, 2000). In this specific study, NSAIDs such as ketoprofen have been considered. Typically, these NSAIDs are applied topically in thick formulations like creams, ointments, or gels. Among these, gel formulations generally provide quicker drug release compared to ointments and creams. However, a significant challenge in transdermal drug delivery is the limited penetration of drugs through the skin, primarily due to the stratum corneum acting as the primary barrier (Jorge et al., 2010).

Improving drug penetration through the skin can be accomplished using vesicular systems like liposomes and niosomes. These systems have garnered attention for delivering dermal drugs due to their biodegradability, non-toxic nature, amphiphilic characteristics, ability to enhance penetration, and efficacy in modulating drug release properties (Jain et al., 2014).Niosomes are small vesicles composed of non-ionic surfactants, featuring an inner aqueous core surrounded by one or more lipid bilayers. These structures have shown the capability to encapsulate both hydrophobic and hydrophilic substances. They can serve as cost-effective alternatives derived from non-biological sources to liposomes or potentially function as a carrier system similar to liposomes in a living organism(Ge et al., 2019). Niosomes, as drug carriers, offer advantages over liposomes, being cost-effective and chemically stable. However, they are associated with physical stability challenges like fusion, aggregation, sedimentation, and leakage during storage. The proniosome approach aims to address these issues by providing dry, easily dispersible formulations that are more stable during storage and sterilization(Khatoon et al., 2017).

In this study, the vesicular system, specifically niosomes, has been chosen to address the undesirable effects of the ketoprofen and optimize its therapeutic benefits. Niosomes are expected to have positive interactions with human skin when used in topical applications, specifically by improving the properties of the stratum corneum, decreasing trans-epidermal water loss, and restoring depleted skin lipids.

2. Material and methods

Materials:

Ketoprofenwas obtained as gift sample from Alembic Pvt. Ltd., India. Spans (Sorbitan Fatty acid esters), Tweens (PolyoxyethyleneSorbitan Fatty acid esters), Cholesterol and all other chemicals and solvents were purchased from Himedia Laboratories, India.All other reagents and solvents used were of analytical grade.

Preparation of KetoprofenContaining Niosomes

Niosomes were prepared by thin film hydration technique as reported by (Yadav et al., 2017). Thin film hydration method involved evaporation of the solvent from the surfactant anddrug solvent system in a round bottom flask. The hydration and entrapping process ismost efficient when the film of dry lipid is thin (Bangham et al., 1965; Kulkarni et al., 1995; Yoshioka et al., 1994).Film formation depends on a suitable combination of solvent system, temperature selection, vacuum, and rotation speed. We worked to determine the best combination of these factors to achieve niosomes with the highest percentage of drug entrapment. Various solvents such as diethyl ether, chloroform, and methanol were tested for preparation. Issues encountered with different solvents included insolubility, uneven film formation, and precipitation of components at different stages of solvent evaporation, as well as inadequate hydration of the formed film or a combination of these issues. Chloroform was identified as the optimal solvent for Ketoprofen in niosome preparation using the thin film hydration method. It was crucial to prepare the vesicles at a temperature exceeding the phase transition temperature of the non-ionic surfactants; Span 60, with a phase transition temperature of 53-57°C, was chosen. Niosomes were hydrated at approximately 60 °C, and a rotary flask speed of 100 to 120 rpm was sufficient for achieving a thin and uniform film. The impact of vacuum on film formation was also considered, and 600 mm Hg was determined to be the ideal pressure for solvent evaporation. Niosome size reduction was accomplished using a probe-type sonicator, with a sonication time of two sessions of thirty seconds each, resulting in niosomes with a size of approximately 5 µm. Further sonication led to smaller sizes but decreased the percentage of drug entrapment.

Optimization of KetoprofenContaining Niosomal Formulations

The optimization of niosome formulations involves numerous formulation and process variables that need fine-tuning to achieve the desired response. Throughout the study, constants such as rotation speed, temperature, applied vacuum, and hydration time were maintained. The study aimed to develop an optimized niosomal formulation, specifically niosomes, with consistent size, shape, and a high percentage of drug entrapment (PDE). To achieve this goal, the impact of independent formulation variables was assessed, with their levels determined based on preliminary study results. The dependent variables chosen were PDE and mean vesicle size (Mean Volume Diameter). A factorial design was employed in this study to optimize the niosomal formulations. Unlike traditional methods that are time-consuming and labor-intensive, a factorial design offers an efficient approach to developing complex formulations in a shorter timeframe and with fewer batches, while also highlighting the relative importance of selected variables and their interactions.

Screening of Variables for Niosomal Formulations

The primary objective of the screening design was to identify significant effects rather than focusing on interactions. Therefore, a Taguchi orthogonal array design (L8; 7 input variables; 2 levels; 8 runs) was employed to screen and optimize formulation and process variables. According to the design matrix, a total of eight formulations were prepared and assessed using Design Expert 10.0 software, considering three response variables: mean vesicle size (nm; Y1), percentage entrapment efficiency (% EE; Y2), and percentage release (Y3). Table 1 illustrates the design matrix, depicting the -1 (low), 0 (intermediate), and +1 (high) levels of material attributes/process parameters utilized for various formulations.

Factorial Design

The Quality by Design (QbD) approach was methodically employed using response surface methodology to develop and analyze second-order polynomial models with a specified number of experimental runs. A 3-factor, 2-level 32 full factorial design was selected and utilized with Design-Expert® software (version 10.0, Stat-Ease Inc., Minneapolis, USA) to identify quadratic response surfaces and independent variables, specifically the molar ratio of Span 60 to cholesterol (X1), drug amount (%, X2), and total lipid concentration (X3). Dependent variables, determined through preliminary screening, included mean vesicle size (Y1) and percentage entrapment efficiency (Y2) of niosomes.

Each variable was categorized as +1, 0, or -1 representing high, medium, and low levels, respectively. The experimental design incorporated all independent and response variables, with their coded and decoded levels considered. A statistical analysis of the experimental design matrix comprising 12 runs (1 run per experiment) for 2 levels and 3 factors using Design of Experiments (DoE) was conducted and evaluated using an appropriate model.

Two-dimensional (2D), three-dimensional (3D) response surface plots, and contour plots were generated using Design-Expert® software to illustrate the relationship between independent and response variables. A total of 12 niosome formulations were prepared based on the factorial design recommendations, and responses were measured in terms of mean vesicle size (Y1) and percentage entrapment efficiency (Y2).

Model generation

The QbD optimization data analysis involved examining the response variables using second-order quadratic polynomial models. ANOVA analysis indicated that the selected quadratic model was highly significant ($p<0.05$), including its individual terms ($p<0.0001$). A specialized polynomial mathematical model with ten coefficients, where β0 serves as the intercept, was proposed. These coefficients account for quadratic and interaction terms, as illustrated in the equation 1.

$Y= \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_4X4 + \beta_5X_1X_2 + \beta_6X_1X_3 + \beta_7X_1X_3 + \beta_8X_1X_2 + \beta_9X_2X_4 + \beta_{10}X_3X_4$ Equation 1.

Where b1–b10 represent the regression coefficients.The coefficients were computed based on the interplay between the response and formulation variables, where X1, X2, X3, and X4 denote the coded formulation variables, and Y represents the measured response. The model established for the response variables proved to be statistically significant ($p<0.0001$), with the r-squared (r2) values indicating a strong fit of the generated polynomials to the response data ($p<0.05$ in all cases). Additionally, all the models exhibited insignificant "lack of fit" values, affirming the appropriateness of the proposed model. The close alignment between the adjusted (Adj) and predicted (Pred) r-squared values with the actual model r-squared further confirms an excellent fit to the data.

Optimization of KetoprofenLoaded Niosomes(KLNs)

To prepare ketoprofenniosomes, Span 40 was chosen as the surfactant, and diethyl ether served as the solvent, ensuring the formation of a uniform surfactant film. The formulation included an equal molar ratio of Span 40 to cholesterol and a hydration medium volume of 5 ml. Ketoprofenniosomes were developed using the thin film hydration method, optimized for total lipid concentration (X1), drug amount (X2), and total lipid concentration (X3), which significantly impact the entrapment efficiency (% EE) and mean vesicle size of niosomes. The independent variables for the study were the molar ratio of Span 40 to cholesterol (X1), drug amount (X2), and total lipid concentration (X3) for preparing ketoprofen-loaded niosome formulations. The different levels of these independent variables are outlined in Table 1. Eight batches of ketoprofenniosomes were prepared using the thin film hydration method according to the experimental design detailed in Table 2, with the model summary statistics for all measured responses provided in Table 3. The study's independent variables included the molar ratio of Span 60 to cholesterol (X1), the drug amount (X2), and the total lipid concentration (X3) for creating Ketoprofen-loaded niosomal formulations. Table 1 displays the various levels of these independent variables. Using the experimental design outlined in Table 1, eight batches of Ketoprofen-loaded niosomes (KLNs) were prepared using the thin film hydration technique. The levels of independent variables, along with their transformed values for the preparation of Ketoprofenniosomes, are detailed in Table 2. Model summary statistics for all measured responses are presented in Table 3.

Table 1: Levels of independent variables with their transformed values in the preparation of KLNs

Independent variables *Here, variable is indicated as +1, 0, and -1 representing high, medium and low levels respectively			
F. No.	**Factor 1** **A: Molar ratio of Span60: cholesterol**	**Factor 2** **B: Amount of drug (mg)**	**Factor 3** **C: : Total lipid concentration (mMol)**
1.	-1 (2:1)	0 (5)	1 (20)
2.	1 (1:2)	1 (7.5)	0 (15)
3.	1 (1:2)	0 (5)	1 (20)
4.	0 (1:1)	1 (10)	-1 (10)

5.	0 (1:1)	-1 (2.5)	-1 (10)
6.	-1 (2:1)	-1 (2.5)	0 (15)
7.	1 (1:2)	0 (5)	-1 (10)
8.	1 (1:2)	-1 (2.5)	0 (15)

Table 2: Levels of independent variables with their transformed values in the preparation of Kitoprofenniosomes.

independent variables	Level		
	Low	Median	High
	-1	0	+1
1.Total lipid concentration (mMol)	10 mMol	15 mMol	20 mMol
2. Amount of drug (mg)	4 mg	6 mg	8 mg
3. Molar ratio of Span40: cholesterol	1:2	1:1	2:1

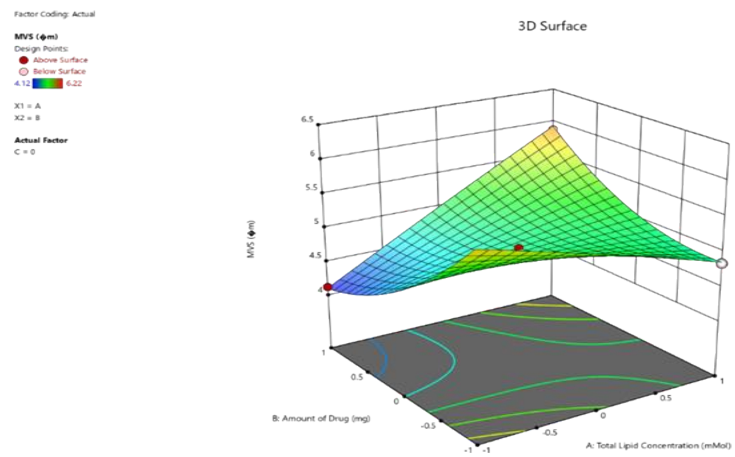
Table3: Design matrix portraying the layout of various obtained experimental runs as per Box-Behnken Design (BBD)

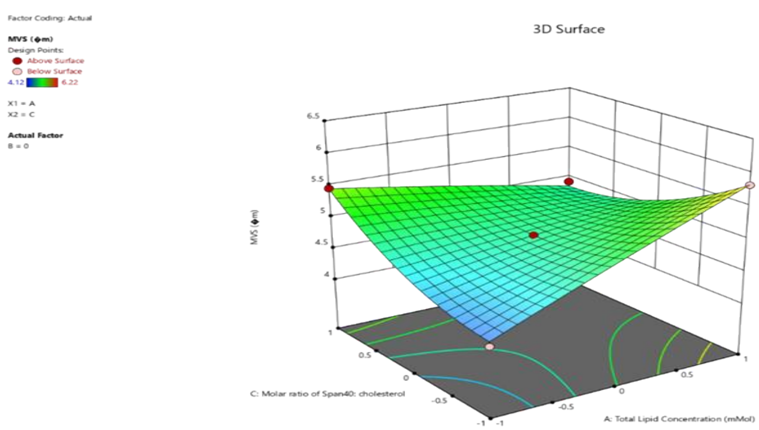
	Independent Variables			Response Variables		
St. Id	A: Total lipid concentration (mMol)	B: Amount of drug (mg)	C: Molar ratio of Span40: cholesterol	Mean vesicle size (µm)	% Entrapment Efficiency (%EE)	% Drug Release (24h)
6	-1 (10)	0 (6)	1 (2:1)	5.82±0.96	74.12±1.22	60.5±3.02
2	-1 (10)	-1 (4)	1 (2:1)	4.84±0.98	75.32±3.02	58.8±3.94
4	0 (15)	0 (6)	1 (2:1)	5.88±0.84	77.12±2.06	60.3±2.84
1	0 (15)	1 (8)	-1 (1:2)	5.84±0.80	68.23±2.95	64.4±3.46
5	-1 (10)	-1 (4)	1 (2:1)	4.24±1.12	64.36±3.64	63.32±3.84
3	0 (15)	1 (8)	-1 (1:2)	4.08±1.05	73.35±3.14	57.5±2.84
7	1 (20)	0 (6)	-1 (1:2)	5.46±0.94	66.32±3.29	64.9±3.64
8	1 (20)	0 (6)	1 (2:1)	4.92±1.06	70.99±2.26	57.5±3.98

Table4: Model summary statistics of all measured responses

Coefficient code	Polynomial coefficients for response variable		
	Mean vesicle size (µm)	% Entrapment Efficiency (%EE)	% Drug Release (24h)
Intercept	+4.89	+66.86	+64.20
A	+0.2250	+3.84	-2.62
B	-0.1538	+0.4625	+0.4975

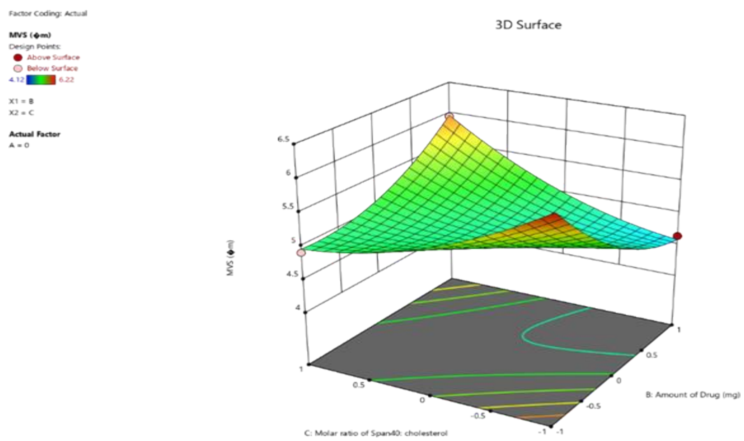
C	+0.0412	-0.4350	-0.4475
AB	+0.6900	+0.5325	+0.1250
AC	-0.5300	-1.27	-1.15
BC	+0.6525	+0.0625	-0.8200
A^2	-0.0212	+2.34	-1.40
B^2	+0.3013	+2.95	-0.5700
C^2	+0.2412	-0.2488	-1.24
r2	**0.9832**	**0.9834**	**0.9874**





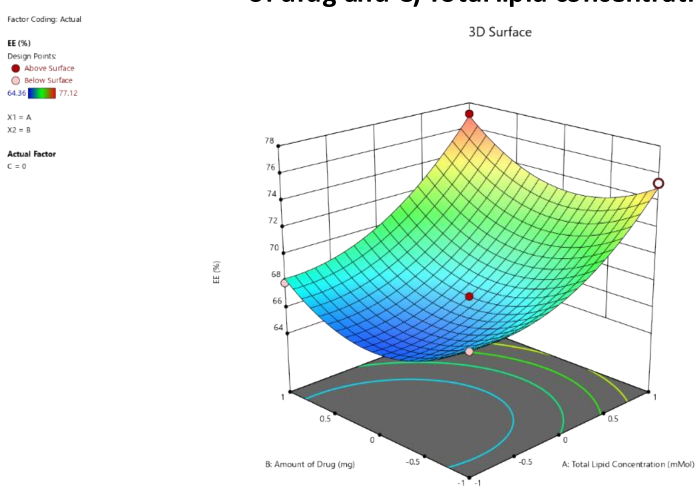
(A) **(B)**

NeuroQuantology | September 2022 | Volume 20 | Issue 9 |Page 7475-7495| doi: 10.48047/nq.2022.20.9.nq44873



(C)

Figure1:Two-dimensional contour plots and corresponding response surface plots depicting the effect of various input variables on mean vesicle size (µm), A) molar ratio of Span 40: cholesterol B) amount of drug and C) Total lipid concentration.



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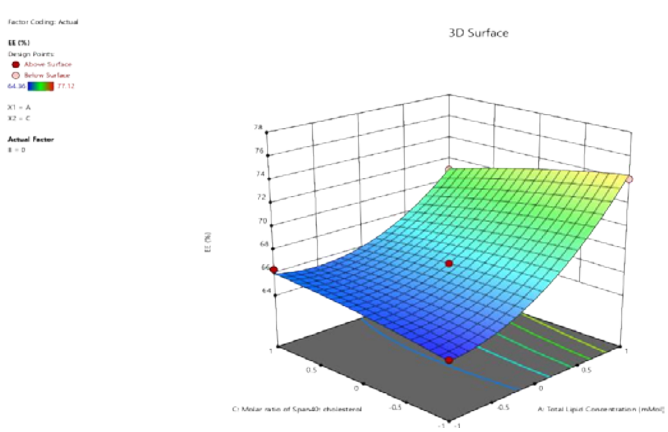

(B)

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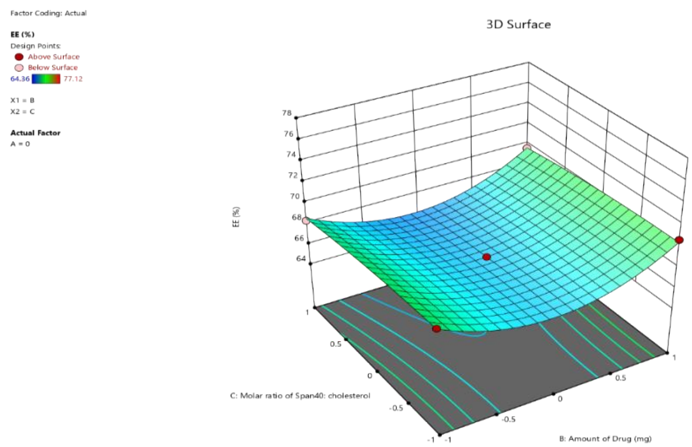

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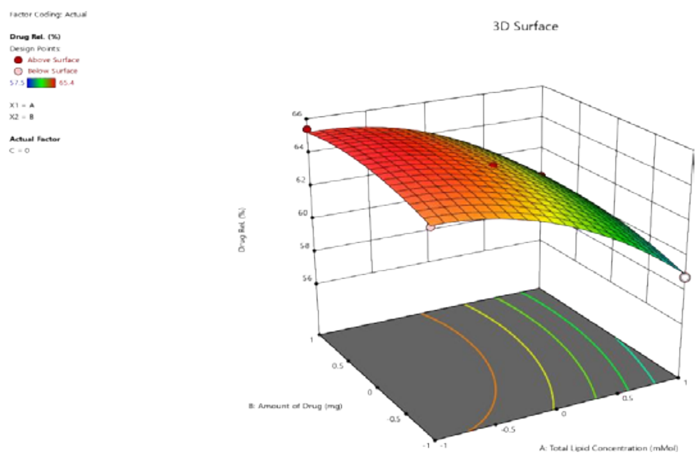
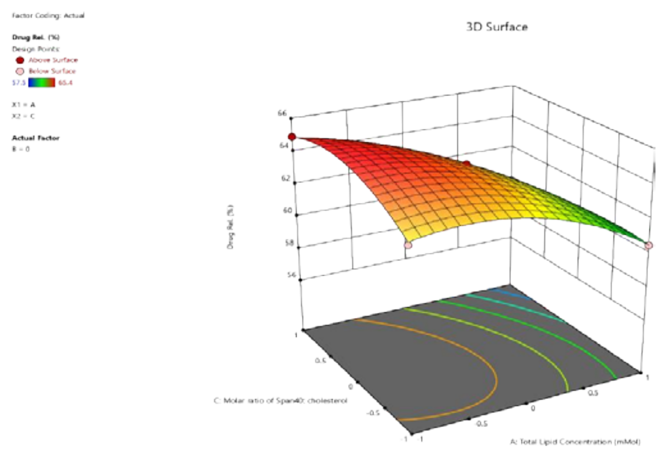
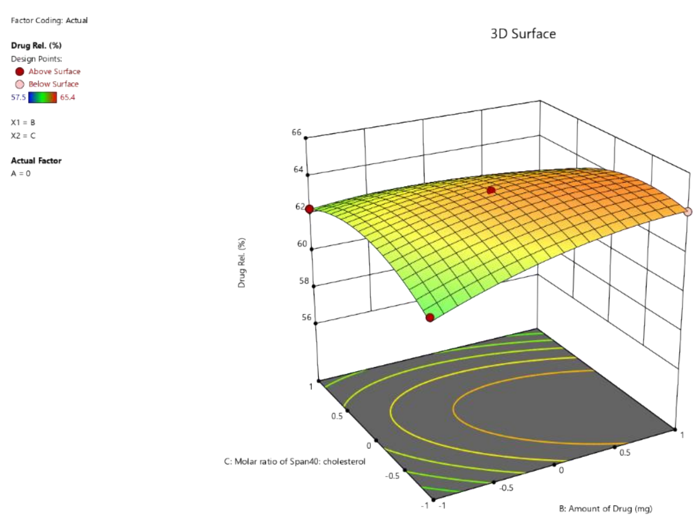
Figure 2:Two-dimensional contour plots and corresponding response surface plots depicting the effect of various input variables on % entrapment efficiency, A) molar ratio of Span 40: cholesterol B) amount of drug and C) Total lipid concentration.



(A)





(B) (C)

Figure 3:Two-dimensional contour plots and corresponding response surface plots depicting the effect of various input variables on % release (24hr), A) molar ratio of Span 40: cholesterol B) amount of drug and C) Total lipid concentration.

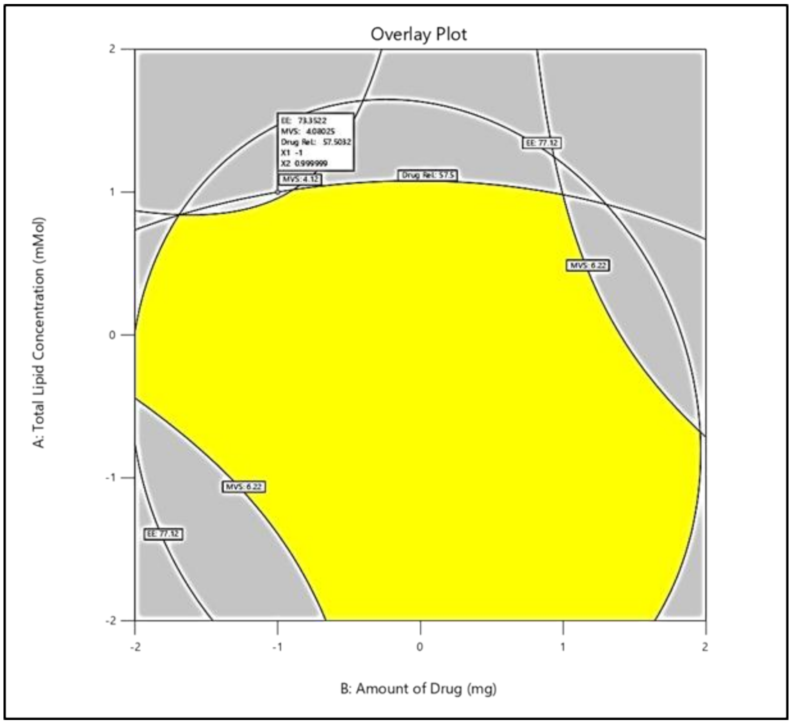


Figure 4:Design space overlay plot depicting the optimized KLNs formulation

3. Characterization of KLNs formulation

Microscopic evaluation

The prepared niosomes were observed under microscope to study their morphology after suitable dilution (Figure 5).

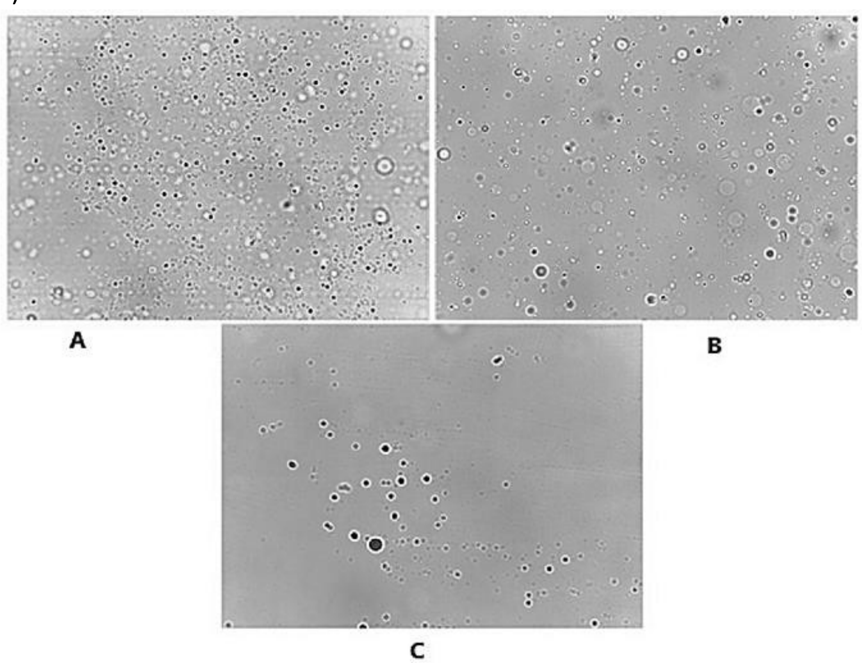


Figure 5: Photomicrograph of Ketoprofen loaded niosomes (Magnification 1200X)

Determination of Mean Vesicles Size

The vesicle sizes (Mean volume diameter) of niosomes were determined by lightscattering based on laser diffraction using particle size analyzer (Sympatec, Germany).The apparatus consists of a He-IMe laser beam of 645.4nm, which was focused with aminimum of 5mW power, using a Fourier lens to a point at the center ofmultielement detector and a small volume sample holding cell (Su cell). The sample wasstirred using a stirrer during detection.

Percentage Drug Entrapment (PDE)

The amount of Ketoprofen trapped within niosomes was measured after eliminating any untrapped drug through dialysis using a dialysis membrane (New, 1990). This process involved placing the niosomal suspension in a dialysis sack (donor compartment) submerged in a beaker containing 400 ml of PBS pH 7.4 (receptor compartment). The unbound drug was thoroughly dialyzed for 4 hours on a magnetic stirrer set at 100-120 rpm. The level of untrapped drug was determined using a UV-Visible Spectrophotometer (Shimadzu, UV 1601). To account for any absorbance caused by the surfactant component, a blank sample was prepared each time using blank niosomes. The percentage of entrapped drug was calculated using the following equation 2:

$$\% \, Entrapment = \frac{(De \; X \; 100)}{Di} \qquad \text{Equation 2}$$

where De is the amount of entrapped drug and Di is the initial amount of drug.

Drug Content

A 10 mL volumetric flask was filled with a niosomal suspension containing 5 mg of drug. The volume was adjusted with a pH 7.4 buffer to disrupt the vesicles using sonication for 40 minutes. Afterward, 1 mL of the resulting solution was transferred to a 50 mL volumetric flask, which was filled with buffer and sonicated again. The drug concentration was then measured using a UV spectrophotometer at 260 nm (Ravalika and Sailaja, 2017). Drug content was assessed for all niosomal formulations containing ketoprofen, with the average of three readings taken into account. The drug content determined by the thin film hydration method for ketoprofenranged from 97.67% to 98.89%.

Table 4: Drug Content of Niosomal formulation containing Ketoprofen

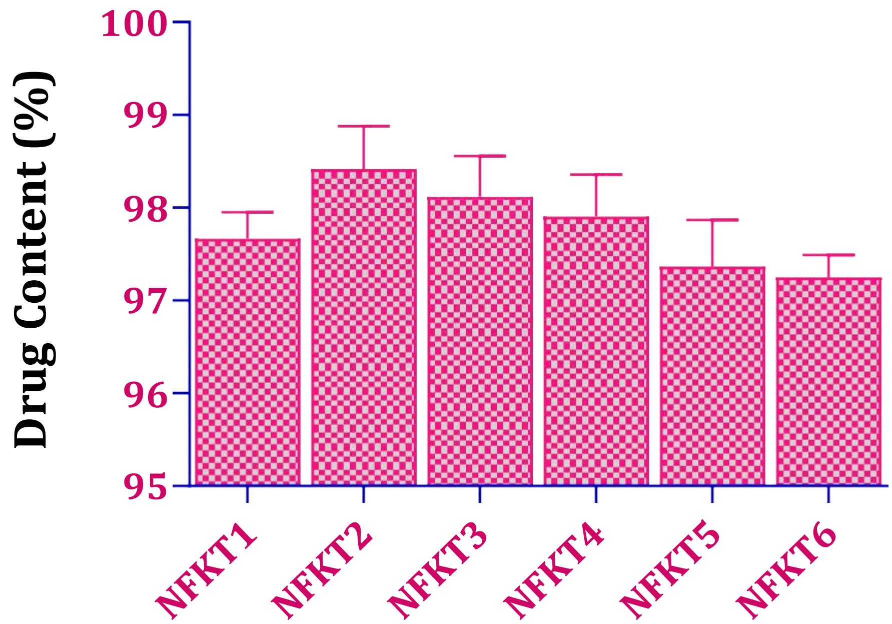


Figure 6: Drug content of Niosomal formulation containing KT

3.5 Drug Release Studies

The release of Ketoprofen from niosomal formulations was assessed using a locally made Franz diffusion cell, which had a receptor compartment capacity of 35 mL. The exposed area between the two compartments was 4.53 cm^2. An eggshell membrane was positioned on the receptor compartment with its outer side facing upward toward the donor compartment. To prevent medium loss, the top of the diffusion cell was covered with paraffin paper. The donor compartment contained the proniosome formulation equivalent to 5 mg of ketoprofen, while the receptor compartment was filled with pH 6.8 phosphate buffer and maintained at a temperature of 37 ± 1°C using a thermostatic hot plate with a magnetic stirrer. Stirring of the receptor fluid was achieved using a Teflon-coated magnetic bead attached to a magnetic stirrer set at 600 rpm. At specific intervals, a 1 mL sample of the receptor medium was withdrawn and promptly replaced with an equal volume of fresh receptor solution. The samples were analyzed using the HPLC method(Kamboj et al., 2014). The samples were analyzed using HPLC method (Figure 4).

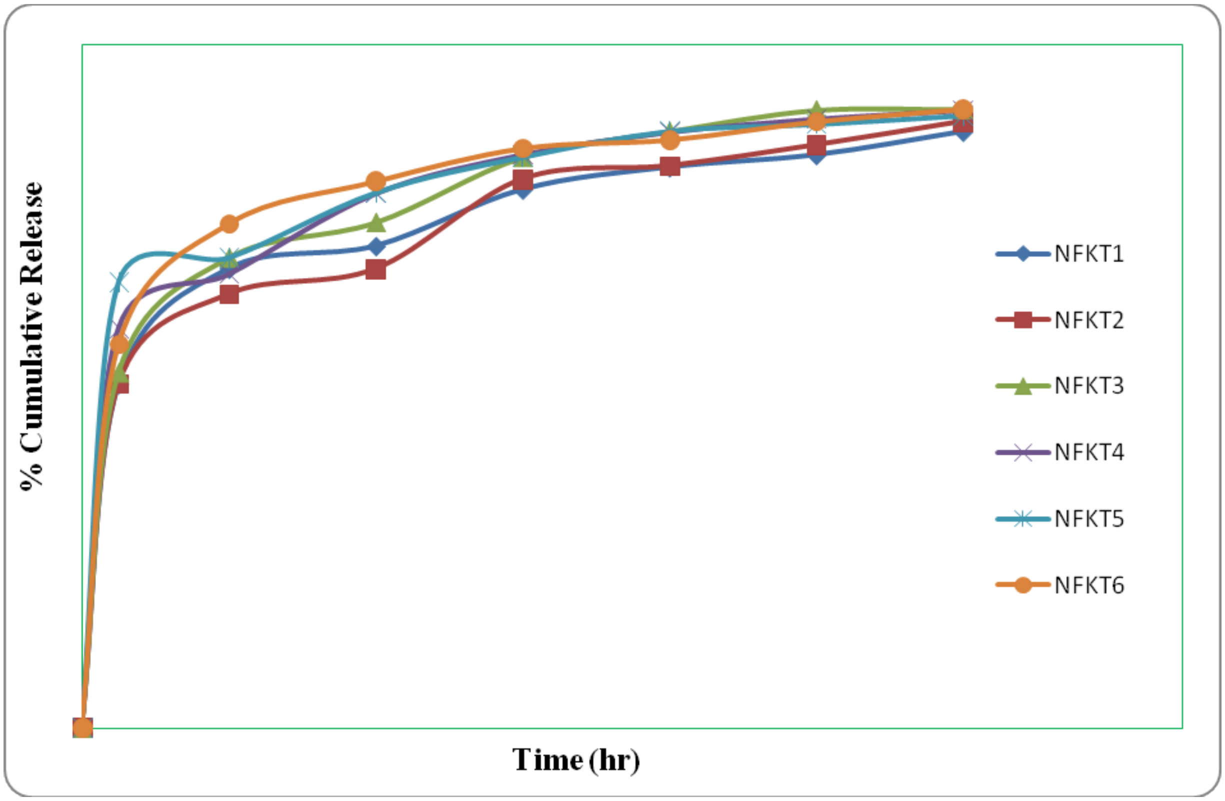


Figure7: ***In Vitro*****drugrelease ofNiosomes formulation containing KT**

4. Preparation of NiosomeBased Gel Formulations

Carbopol 980, acting as a gelling agent at a concentration of 0.9% w/w, was dispersed in distilled water to create an aqueous dispersion. This dispersion was allowed to hydrate for 4-5 hours. Optimized batches of ketoprofen-loaded niosomes (KLNs) were then centrifuged at 20,000 rpm and 4°C for 30 minutes to collect the pellets. These pellets were mixed into the aqueous dispersion of Carbopol, and after thorough mixing, the resulting dispersion was neutralized and thickened by adding triethanolamine (5% w/v) until a translucent gel was obtained. Additionally, a plain gel was prepared.

5. Characterization of KetoprofenNoisomal Gel Formulation

On the basis of results obtained from entrapment efficient, drug content and *in vitro* diffusion studies of niosomal formulation containing ketoprofen. It was found that the formulation code NFKT2 was found to have highest entrapment efficiency, drug content and % drug release, therefore thisformulation was taken and incorporated to form niosomal gel. All the prepared formulations were evaluated. The results were presented below.

Physical Examination

All the prepared gels were examined for physical appearance and results of the physical examination of the gel are shown in table 5 for ketoprofen. Plain gel containing ketoprofenand niosomal gel containing ketoprofenbearing niosomeswere found to be white in color, were homogenous, and had smooth texture.

pH Measurement

pH of the niosomal gel formulations containing ketoprofenand plain gel containing ketoprofenas shown in table 5 for KT, lied in the normal pH range of skin to avoid any risk of irritation upon the application to the skin.

Viscosity Measurements

The viscosities of the gel formulations are optimum as they provide good *in vitro* release of drug through the gel and results were shown in table 5for ketoprofen.

Spreadability

Spreadability of the plain gel containing ketoprofenand niosomal gel formulation containing ketoprofenwere found to be better as compared to plain gel as shown in 5. This could be because of loose gel matrix of gel due to presence of vesicles

Drug content

The drug content of the plain gel containing ketoprofenand niosomal gel formulation containing ketoprofenwas recorded in table 5.

Table 5: Physical examination and other characterization of plain and niosomal gel formulation containing ketoprofen

Formulation Code	Color	Homogeneity	Texture	pH	Viscosity (cps)	Spreadability	Drug Content
PGKT	White	Homogeneous	Smooth	6.62	6082	14.25	97.24±0.20
NFKT	White	Homogeneous	Smooth	6.81	6238	16.42	98.91±0.25

***In vitro* release study**

In vitro release studies were carried out by taking 1 gram of gel formulations into dialysis tubing and placed in a beaker containing 250 ml PBS pH 7.4 at 37 ± 1°C. The beaker was placed over a magnetic stirrer and stirred at constant speed. Aliquots of samples were withdrawn at specified time intervals and replaced with equal volume of fresh receptor fluid every time. Samples withdrawn were analyzed by UV spectrophotometer.

In vitro release rate studies were done for different formulations and effects of variation in composition on release rate were studied. In order to evaluate drug release from plain gel containing ketoprofen .Figure 6.1 shows the comparative in vitro release profile of the ketoprofen from the different niosomalgel formulations. It was found that only about 81% to 90% of the encapsulated drug released during a period of 12 hrs from the various niosomal gel formulations (Figure 8).

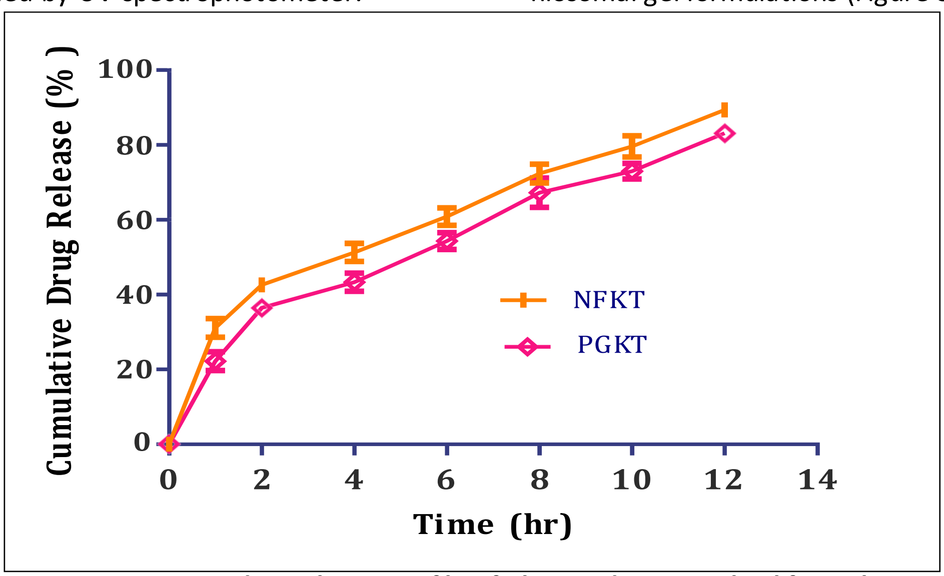


Figure 8: Comparative *Invitro*drugrelease profile of plain and niosomal gel formulation containing Ketoprofen

***In vitro* skin permeation studies**

White albino rats of either sex weighing 220 ± 20 g (7-9 weeks old) were selected for the study because they offer several advantages as animal model for anti-inflammatory studies. All animals were housed in polypropylene cages with free access to palletized chow and tap water.

The drug permeation from niosome-based gel formulations was assessed using a Franz diffusion cell. Rat skin, excised and mounted with the stratum corneum facing upwards towards the donor compartment, was placed on the receptor compartment. The donor compartment was filled with the niosome-based gel formulation, while a 20 ml solution of pH 7.4 phosphate buffer served as the receptor medium to maintain a sink condition. The available diffusion area of the cell was 2.84 cm². The receptor compartment was kept at 37°C with magnetic stirring at 600 rpm. Samples were withdrawn from the receptor compartment at set time intervals and promptly replaced with fresh buffer solution to ensure a constant sink condition, confirmed through initial experiments.

These withdrawn samples were then analyzed using a UV spectrophotometer. The permeability coefficient (Kp) was calculated based on the steady-state flux and the concentration in the donor compartment (Cdonor) using a specific equation. Permeation kinetic parameters such as permeation flux, permeability coefficient, and enhancement ratio were derived from this analysis.

Table 6: Permeation Kinetic parameters of ketoprofen loaded niosomal gel

Formulation	Transdermal Flux Jss (µg/cm) 2hr	Lag time (h)	Permeability Coefficient (Kp)	Diffusion Coefficient (D) (cm/h×10-8)	Enhancement Ratio
NFKT	171.28	1.30	1.70×10-5	0.0284	1.352

Skin membrane preparation

The abdominal hair of albino rats (wistar strain), weighing 220 ± 20 gm, was shaved using hand razor. After anesthetizing the rats with ether, the abdominal skin was surgically removed from the animal, and adhering subcutaneous fat was carefully cleaned. To remove extraneous debris and teachable enzymes, the dermal side of the skin was kept in contact with a saline solution for 1 h before starting the permeation experiment.

Pharmacological response

The comparative assessment of anti-inflammatory activity was conducted using the carrageenan-induced rat paw edema method. Albino rats (Wistar strain) of either sex, weighing 220 ± 20 g, were selected for the study. Each group consisted of three animals, chosen to ensure similar average body weights among the groups. Inflammation was induced in the rats by injecting 0.1 ml of 1% w/v carrageenan suspension in saline into the plantar surface of the left hind paw. Thirty minutes later, 0.8 gm of the gel formulation was topically applied to the edematous paw by gently rubbing with an index finger.

The topical activity of the various formulations was evaluated by measuring an increase in hind paw thickness using digital calipers before (Zero time) and 1, 2, 3, 4, and 6 hours after carrageenan administration. The percentage increase in paw thickness from Zero time was calculated and compared with that of animals treated with carrageenan alone, serving as the control group (untreated).

The in vivo anti-inflammatory activity was determined using the carrageenan-induced rat paw edema method, with paw volume measured at 1, 2, 3, 4, 5, and 6 hours, and the results were tabulated in Table 7. The percentage inhibition of edema was calculated for different formulations, and the results were presented in Table 8.

Table 7: Comparative anti-inflammatory activity of optimized niosomal gel formulation containing ketoprofen and plain gel of ketoprofen

Group	Formulation Code	Right hind paw volume (mL)					
		1 h	2h	3h	4h	5h	6h
I	Control	1.70±0.01	1.82±0.00	1.72±0.00	1.66±0.01	1.66±0.00	1.64±0.00
II	PGKT	1.31±0.02	1.22±0.01	1.13±0.02	1.06±0.01	1.00±0.01	0.86±0.02
III	NFKT2	1.20±0.01	0.83±0.01	0.77±0.02	0.73±0.01	0.73±0.01	0.60±0.01
IV	NFET2	1.23±0.02	1.20±0.01	1.03±0.04	1.02±0.01	0.91±0.91	0.77±0.00

*Values are expressed as X (Mean) ±SEM, n=6. (One way ANOVA followed by Dunnett Multiple Comparison Test). Statistically significance *P<0.01 in comparison to control.*

Table 8: Comparative Percentage inhibition of optimized niosomal gel formulation containing ketoprofenand plain niosomes of ketoprofen

Group	Formulation Code	Percentage inhibition of edema at different interval					
		1 h	2h	3h	4h	5h	6h
I	Control	-	-	-	-	-	-
IV	**PGKT**	22.94±1.01	32.96±1.20	34.30±0.91	36.14±0.86	39.75±2.25	47.56±2.15
VII	**NFKT**	54.39±2.56	55.232.67	55.86±3.21	56.02±4.25	56.08±3.25	63.41±4.56

*Values are expressed as X (Mean) ±SEM, n=6. (One way ANOVA followed by Dunnett Multiple Comparison Test). Statistically significance *P<0.01 in comparison to control.*

RESULTS AND DISCUSSION

Screening studies were conducted to identify significant formulations and process variables crucial for quality formulation development. The chosen design minimized the number of runs required for numerous input variables, particularly beneficial for cost-effective experimentation. The resulting mathematical model was statistically significant (p<0.001) for each response variable. Through ANOVA analysis and half-normality plots, the most significant variables were identified and incorporated into the equations. Pareto charts and half-normal plots of mean vesicle size (µm), entrapment efficiency, and 24-hour % drug release were utilized to assess the impact of each factor on these variables. Notable factors such as the molar ratio of surfactant to cholesterol, amount of drug, and total lipid concentration exhibited significant influence on the studied responses, evident from their high magnitude compared to the t-value limit in the charts.

KLNs were prepared using a well-established method. Various process and formulation variables, including the molar ratio of Span 40 to cholesterol, drug amount, and total lipid concentration, were optimized using a 33 Box-Behnken Design to achieve suitable hydrodynamic diameter, PDI, Zeta potential, and maximum drug entrapment. The average mean vesicle size of niosomes was determined using photon correlation spectroscopy, with KLNs demonstrating a mean hydrodynamic diameter of approximately 5µm.

The optimized formulations were assessed by evaluating various response variables using a second-order quadratic polynomial model. Data analysis through ANOVA indicated that the selected quadratic model was highly significant (p<0.0001). The nanocomposite formulations, prepared following the 33 Box-Behnken Design, involved multiple independent and response variables. The polynomial equations generated by the design for the studied response variables—

mean vesicle size (µm), entrapment efficiency, and 24-hour % drug release—are summarized in Table 3.

The R2 values of 0.9750, 0.9533, and 0.9482 for all studied response variables suggest an excellent fit of the generated polynomials to the response data, which is statistically significant (p<0.0001). The combinations of various factor levels from the Box-Behnken Design matrix resulted in KLNs with an average mean vesicle size ranging from 4.08±1.05 to 5.88±0.84 µm. These size variations indicate a strong influence of the selected independent variables on the vesicle size. The quadratic model's significance (p<0.0001) and the predicted R2 value of 0.9832 demonstrate a good correlation between experimental and predicted results. The minimal difference (less than 0.2) between the predicted and adjusted R2 values confirms the suitability of the chosen model for predicting responses. Adequacy precision, with a signal-to-noise ratio greater than 4, further supports the model's effectiveness.

Figure 1 (A) represented the 3-D surface graphs for mean vesicle size. A curvilinear slope was obtained for the value of particle size with increasing the cholesterol and total lipid content. Data represents the effects of individual and combined variables on mean vesicle size of Ketoprofenniosomes. Coefficient values for all the terms except X, having *P* value less than 0.05 indicates their non-significant effect on the MVD of Ketoprofenniosomes. A positive value of *Xi* term indicates that vesicle size increase with increase in the cholesterol content. This may be due to the Cholesterol in a mixed molecular bilayer occupies an alternate position resulting in increased in size. This observation is in agreement with the results reported earlier, who reported that cholesterol increases the width of phospholipid bilayers.

Figure 2 displays 3D surface graphs illustrating the entrapment efficiency. These graphs demonstrate that higher and lower levels of both X1 and X2 independent variables lead to increased entrapment efficiency, indicating an upward trend. The significant F-value (p<0.0001) from the quadratic model indicates its significance, with only a 0.01% chance of F-value variation due to noise. This underscores the quadratic model's utility in correlating entrapment efficiency findings. The higher predicted R2 value aligns reasonably well with the correlation between experimental and predicted results. The minimal difference (less than 0.2) between predicted R2 and adjusted R2 values confirms the model's adequacy for response prediction. Adequacy precision, with a signal-to-noise ratio greater than 4, further supports the model's suitability. Consequently, this model is suitable for navigating the design space.

The contour plot reveals that the percentage drug entrapment (PDE) decreases with an increase in the molar ratio of Span 40 to cholesterol. This observation is consistent with Baillie and Florence's findings, indicating that entrapment efficiency increases with higher concentrations of hydrophobic surfactant, while cholesterol intercalation in the bilayers reduces the entrapment volume and thus the efficiency. The contour plot also indicates that PDE decreases with an increase in the amount of drug, possibly due to excess drug remaining free after the hydrophobic interior of vesicles saturates.

Figure 3 depicted the impact of independent variables on the % release of Ketoprofen from KLNs across various factor level combinations within 24 hours. The 3-D surface graphs indicated that input independent variables had no significant influence on the % release of Ketoprofen from KLNs across different factor level combinations within 24 hours. The rapid release properties of niosomes could be attributed to destabilization. The quadratic model's p-value (p<0.0001) and higher model F-value suggested the model's significance. The minimal difference (≥0.2) between the predicted R2 value and adjusted R2 value indicated the suitability of the chosen model for response prediction.

Numerical optimization was conducted in both formulations using graphs to find the optimal formulation that meets the desired objectives based on response variables. The entire experimental domain was explored using the desirability function, aiming for 'r' values near or equal to 1, signifying the best fit model with a desirable composition. The optimal formulation was selected based on criteria such as minimum mean vesicle size, maximum % entrapment efficiency, and maximum release. These parameters were chosen as checkpoints to validate the Design of Experiments (DoE). Linear correlation plots between actual and predicted response variables showed high r2 values, indicating an excellent fit. Overall, the results clearly demonstrated the accuracy and validity of the proposed model in formulating the polynomial model and predicting response variables. The residual plots displayed uniform, relatively narrow, and randomly scattered data around the zero-axis, indicating well-regulated outcomes.

The ketoprofenniosomal formulations underwent characterization for particle size, shape, entrapment efficiency, and in vitro drug release profile. Optical microscopy analysis (Figure 5) revealed that all batches of ketoprofenniosomes were spherical in shape.

The mean particle size of the ketoprofenniosomal formulation ranged from 4.00 to 7.00 µm. Increasing the cholesterol content led to an increase in the particle size of the niosomal formulations. Cholesterol content enhances the strength of the nonpolar tail of the nonionic surfactant. Lower cholesterol content results in tighter packing of cholesterol and nonionic surfactant, leading to increased curvature and reduced size. On the other hand, higher cholesterol content reduces surfactant content and increases the hydrophobicity of the bilayer membrane, resulting in larger vesicle radius for a more thermodynamically stable structure. The rigid structure of the bilayer membrane due to cholesterol content also resists size reduction during sonication, leading to larger vesicles.

The drug content of the ketoprofen-containing niosomal formulations was determined, with an average of three readings considered. The drug content ranged from 97.67% to 98.89% using the thin film hydration method. The drug content in niosomal formulations of ketoprofen was influenced by varying the amount of surfactant. Increased surfactant content led to higher drug content, likely due to reduced drug leakage, enhancing entrapment efficiency and drug content. Notably, niosomal formulations with Tween showed higher drug content compared to those with spans, attributed to the hydrophilic nature of tweens and their higher HLB value, enabling better entrapment of hydrophobic drugs like ketoprofen compared to spans, thus resulting in higher drug content.

Entrapment efficiency in all niosomal formulations containing ketoprofen was assessed using a centrifugation method at varying ratios of cholesterol and surfactant. Figure 2 data clearly demonstrated an increase in entrapment efficiency with higher cholesterol ratios in the niosomal formulation. This can be attributed to two factors: first, increased cholesterol ratio enhances the hydrophobicity and stability of bilayer vesicles while reducing permeability, leading to more efficient trapping of the hydrophobic drug within the bilayers as vesicles form. Second, a higher cholesterol amount may compete with the drug for packing space within the bilayer, potentially excluding the drug as amphiphiles assemble into the structure.

All niosomal formulations of KT and tweens exhibited higher entrapment efficiency compared to spans. This is because tweens are hydrophilic in nature with a higher HLB value than spans, making them better suited for entrapping hydrophobic drugs compared to spans.

The thin film hydration technique was chosen for preparing niosomes containing ketoprofen due to its simplicity and reproducibility. Percentage drug entrapment (PDE) results were compared among niosomes prepared with different non-ionic surfactants, with Span 40 showing the highest PDE for ketoprofenniosomes. This difference may be attributed to the varying lipophilic/hydrophilic character (log P values) of the drugs and the different HLB values of Span 60 (4.7) and Span 40 (6.7).

Niosomes containing all three drugs exhibited elevated PDE values and effective drug entrapment when the surfactant and cholesterol were maintained at an equal molar ratio. This underscores the importance of an optimal cholesterol level for successful niosome preparation. The PDE value decreased with increasing drug amount during niosome preparation, indicating the need for an optimal drug quantity to achieve maximum PDE within the niosomes. This can be attributed to the drug's entrapment within the niosomes and membrane, depending on its lipophilic/hydrophilic nature, until saturation is reached, beyond which excess drug remains unentrapped.

The variation in PDE within niosomes is attributed to the drug's interaction with the non-ionic surfactant and depends on the drug's lipophilic/water characteristics and the non-ionic surfactant used.

The conventional thin film hydration technique was deemed suitable for laboratory-scale preparation of niosomes containing Span and cholesterol, capable of entrapping amphiphilic drugs like ketoprofen. PDE and vesicle size were influenced by the molar ratio of surfactant to cholesterol, total lipid concentration, drug amount, and hydration volume. Utilizing 32 factorial designs, derived polynomial equations, and two-dimensional contour plots optimized the formulation variables for niosome preparation. Contour plots facilitated the preparation of niosomes with predetermined PDE, reducing the number of experiments and development costs.

Optimized batches of all three drugs exhibited high PDE values, significant drug entrapment, and reduced vesicle size. However, niosomes were found to be unstable under room temperature and refrigerated storage conditions. Various approaches were explored, including the incorporation of negatively charged molecules like dicetyl phosphate and dry granular proniosome formulations, to enhance niosome stability.

The release profile of ketoprofen from the studied gel formulations demonstrates a direct correlation with time, indicating zero-order drug release kinetics in niosome-based gel formulations. This suggests that niosomes function as reservoirs. Comparing the release patterns among different niosomal gels, these formulations exhibit an initial high drug release. This initial burst release can be attributed to the relatively higher permeability of niosomes, especially in batches with lower cholesterol content. The subsequent slow release and lower cumulative release from these gels may be due to the drug's affinity for the lipid environment, which restricts further drug molecule release.

As ketoprofen is an amphiphilic molecule (log P = 0.98), it can reside in both the aqueous core and lipid layer of niosomes. Therefore, the lipid layer of niosomes can act as a barrier to diffusion or release for ketoprofen. The release of ketoprofen is influenced by the lipid concentration and composition of niosomes, leading to variations in bilayer thickness and drug partitioning from the lipid layer to the release medium. Higher lipid concentration in niosomal gel formulations is observed to retard drug release.

In vitro permeation of the drug through rat skin was significantly lower compared to its release through synthetic membrane, indicating the skin's barrier properties. A plain gel was also prepared by directly mixing the drug with carbopol dispersion and evaluated for in vitro permeation. Various mechanisms can explain how niosomes modulate drug transfer across the skin, including adsorption and fusion at the stratum corneum surface, acting as penetration enhancers to reduce barrier properties, and the lipid bilayers of niosomes acting as rate-limiting barriers for drug diffusion. Therefore, understanding the interaction between the skin and vesicles is crucial for enhancing transdermal drug delivery.

The cumulative release of ketoprofen from the investigated gel formulations displays a linear correlation with time, indicating zero-order drug release kinetics in niosome-based gel formulations, suggesting that niosomes function as reservoirs.

The efficacy of the niosome-based gel formulation was assessed using the carrageenan-induced rat paw edema method, comparing it with plain drug and marketed formulations. The increase in paw thickness in rats treated with niosomal gel batches was significantly lower than those treated with plain and marketed gels, as observed at different time intervals (see Table 7). In vitro skin permeation studies of the gel formulations yielded similar results to the in vitro release studies. Moreover, the in vivo studies showed a significant reduction in paw thickness increase after carrageenan injection with the gel formulation compared to plain niosomes. When comparing different niosome-based gel formulations in vivo, the group treated with niosomal gel containing KT exhibited the greatest reduction in paw thickness across all time points.

Evaluation of *in vitro* permeation and *in vivo* reduction in paw thickness of ketoprofen from niosome-based gel formulations with varying contents of non-ionic surfactants and drug types was conducted. The drugs in the niosome-based gel formulations were entrapped within lipid bilayers and the aqueous core. Experimental and theoretical analyses support the idea that direct drug transfer from vesicles to the skin occurs only when the drug is intercalated within the bilayers. Two types of interactions between the skin and niosomes may enhance penetration in niosome-based gel formulations: adsorption and fusion of drug-entrapped niosomes on the skin's surface leading to a high thermodynamic activity gradient at the drug-stratum corneum interface, and the effect of niosomes on the stratum corneum causing changes in drug permeation kinetics due to impaired barrier function. These studies demonstrate that niosome-based gel formulations containing NSAIDs significantly enhance drug penetration compared to plain gel formulations. Niosome-based gel formulations show promise for topical NSAID delivery, offering enhanced permeation, stability, high entrapment efficiency, and scalability due to their simple process. Clinical studies will further validate their efficiency in NSAID delivery.

Conclusion

These studies clearly demonstrate that niosome-based gel formulations containing NSAIDs significantly improve drug penetration compared to plain gel formulations. Niosome-based gel formulations have exhibited substantial potential for NSAIDs in topical applications. In addition to enhancing the permeation of NSAIDs, this proposed system offers advantages such as high entrapment efficiency and scalability due to its straightforward process. Therefore, niosome-based gel formulations hold promise as effective vehicles for delivering NSAIDs via the topical route. Further validation through clinical studies will confirm their efficiency in NSAID delivery.

Conflict of Interest

None

References

Bangham, A. D., Standish, M. M., and Watkins, J. C. (1965). Diffusion of univalent ions across the lamellae of swollen phospholipids. Journal of molecular biology *13*, 238-IN227.

Bindu, S., Mazumder, S., and Bandyopadhyay, U. (2020). Non-steroidal anti-inflammatory drugs (NSAIDs) and organ damage: A current perspective. Biochemical pharmacology *180*, 114147.

Ge, X., Wei, M., He, S., and Yuan, W.-E. (2019). Advances of non-ionic surfactant vesicles (niosomes) and their application in drug delivery. Pharmaceutics *11*, 55.

Jain, S., Jain, V., and Mahajan, S. (2014). Lipid based vesicular drug delivery systems. Advances in Pharmaceutics *2014*, 1-12.

Janssen, N. M., and Genta, M. S. (2000). The effects of immunosuppressive and anti-inflammatory medications on fertility, pregnancy, and lactation. Archives of internal medicine *160*, 610-619.

Jorge, L. L., Feres, C. C., and Teles, V. E. (2010). Topical preparations for pain relief: efficacy and patient adherence. Journal of pain research, 11-24.

Kamboj, S., Saini, V., and Bala, S. (2014). Formulation and characterization of drug loaded nonionic surfactant vesicles (niosomes) for oral bioavailability enhancement. The scientific world journal *2014*.

Khatoon, M., Shah, K. U., Din, F. U., Shah, S. U., Rehman, A. U., Dilawar, N., and Khan, A. N. (2017). Proniosomes derived niosomes: recent advancements in drug delivery and targeting. Drug delivery *24*, 56-69.

Kulkarni, S., Betageri, G., and Singh, M. (1995). Factors affecting microencapsulation of drugs in liposomes. Journal of microencapsulation *12*, 229-246.

McGettigan, P., and Henry, D. (2013). Use of non-steroidal anti-inflammatory drugs that elevate cardiovascular risk: an examination of sales and essential medicines lists in low-, middle-, and high-income countries. PLoS medicine *10*, e1001388.

New, R. R. (1990). Liposomes: IRL at Oxford University Press).

Ravalika, V., and Sailaja, A. K. (2017). Formulation and evaluation of etoricoxib niosomes by thin film hydration technique and ether injection method. Nano Biomedicine and Engineering *9*, 242-248.

Yadav, N., Nanda, S., Sharma, G., and Katare, O. (2017). Systematically optimized ketoprofen-loaded novel proniosomal formulation for periodontitis: in vitro characterization and in vivo pharmacodynamic evaluation. AAPS PharmSciTech *18*, 1863-1880.

Yoshioka, T., Sternberg, B., and Florence, A. T. (1994). Preparation and properties of vesicles (niosomes) of sorbitan monoesters (Span 20, 40, 60 and 80) and a sorbitan triester (Span 85). International journal of pharmaceutics *105*, 1-6.