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Research Article

Permeability Enhancement Approach for Dexamethasone using Niosomal Gel for Treating Keloids

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ABSTRACT

Background: Niosome are surfactant based vesicular drug delivery system that improves the residence time of drugs by overcoming the inherent anatomical barrier. Dexamethasone is used as an intralesional injection for treating keloids, which is formulated as niosomal gel for the inhibition of keloid fibroblast. This study aimed to formulate the Dexamethasone niosome suspension and the suspension was incorporated into the gel. Niosomal suspension of Dexamethasone was formulated by thin-film hydration method using Tween 20, Span 60, and Tween 80. Tween 80 niosome formulation was optimized using Design of Expert software (DOE). Optimized niosome were characterized for particle size, zeta potential, Atomic Force Microscopy (AFM), entrapment efficiency, *in vitro* release, cell proliferation studies, and cell permeation studies. The optimized tween 80 formulation was incorporated into the carbopol gel base and evaluated.

Results: The desirable quantity of tween 80 was 86.2 µM and cholesterol was 38.9 µM. Spherical shaped vesicle with particle size of tween 80 suspension was 498.1 ± 1.1710 nm, entrapment efficiency was 85.2% ± 2.851, and *in vitro* release was 96.5% ± 2.88 at the end of 6 hr. Cell proliferation studies were performed to confirm the anti-keloid activities using MTT assay which decreased the cell viability in the range between 49% to 58%. Cell permeability studies were performed using Caco-2 cells showed a low Trans Epithelial Electrical Resistance (TEER) value after 2 hr treatment indicating more permeation than the drug solution.

Conclusion: The results confirm niosomal gel with Tween 80 enhances the skin permeation of Dexamethasone.

Keywords: Transdermal drug delivery; Dexamethasone; Keloids; Niosome; Gel

Abbreviations: FT-IR: Fourier Transform Infrared Spectrophotometer; HLB: Hydrophilic and lipophilic balance; PDI: Polydisperse Index; PS: Pascals

BACKGROUND

A fibro-proliferative disease is a keloid. It is the creation of a scar that is mostly made up of collagen. It is an extravascular tissue expansion [1]. Keloids are solid, rubbery, or glossy lesions that can range from pink to purple and cause itching and pain. Eyelids, palms, cornea, mucous membrane, genitalia, and soles are less impacted than the anterior chest, earlobes, shoulders, upper arm, and cheeks. People with dark skin are adversely affected [2]. Injury, surgery, immunization, skin piercing, acne, and other factors are common causes of keloids [3].

Keloids are difficult to treat and remain a therapeutic challenge for all researchers. There has been no proof or commonly accepted treatment. Excision surgery, cryotherapy, radiation, laser therapy, pressure therapy, and silicone gel sheeting are some of the current treatments for keloids. Therapeutically active drugs used to treat keloids are intralesional injection, imiquimod cream, interferon injection, verapamil, 5-flurouracil, and bleomycin [4].

Frequent injection of steroids leads to inconvenience, pain, and side effects to patients [5]. As a result, transdermal drug delivery is essential for more effective keloid therapies.

Dermatological formulations applied topically have greater bioavailability, targeted local administration, low systemic uptake, increased patient compliance, and so on. Topical formulations have a higher efficacy and safety profile than systemic administration. Superficial Stratum Corneum (SC) or below the epidermis is the target site for the topical application which may be depends on the

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type of condition [6].

Niosomes are vesicular drug delivery system, which can be used for the targeted, controlled, transdermal as well as sustained delivery of drugs [7]. Niosomes are made up of non-ionic surfactants they can be unilamellar, oligolamellar, or multilamellar. Niosomes are in a size range between 10 to 1000 nm [8]. Along with non-ionic surfactants, it may contain cholesterol or soya phosphatidylcholine and charged molecules like cetyl pyridinium chloride, diacetyl phosphate, etc. Cholesterol offers rigidity to the niosome structure and the charged molecule keeps the preparation stable [9]. The niosomes are amphiphilic, which allows entrapment of both hydrophilic and lipophilic drugs.

Niosomes can improve drug penetration, form drug depots, and release drugs in a controlled or sustained manner. Niosomes are to enhance dermal drug absorption by two mechanisms. A) Niosomes reduce Tran's epidermal water loss by altering the properties of the stratum corneum, which leads to increases in the stratum corneum hydration. B) Nano size of the niosomes improves drug transfer across the stratum corneum [10].

Niosome gel has an advantage over other semisolid dosage forms because it has a well-behaved rheological behaviour, a long residence period in the skin, and a higher drug concentration in the skin [11]. The present investigation was undertaken to increase the topical permeability of Dexamethasone for anti-keloid treatment through a niosomal gel.

MATERIALS AND METHODS

Dexamethasone was obtained as a gift sample from Fourt's India Lab. Pvt. Ltd, Chennai. Cholesterol, span 60, tween 20, tween 80, carbopol, methanol, and chloroform were obtained from Loba Chemie Pvt Ltd, Mumbai, India. Triethanolamine was obtained from Rankem Pvt Ltd, New Delhi, India.

Pre-formulation studies of dexamethasone

The melting point of Dexamethasone was determined by capillary tube method using melting apparatus. The lambda max of Dexamethasone in phosphate buffer pH 7.4 with the concentration of 1 µg/ml was scanned between 200-400 nm by UV-Visible spectrophotometer.

FT-IR drug-excipients compatibility study

The KBr pellet method was used to record the infrared spectra of the drug (Dexamethasone) with excipients (tween 80 and cholesterol) using a fourier transform infrared spectrophotometer (Jasco, Japan). Following a baseline correction with dried KBr, spectra of dried drug-excipient mixtures were recorded. The samples were compressed at a rate of 6 ton/nm^2 to make KBr pellets. The wavelength ranges were selected between 500-4000 cm^{-1}.

Preliminary studies for the dexamethasone noisome

Dexamethasone niosome was formulated by a thin-film hydration method using a rotary evaporator (Superfit, Rotavap, and Mumbai). Different concentrations of cholesterol (15 µM, 30 µM, 50 µM) and non-ionic surfactants (Span 60, Tween 20, and Tween 80) were used in this preparation (Table 1). Non-ionic surfactant and cholesterol were dissolved in a mixture of 10 ml of chloroform and 10 ml of methanol in the molar ratio of 1:1. Chloroform and methanol were evaporated under reduced pressure at 60°C for 2 hrs using a rotary evaporator. A thin film was hydrated using phosphate buffer pH 7.4 of 30 ml containing 12 mg of Dexamethasone. The formed niosome was subjected to sonication for 10 min using a bath sonicator (PCI Mumbai). The particle size, entrapment efficiency, and *in vitro* release of the Dexamethasone niosome were all measured.

Table 1: Quantities of surfactant and cholesterol used in the pre-formulation studies of niosome formulations.

Formulation code	Amount of surfactant (µM)		Amount of cholesterol (µM)	Amount of chloroform (ml)	Amount of methanol (ml)
F1	Span 60	100	15	10	10
F2		100	30		
F3		100	50		
F4	Tween 20	100	15	10	10
F5		100	30		
F6		100	50		
F7	Tween 80	100	15	10	10
F8		100	30		
F9		100	50		

Note: µM is micromole, ml is a milliliter.

Screening studies

For statistical optimization of Dexamethasone-loaded niosome, a 22 factorial design was utilized (Table 2). For the development of the mathematical relationship between dependent and independent variables, Design-Expert® software (Version 13, Stat-Ease) was used. Tween 80 was used to formulate several formulations of Dexamethasone niosome based on the runs. As a result, four experiments were conducted for the 22 factor design. Based on the preliminary tests, the amounts of tween 80 (X1) and cholesterol (X2) were chosen as independent variables that were varied at two levels (low and high). The dependent variables are particle size (Y1), entrapment efficiency (Y2), and drug release (Y3). The resulting data were entered into the software and analyzed.

Table 2: Factorial design screening parameter.

Factors (Independent variable)	Levels	
	Low	High
X1: Tween 80	50 µM	100 µM
X2: Cholesterol	30 µM	50 µM
FigRuns (TF)	**Tween 80(µm)**	**Cholesterol(µm)**
TF1	100 µM	30 µM
TF2	100 µM	50 µM
TF3	50 µM	30 µM
TF4	50 µM	50 µM
Response (Dependent variable)	**Desirability characteristics**	
Y1: Particle size	Minimize	
Y2: Entrapment efficiency	Maximize	
Y3: Drug release	Maximize	

Note: µM is micromole, TF- Tween formulation

Preparation of optimized dexamethasone noisome

Tween 80 (38.9 µM) and cholesterol (86.2 µM) were dissolved in a mixture of 10 ml of chloroform and 10 ml of methanol. Chloroform and methanol were evaporated under reduced pressure at 60°C for 2 hrs using a rotary evaporator. A thin film

was hydrated using phosphate buffer pH 7.4 of 30 ml containing 120 mg of Dexamethasone. The formed niosome was subjected to sonication for 10 mins. The optimized Dexamethasone niosome were characterized for particle size, zeta potential, Atomic Force Microscopy (AFM), entrapment efficiency, *in vitro* release, cell proliferation studies, and cell permeation studies.

Characterization of noisome

Analysis of particle size and zeta potential: A zeta sizer (Nano ZS90) were used to determine the particle size and zeta potential of Dexamethasone niosome at room temperature. After being diluted with distilled water, the samples were kept in a polystyrene cuvette and the measurements were computed.

Surface morphology: Atomic Force Microscopy (AFM) was used to examine the surface morphology, roughness, and diameter of the optimized Dexamethasone niosome. The sample was placed onto a slide and air-dried for 24 hrs at room temperature, and then AFM was imaged.

Determination of entrapment efficiency: Ultracentrifugation technique was used to determine the entrapment efficiency of the prepared niosome. Accurately 1 ml of the formulation was disrupted using 0.1 ml of 0.1% Triton X-100 in 2 ml of phosphate buffer pH 7.4 [12]. The solution was centrifuged at a speed of 3000 rpm for 20 min. The supernatant was collected and the amount of Dexamethasone was determined by UV-Visible spectroscopy at 241 nm. Entrapment efficiency was calculated by:

$$Entrapment\ efficiency = \frac{Total\ amount\ of\ drug - Amount\ of\ drug\ unentrapped}{Total\ amount\ of\ drug} \times 100$$

***In vitro* release studies:** *In vitro* release studies of niosomal formulation and the drug solution were performed by dialysis bag method using phosphate buffer pH 7.4. Accurately 1 ml of niosomal suspension was taken in a dialysis bag and placed in a 250 ml beaker containing 100 ml of phosphate buffer solution pH 7.4. At 40 rpm the solution was magnetically agitated at 37°C. Dialysate aliquots of 5 ml were taken until 6 hrs and the sink conditions were maintained by replacing the same volume of buffer. The samples were analyzed using UV spectroscopy at 241 nm [12]. The same procedure was carried out for the drug solution.

Cell proliferation studies: The cell culture (3T3L1) was centrifuged, and using DMEM (Dulbecco's Modified Eagle's Medium) containing 10% Fetal bovine serum, the cell count was adjusted to 1.0×10^5 cells/ml. 100 µL of the diluted cell suspension (approximately 10,000 cells/well) was added to each well of a 96 well flat bottom microtitre plate. After 24 hrs, when the cell population was found adequate, the cells were centrifuged, and the pellets were suspended with 100 µL of Dexamethasone niosome (12.5, 25, 50, and 100 µg/ml) and the drug solution (12.5, 25, 50, and 100 µg/ml) prepared in maintenance media. The plates were then incubated at 37°C for 48 hrs in a 5% CO_2 environment, with microscopic examination and observations recorded every 24 hrs. After 48 hrs, 20 µL of MTT (2 mg/ml) in MEM-PR (MEM without Phenol Red) was added. The plates were gently shaken and incubated for 2 hrs at 37°C. The 100 µL of DMSO (Dimethyl sulfoxide) was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a micro plate reader at a wavelength of 540 nm after 3 days [13].

Cell permeation studies: The effect of permeation of dexamethasone niosome and the drug solution were studied on the Caco-2 cell monolayer. Caco-2 cells are widely used as an *in vitro* model for predicting human drug absorption and prediction of intestinal permeability [14]. Caco-2 cells are generated from a human colorectal cancer and spontaneously differentiate into polarized entrecote monolayers when grown. Before the experiment, the cells were seeded on multiwall-insert plates and allowed to form a confluent monolayer for a period of 20 days. On day 20, the test compound (Dexamethasone niosome-50 µg/ml, 100 µg/ml and the drug solution-50 µg/ml,100 µg/ml) were added to the apical side of the membrane and the permeability coefficient (Papp) of the compound across the monolayer is monitored over 2 hr. The barrier integrity of the Caco-2 cell was ensured by TEER (Transepithelial electrical resistance) measurement using EVOM (Epithelial volt-ohm meter) machine using the following equation:

$$R_{Tissue}(\Omega) = R_{Total} - R_{Blank}$$

$$TERR = R_{Tissue}(\Omega) \times M_{Area}(cm^2)$$

Loading of optimized dexamethasone niosomal suspension into a gel

The gels (1%) was prepared by dissolving 1000 mg of carbopol 934 in 88 ml of distilled water, then add 5 ml of glycerol and stir the mixture with a magnetic stirrer until thickening occurred. The suspension was added to the gel base at a concentration of 4 mg/gm, and then neutralized with triethanolamine until a translucent gel appeared [15].

Evaluation of gel

Physical appearance: The physical appearance of the gel was observed by visual inspection.

Viscosity: Brookfield Viscometer (MIDDLEBORO, MA 02346, U.S.A) was used to determine the viscosity of the niosomal gel. Accurately 50 gm of gel was taken into a beaker and the spindle was dipped into the gel, viscosity of the gel was measured by rotating the spindle (s64) at 5 rpm [16].

pH: A digital meter was used to measure the pH of the gel. About 1 gm of the gel was dispersed in 25 ml of distilled water and set aside for 1 hr. In a beaker, the electrode of a pH meter (SYSTRONICS pH system 361, Ahmedabad) was placed and measured.

Spreadability and extrudability: The spreadability and extrudability of the gel were analyzed by using Texture Analyzer (TAXT.Plus, Stable Microsystems, and U.K).

***Ex vivo* permeation studies:** The skin of a goat's ear was obtained from a slaughterhouse [17]. The extra hair was trimmed using scissors and washed with cold water. The skin was then soaked in saline phosphate buffer pH 7.4 for 12-18 hrs. The prepared goat ear skin was tied to one end of an open-ended glass cylinder with a diameter of 1.25 cm so that the stratum corneum of the skin would place above the saline buffer. The skin was applied with a 4 mg equivalent amount of the Dexamethasone niosome. The cylinder was then placed in a 250 ml beaker containing 100 ml of saline phosphate buffer pH 7.4, which would serve as a receptor compartment. At 37°C, the medium was agitated using a magnetic stirrer at 40 rpm. 5 ml of aliquots were taken until 6 hrs and the sink conditions were maintained by replacing the same volume of buffer. The withdrawn sample was analyzed by UV spectroscopy at 241 nm. The same procedure was carried out for the drug solution. Steady-state flux (Jss) was calculated from the slope of the linear regression line. The apparent permeability coefficient (Papp) was

calculated according to the following equation:

$$P_{app} = J_{SS} / C_0$$

Where Co is the initial drug concentration.

Drug release kinetics

The zero order, first order, higuchi, and korsmeyer-Peppas equation models were used to analyze *in vitro* drug release data using the DD solver excel programme.

Stability studies

According to ICH guidelines, the stability studies for niosomal formulation were performed. The formulation was kept for 3 months at room (ambient) temperature (25°C-27°C) and in the refrigerator (3°C-5°C). The appearance, particle size, and zeta potential were examined.

Statistical analysis of data

The particle size, entrapment efficiency, and *in vitro* release data were statistically analyzed by ANOVA (one-way analysis of variance) using GraphPad Prism 9 software (Inc, San Diego, CA, USA). The value were considered to be significant if the P value <0.05.

RESULTS

Pre-formulation studies

The melting point of Dexamethasone was found to be 263°C-283°C. The concentration (1 µg/ml) was chosen for the determination of max which shows the maximum absorption at 241 nm.

FT-IR drug-excipients compatibility study

FT-IR spectra of drug-excipients mixture retained the characteristic functional peaks of the drug and it ensures that there were no interactions between the Dexamethasone and excipients.

Preliminary studies

The result of preliminary studies of Dexamethasone using different concentrations of cholesterol (15 µM, 30 µM, 50 µM) and non-ionic surfactants (Span 60, Tween 20, and Tween 80) were shown in Figure 1 and Table 3.

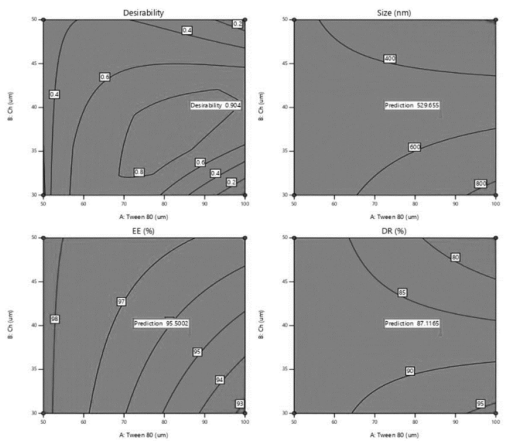

Figure 1: Desirability of optimized niosome using tween 80 and cholesterol.

Table 3: Particle size, entrapment efficiency, and *in vitro* release of preliminary niosome formulations.

Formulations	Particle size (nm)	PDI	Entrapment efficiency (%)	*In vitro* release (%)
F1	190 ± 0.12	0.502 ± 0.56	96.75 ± 1.45	36.25 ± 0.98 at 3 hr 30 mins
F2	621 ± 0.23	0.4 ± 0.39	98 ± 0.6	93.75 ± 1.96 at 2 hr 30 mins
F3	609 ± 1.12	0.6 ± 1.49	98.7 ± 1.45	33.75 ± 2.39 at 2 hrs
F4	236.8 ± 0.85	0.302 ± 1.02	92.75 ± 1.08	72.3 ± 1.78 at 2 hrs
F5	261 ± 0.24	0.313 ± 0.85	97 ± 1.28	90 ± 0.94 at 3 hr 30 mins
F6	539.3 ± 0.96	0.961 ± 1.04	96.75 ± 1.85	53.75 ± 2.45 at 2 hr 30 mins
F7	171.9 ± 1.39	0.459 ± 0.96	79.5 ± 0.78	95 ± 1.56 at 3 hrs
F8	853 ± 2.45	0.962 ± 1.28	92.75 ± 2.45	96.25 ± 1.96 at 4 hr 30 mins
F9	187.9 ± 1.28	0.43 ± 0.96	96.62 ± 1.12	75 ± 2.45 at 3 hrs

Note: nm is nanometer, hr is an hour, min is minute. Results are shown as mean ± SD(n=3)

Screening studies

Tween 80 was selected as a non-ionic surfactant for the screening studies due to the lesser particle size and higher *in vitro* release. Tween 80 has played a significant role in particle size with 171 nm to 853 nm, entrapment efficiency with 79% to 96%, and *in vitro* release of 75% to 96%.

The evaluation of different concentrations of cholesterol and tween 80 were subjected for screening studies (Table 4). Whole experimental data were fitted into 22 factorial designs and the results of multiple linear regression analysis and its effect were interpreted.

Table 4: Evaluation of screening studies using tween 80 as surfactant.

Runs	Particle size (nm)	PDI	Entrapment efficiency (%)	*In vitro* release (%)
TF1(100:30)	853 ± 0.623	0.862 ± 0.010	92.75 ± 1.60	96.25 ± 1.59
TF2(100:50)	187 ± 0.484	0.437 ± 0.015	96.62 ± 1.06	75 ± 0.65
TF3(50:30)	480.9 ± 0.951	0.836 ± 0.013	98.25 ± 2.34	87.5 ± 1.94
TF4(50:50)	430.9 ± 0.585	0.898 ± 0.012	98.15 ± 1.15	88.75 ± 1.23

Note: nm is a nanometer. Results are shown as mean ± SD(n=3)

Evaluation of optimized noisome

The optimized Dexamethasone niosome was formulated using a thin-film hydration method with particle size of 498.1 ± 1.710 nm and PDI -0.803 ± 0.0101. The zeta potential was used to measure the stability of the niosome. Optimized niosome showed negative values -5 mV is considered to be stable to refuse the aggregation of niosome and fusion. Atomic force microscopy was used to examine the surface morphology of optimized Dexamethasone niosome and it was in spherical shape (Figure 2). The entrapment efficiency was measured using ultracentrifuge and it was found to be 85.2% ± 2.851 and *in vitro* drug release was carried out by dialysis bag method and the percentage drug release was calculated. As compared to drug solution, niosome showed more release at 6 hrs with 96.5%. The release was shown in Figure 3.

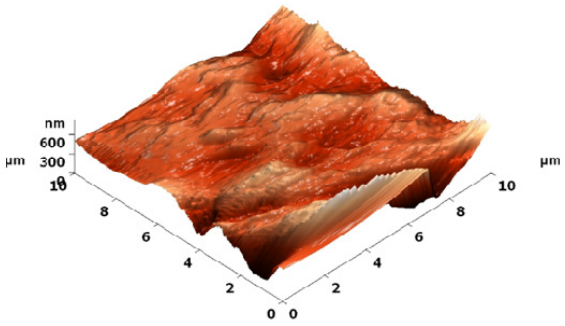

Figure 2: Atomic force microscopy.

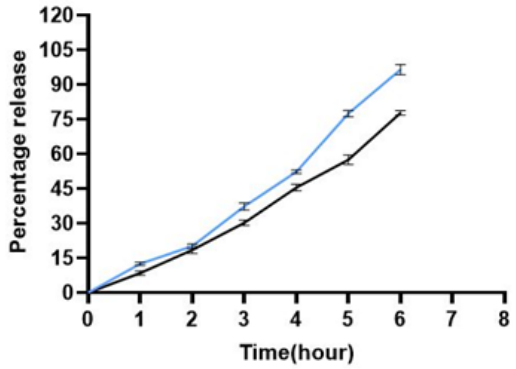

Figure 3: *In vitro* release profile of niosome and the drug solution. Note: (—) Niosome, (—) Drug solution

Cell proliferation studies: The cell proliferation was studied using MTT assay by treating Dexamethasone niosome and the drug solution in 3T3L1 cell line. The Dexamethasone niosome decreased the fibroblast viability in the range between 49%-58%, IC50 was 23.361 μg/ml and the drug solution was found in the range between 54%-63%, IC50 was 30.742 μg/ml respectively after 3 days (Figure 4).

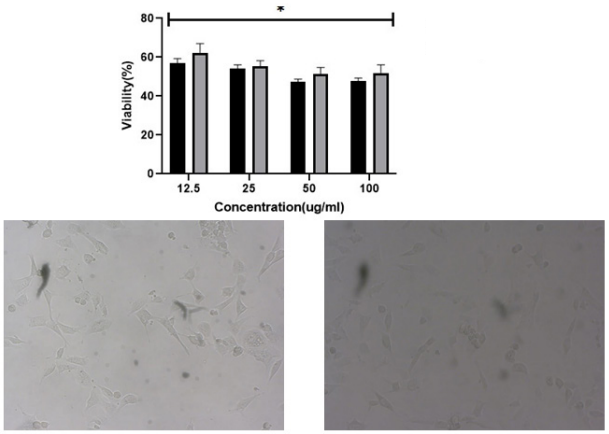

Figure 4: Effects of niosome and the drug solution on the proliferation of the keloid fibroblast after 3 days. **Note:** (▬)Dexamethasone niosome, (▬) Drug solution.

Cell permeation studies: The result of cell permeation studies shows that the niosome affects the paracellular permeability of Caco-2 cells by lowering the TEER values with the opening of tight junctions. It shows the TEER value induced by 2 hrs of niosome at the concentration of 50 μM and 100 μM and the drug solution 50 μM and 100 μM (Figure 5).

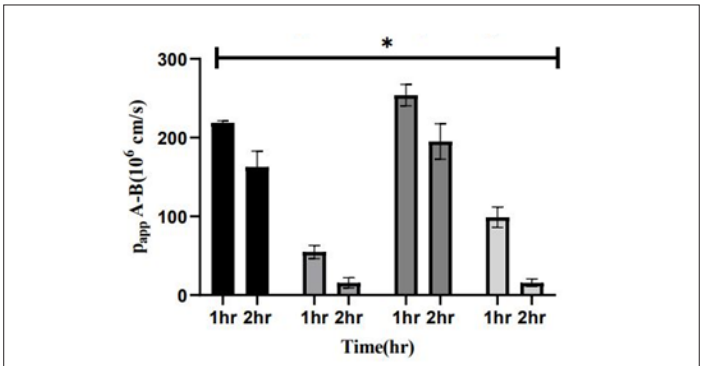

Figure 5: Cell permeation studies of niosome and the drug solution. **Note:** (▬)Nio(50), (▬)Nio(100), (▬)Soln(50), (▬)Soln(100).

Evaluation of the gel

Dexamethasone niosomal gel and the drug solution gel was formulated using Carbopol 940(1%) gel base. The gel was white with desired consistency, homogeneity, and free from grittiness. The pH was found to 5.5 ± 0.277 and the viscosity was found to be 113 × 103 ps ± 1.12. The spreadability of the gel formulation was determined as 504.4 gm.sec. The extrudability of the gel was 2927.14 gm.

A skin permeation study was performed using the goat ear skin on an open-end cylinder set up with Dexamethasone niosomal gel and the drug solution gel and estimated the permeation of the drug in the skin for 6 hrs. The result shown in Figure 6 and Table 5.

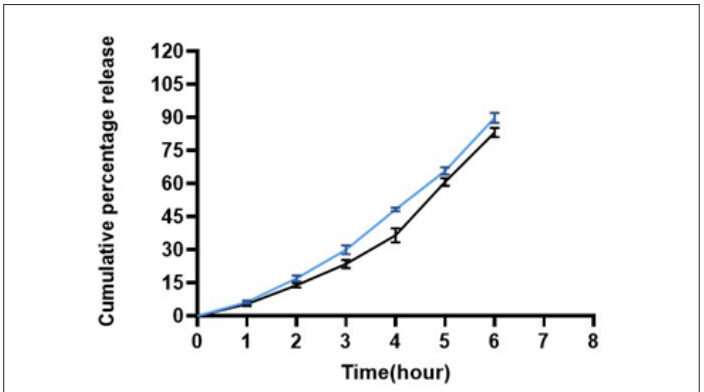

Figure 6: *Drug Release profile of niosomal gel and the drug solution gel.* Note: (—) Niosome, (—) Drug solution

Table 5: Flux and Permeability coefficient of the gel.

Formulations	Flux (mg/cm²/hr) (mean ± SD)	Permeability coefficient (cm/hr) (mean ± SD)
Dexamethasone niosomal gel	16.44 ± 0.969	4.111 ± 0.242
Drug solution gel	15.486 ± 0.085	3.871 ± 0.537

Note: mg is milligram, cm is centimeter, hr is an hour. Results are shown as mean ± SD(n=3)

Drug release kinetics

The obtained cumulative percentage release of dexamethasone niosomal gel and the drug solution gel data were fitted into kinetic models to detect the mechanism of release from the gel. The obtained results were shown in Table 6.

Table 6: Drug release kinetics of Dexamethasone niosomal gel and the drug solution gel.

Formulations	Zero -order(r^2)	First-order(r^2)	Higuchi(r^2)	Korsmeyer-peppas(n)
Dexamethasone niosomal gel	0.9302	0.7967	0.6466	1.525
Drug solution gel	0.8817	0.7606	0.5908	1.789

Stability studies

Stability studies were performed at room (ambient) temperature (25°C-27°C) and in a refrigerator (3°C-5°C) over a period for 3 months. The appearance, particle size, and zeta potential were evaluated every month (Table 7).

Table 7: Stability data of Dexamethasone noisome.

Month		Appearance	Particle size (nm)	PDI	Zeta potential (mV)
0 Month	-	Clear liquid	140.57 ± 0.08	0.266 ± 0.4	-5.42 ± 0.96
1 Month	Room temperature	Clear liquid	188.8 ± 1.23	0.530 ± 0.9	-8.7 ± 1.02
	Refrigerator	Clear liquid	157.6 ± 0.56	0.343 ± 0.23	-10.3 ± 0.96
2 Month	Room temperature	Clear liquid	242.1 ± 1.56	0.662 ± 1.2	-12.4 ± 1.9
	Refrigerator	Clear liquid	171.85 ± 0.7	0.387 ± 0.3	-14.1 ± 0.76
3 Month	Room temperature	Viscous	372.4 ± 2.39	0.779 ± 1.17	-20.3 ± 2.49
	Refrigerator	Clear liquid	201.5 ± 1.39	0.582 ± 1.08	-17.2 ± 0.9

Note: nm is nanometer, mV is millivolt. Results are shown as mean ± SD(n=3)

DISCUSSION

The melting point of Dexamethasone was found to be 263°C-283°C. FT-IR studies showed that the Dexamethasone shows intense bands at 3390-1268 cm^{-1} was due to stretching vibration of O-H and C-F. The bands at 1693 cm^{-1} and 1607 cm^{-1} was due to C=O bands [18]. Tween 80 shows intense bands at 2799 cm^{-1} was due to CH_2 and 1693 cm^{-1} was due to C=O. Cholesterol shows intense bands at 3412 cm^{-1} was due to O-H and 2932 cm^{-1} was due to CH_2.

Preliminary studies resulted that the particle size of the prepared Dexamethasone niosome was in the range of 187 nm to 853 nm. The particle size of the prepared niosome using span 60 was in the range of 190 nm to 621 nm which was larger than reported by Mavaddati et al., who prepared the same drug with the different molar ratio (100:50, 125:25, 75:75) of cholesterol and span 60. The particle size of the formulations was dependent on cholesterol. At small concentrations, cholesterol leads to close packing of surfactant monomers with reducing size. Rising cholesterol concentration reduces non-ionic surfactant content and increases the hydrophobicity of the bilayer membrane. This may disrupt the vesicular membrane [12]. The entrapment efficiency was carried out to find out whether the drug is entrapped in the niosome. The lower entrapment efficiency of Dexamethasone was found in formulation F7 (100 µM of Tween 80 and 15 µM of Cholesterol) and the highest entrapment efficiency of Dexamethasone was found in formulation F3 (100 µM of Span 60 and 50 µM of Cholesterol). Increasing cholesterol concentration increases the entrapment efficiency of Dexamethasone due to two different factors: (1) when cholesterol levels rise, bilayer hydrophobicity and stability improve, but permeability decreases, allowing lipophilic drugs to be effectively entrapped. (2) However, increased levels of cholesterol may compete with the drug for packing space within the bilayer, causing the drug to be excluded as the amphiphiles form vesicles. Cholesterol and non-ionic surfactants were investigated for their effects on the permeability and stiffness of the niosomal membrane. Exceeding a certain level of cholesterol (75 µM) can cause the niosomal membrane's linear form to be disrupted. Increasing the molar ratio of cholesterol from 15 µM to 30 µM increased the release of Dexamethasone. Cholesterol and tween 80 in the molar ratio (30 µM:100 µM) inhibited the formation of transient hydrophilic holes, which are responsible for drug release, by decreasing membrane fluidity [19]. The formulation F8 (100 µM of Tween 80 and 30 µM of Cholesterol) showed a maximum *in vitro* drug release of 96.25% at the end of 4 hr 30 mins. The formulation F3 (100 µM of Span 60 and 50 µM of Cholesterol) showed a minimum *in vitro* drug release of 33.75% at the end of 2 hrs. Tween 80 showed good release than span 60 and tween 20 formulations. The difference in the release rate may be due to the difference in the lipophilicity of surfactants. Tween 80 being hydrophilic allows easy penetration of drug to the aqueous medium.

Screening studies using DOE software interpreted as

The polynomial equation of particle size was

$$Y1(PS) = +489 + 31X1 - 180.50X2 - 152.50X1X2$$

Co-efficient X1 has a positive sign, indicating that the increasing tween 80 concentration causes particle size to rise, whereas co-efficient X2 has a negative sign, indicating that the increasing cholesterol concentration causes particle size to decrease.

The polynomial equation of entrapment efficiency was

$$Y2(EE) = +96.44 - 1.76X1 + 0.9425X2 + 0.99X1X2$$

Co-efficient X1 has a negative sign, indicating that the increasing tween 80 concentration leads to a decrease in entrapment efficiency, while X2 has a positive sign indicating that the increasing cholesterol concentration leads to an increase in entrapment efficiency.

The polynomial equation of drug release was

$$Y3(DR) = +86.88 - 1.25X1 - 5.0X2 - 5.63X1X2$$

Both co-efficient X1 and X2 having a negative sign indicates increasing the concentration of either tween 80 or cholesterol leads to a decrease in the release of the drug.

Pearson correlation coefficient for TF1, TF2, TF3, and TF4 formulations of *in vitro* release were determined by using SPSS software (22.0 version), which shows the positive correlation indicating an increase in time, increase the amount of drug release. TF3 formulation shows high positive correlation with r value=0.997 when compared to other formulations.

Following the application of desirability constraints on particle size, entrapment efficiency, and *in vitro* release, Design-Expert® software performed a numerical optimization study utilizing the desirability function to generate the optimized niosome formulation with the desired results (Figure 1). The overall desirability of the optimized niosome formulation was 0.904, and it was proposed that tween 80 of 38.9 µM (X1) and cholesterol of 86.2 µM (X2) be used to prepare the niosome formulation.

The results of the optimized niosome show that the average height of the niosome was 480.7 nm and the average diameter was 417.5 nm. There was no much difference in the height and diameter of the niosome which confirms that the niosome is spherical. The root mean square and average roughness of the niosome was 82.48 nm and 57.66 nm shows that there was a roughness in the niosome surface. In the histogram, the surface skewness has a negative value indicating that the niosome are planar and the kurtosis moments are more than 3 suggesting that the niosome have sharper and higher peaks [20].The entrapment efficiency was found to be 85.2% ± 2.851 which shows more entrapment compared with Yaghoobian et al., formulated repaglinide niosome using tween 80 resulted as 71.5% ± 4.1 [21]. Li et al. Fabricated the electrospun ultrafine fiber of Dexamethasone and green tea polyphenols for treating keloid there *in vitro* drug release was 40% to 65%, while our study has shown more release 96.5% [22]. The release was shown in Figure 3. The student T-test revealed a statistically significant difference (p=0.0278) in the *in vitro* release of Dexamethasone niosome and the drug solution.

Cell proliferation studies results that both Dexamethasone niosome and the drug solution inhibited fibroblast proliferation. The decreased viability of the niosome may be the presence of non-ionic surfactant which permeates more into the cell and cause decreased proliferation. The Student T-test was used to compare the two formulations (Dexamethasone niosome and the drug solution), and the difference was found to be statistically significant with a p-value of 0.0242.

The results of cell permeation studies prove that the Dexamethasone niosome shows one-fold increased permeation than the drug solution. This seems that tween 80 affect the tight junctions and thus more efficiently enhance the permeability of Dexamethasone. The Student T-test was used to compare the two formulations (Dexamethasone niosome and the drug solution), and the difference was found to be statistically significant with a p-value of 0.0272.

The gel was white consistency. Gel therapeutic effectiveness was determined by their spread, which aids in the gel even topical application. The highest positive peak shows firmness, while the highest positive area suggested shear work to spread the gel. The smallest negative peak observed suggested that the gel was less sticky which means it takes less force to remove the probe, resulting in a smaller negative area. The extrudability of gel was also evaluated to determine the amount of compression force needed for the piston to extrude a product through a standard-size outlet into the container's base. An extrudability result suggests that a greater force needs to be given for it to extrude through the outlet [23]. The higher flux value and permeability coefficient of niosomal gel suggest that there is a slight increase in the skin permeation of the drug due to the non-ionic surfactant (Tween 80) in the niosomal formulation which also acts as a penetration enhancer, thus improve Dexamethasone permeation [24]. Both gel formulations (Dexamethasone niosomal gel and the drug solution gel) were statistically compared using the Student T-test, and the difference was found to be statistically significant with a p-value of 0.0078.

Both the gel formulation (Dexamethasone niosomal gel and the drug solution gel) follow zero-order release, where the regression coefficient value of zero-order kinetics was higher than first-order kinetics. The slope value of Korsmeyer-Peppas was found to be 1.525 (Dexamethasone niosomal gel) and 1.789 (Drug solution gel) which confirms that both the gel follows Super case-II transport (Sorption).

The particle size and zeta potential for the room temperature was increased by 2.6 times (particle size) and 3.74 times (zeta potential) while the refrigerator was increased by 1.4 times (particle size) and 3.17 times (zeta potential). The result indicates that the Dexamethasone niosome formulation is relatively stable in refrigerator conditions (3°C-5°C).

CONCLUSION

The present research work was aimed to enhance the permeability of Dexamethasone by preparing it into niosomal gel. Dexamethasone-loaded niosome was prepared by thin-film hydration method. Different concentrations of cholesterol (15 µM, 30 µM, 50 µM) and non-ionic surfactant (Span 60, Tween 20, Tween 80) were used to obtain an optimized formulation with desired particle size, entrapment efficiency, and *in vitro* release. Among this formulation, Tween 80 was selected for the optimization technique using the design of expert software with minimal particle size, high entrapment efficiency, and high *in vitro* release. It could be anticipated that increased HLB of a non-ionic surfactant and decreased cholesterol concentration might have enhanced the permeability of the lipophilic drug (Dexamethasone). The optimized formulation was to be at the concentration of tween 80 (38.9 µM) and cholesterol (86.2 µM) with the particle size of 498.1 ± 1.710 nm and PDI -0.803 ± 0.0101, entrapment efficiency of 85.2% ± 2.851 and *in vitro* release of 96.25% at the end of 6 hrs. The niosome and drug solution were compared at the end of 6 hrs by dialysis whereas the drug solution release was 75% released at 6 hrs. Niosome showed better release than drug solution. The MTT assay was performed for its keloid activities. Niosome has by decreasing the cell viability between 58% to 49% while drug solution decreased between 63% to 54%. Niosome decreased proliferation more than drug solution due to the presence of non-ionic surfactant by permeating more into the cells and decreased the proliferation. Cell permeability studies of Dexamethasone niosome shows one-fold increased permeation than the drug solution by decreasing the TEER value after 2 hrs treatment in Caco-2 cells. This seems that tween 80 affect the tight junctions and thus more efficiently enhance the permeability of Dexamethasone.

The optimized tween 80 niosome was loaded into 1% Carbopol gel base for topical application 0.1%. *Ex vivo* diffusion study for Dexamethasone loaded niosomal gel and the drug solution gel was performed by using goat ear skin for 6 hrs. It depicted that Dexamethasone-loaded niosomal gel has enhanced drug release of about 70% than the drug solution gel with 63.75% at the end of 6 hrs.

Further, *in vitro* release pattern of the formulation was determined by kinetic modeling using the DD solver program which revealed that both the Dexamethasone-loaded niosomal gel and the drug solution gel follow zero-order kinetics and super case-II model. In other words, the release of the drugs from the formulation occurs by sorption.

Comprehensively, this study revealed that niosome is a promising delivery system, to enhance the permeability of the drug. Hence from the results, it can be concluded that niosomal gel containing dexamethasone has potential application in topical delivery. This study warrants *in vivo* study to confirm the activity.

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COMPETING INTERESTS

The authors do not report any computing interest in this work.

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