RESEARCH PAPER

Development and *In Vivo* Characterization of Calcipotriol Niosomal Gel for Psoriasis Management

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Abstract
Objective: Topical therapy is the mainstay of treatment for mild to moderate psoriasis and serves as a useful adjunct support to systemic therapy in severe disease. To enhance the permeability and effectiveness of calcipotriol, it is loaded in niosomes for the management of psoriasis. The objective of the study was to evaluate the viability of niosomal gel as carrier system for calcipotriol in the treatment of psoriasis.
Method: The study was conducted by synthesizing total nine formulations labelled as NF1 to NF9 of calcipotriol niosomes by thin film hydration technique using different ratio of span 20, span 60 and span 80. From these formulations, NF5 with highest entrapment efficiency and optimum vesicle size was found best for further study, NF5 was used to make niosomal gel formulation labelled as F1 to F10, with gelling agents HPMC, sodium alginate, carbopol, xanthan gum and guar gum.
Result: The formulation F5 with entrapment efficiency 98.68 % and vesicle size 279.7 nm was used to make niosomal gel formulation labelled as F1 to F10 with different gelling agents was further characterized. From these, formulation F6 was homogeneous, clear and spreadable with viscosity value of 8166 cps, and was taken further for skin irritation test as well *in vivo* anti-psoriatic activity analysis in the mice. In the anti-psoriatic analysis, the average PASI score was found to be about 0.5, indicating the effectiveness of the prepared niosomal gel formulation of calcipotriol.
Discussion: The outcome of the study showed that topical delivery of the calcipotriol encapsulated in niosomal gel, demonstrated improved performance compared to blank gel and showed better consistency.
Keywords: Niosomes, Calcipotriol, Topical drug delivery, Anti-psoriatic activity, Erythema, Psoriasis management, Psoriasis area and severity index.
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Introduction: Topical drug delivery involves drug transport from drug delivery system to the skin then to local target site and clearance by diffusion, metabolism, and the dermal circulation to the rest of the body and deeper tissues. The effectiveness and toxicity of topical products are influenced by four factors. These are (a) percutaneous absorption of drug from formulation; (b) the application conditions used; (c) the skin physiology of patient; and (d) product perception by the patient [1]. The penetration enhancers are used to enhance the penetration of the drug for fast action and better efficacy. Among the known skin penetration enhancing agents, fatty acids occupy a special place. For instance, the extent of the alkyl chain and the saturation percentage together with the structure of the alkyl group deeply influence the effect [2]. Formulating gels with the permeation enhancers may enhance the drug penetration efficiency and is multifaceted. The mechanism of fatty acids as permeation enhancers was found to be mainly through the enhancement of permeant partitioning into the stratum corneum intercellular lipid domain [3]. Tacrolimus has been formulated as a niosomal gel together with HPMC, xanthan gum, involved in penetration enhancer activity. It was proved that these improved the diffusion of the drug and thereby the gels prepared met the criteria effectively [4].

Polymeric gels are soft materials, and are defined as semi-solid formulations that are composed of at least two components, i.e., a liquid solvent and a solid gelling substance. The solvent forms an external phase and can be hydrophobic or hydrophilic. The solvent is immobilized within the space of a three-dimensional network structure of the gelling agent [5]. Gels are divided into following categories: organogels (containing an apolar solvent), hydrogels (containing a polar solvent), xerogels (solid-formed gels).Another classification criterion for polymeric gels is its cross-linking character. The following types are determined: physical gels, covalently cross-linked gels, and entanglement network gels. Polymeric gels have following properties: infinite molecular weight, insoluble, infusible and ability to reversibly swell or shrink [6].

The application of hydrogels in the management of psoriasis has many advantages, like reducing the side effects of drugs due to higher bioavailability of medicines. This allows the use of a lower dose, protection against degradation, and highly controlled release of drug from the formulation. Unlike typical conventional dosage forms like creams, ointments or lotions, hydrogels can effectively promote the penetration of many medical substances, which often have different molecular weight controlling their retention in skin layers and target tissues. Moreover, a positive effect of treatment with hydrogels may also be the restoration of the skin barrier, hydration of the epidermal layer and the reduction of the mitotic activity of the epidermis in psoriatic plaques [7].

Hydrogel increases the viscosity of the system, and this influences the applicability and sensory properties as well as prolongs contact time with the skin. Moreover, the nanocarrier/hydrogel matrix systems have increased controlled release, which prolongs the exposition of the skin to the active agent. When hydrogels are used as the carriers of lipid nanostructures, it is possible to obtain a

preparation for topical application with methotrexate immunosuppressant [8].

Niosomes are non-ionic surfactant vesicles, which are formulated with non-ionic amphiphiles in certain aqueous solutions by self-assemble technology; they were first used in the development of cosmetics. Structure of niosomes are usually multilamellar or unilamellar vesicles which possess closed bilayers with hydrophilic cavity and hydrophobic shell as the outer layer to accommodate the active agent, it can encapsulate both hydrophilic and hydrophobic drugs[9].Compared with liposomes, niosomes have advantages such as good stability, low cost, easy to be formulated and scaling-up. Niosomes are much more stable because the forming materials the non-ionic surfactants, these are more stable than those of the lipids both in terms of physical and chemical stability [10].

Niosomes offers better patient compliance and therapeutic effect with greater bioavailability compared to conventional dosage forms. The depot formation of noisome shows controlled and sustained release of drugs. The remarkable capability of noisome is to entrap hydrophilic drugs, lipophilic drugs as well as amphiphilic drugs. The size, shape, fluidity, composition, of drug-containing niosomes can be controlled whenever necessary [11].

Entrapment efficiency is an important parameter for the characterization of niosomal vesicles. The percentage entrapment efficiency of drug in niosomal vesicles varies with type of surfactant used and its concentration. The percentage entrapment efficiency increased with increasing the cholesterol concentration in formulation. The incorporation of cholesterol leads to increase the viscosity of the formulation, indicating more membrane rigidity and good physical stability. The percentage of drug entrapment was found to be good in all grades of surfactants but sorbitan monostearate showed the highest drug entrapment as compared to other surfactants [11].

Calcipotriol is a synthetic derivative of vitamin D3, frequently used alone or in combination for psoriasis. Keratinocytes of the lower epidermal region express D-vitamin receptor and thus, remains the target site for calcipotriol. Excessive proliferation and differentiation of epidermal cells during psoriasis is controlled by the interaction between calcipotriol and D-vitamin receptor [12].

Calcipotriol has been reported to present a better therapeutic response than potent corticosteroid, calcitriol, coal tar, and dithranol. However, the lipophilic nature and low penetration of calcipotriol through impervious stratum corneum remain a major issue for topical delivery of calcipotriol.

So, to overcome the disadvantage of conventional drug delivery system in management of psoriasis, encapsulation of calcipotriol in nano vesicle specially niosome would show drug entrapment in lipophillic domain and may enhance skin penetration. This drug delivery approach would facilitate enhanced drug absorption and provide the successful delivery of calcipotriol to the target site or affected skin. Furthermore, skin irritation issues could be resolved as drug encapsulated inside the nanocarrier would avoid direct contact of therapeutic moiety with skin [12].

Pradhan M *et al*. Fabricated calcipotriol fused nanostructured lipid carrier enriched nanogel_for topical treatment of psoriasis using a modified emulsification–ultrasonication method. *In vivo* study on mice tail animal model revealed significantly higher anti-psoriatic efficacy of nanogel formulation in terms of enhancement in percent orthokeratosis of skin and percent drug activity. The study revealed that the newly developed formulation is an expectant modality for the cure of psoriasis[12].

The research aims to prepare calcipotriol loaded noisome gel for topical application in psoriasis management. In the study Calcipotriol was incorporated into lipophillic domain of niosomes in order to enhance its skin permeation for better patient compliance and to get better encapsulation of drug in vesicle for psoriasis management. Niosomes with different concentration of sorbitan laurate (Span 20), sorbitan monostearate (span 60) and sorbitan oleate (span 80) were formulated, the formulation with highest entrapment efficiency was further used to add gelling agents, to formulate calcipotriol topical gel. Characterization of the prepared calcipotriol loaded niosomes involved measurement of particle size, entrapment efficiency, *in vitro* release profile and skin irritation test on hairless mice skin.

The limitations of the conventional calcipotriol delivery systems include poor skin penetration, greasy formulation with skin irritation, poor drug retention requiring frequent application, and rapid degradation of the drug, which leads to unpredictable therapeutic efficacy. Technology transfer is the major research gap. Preparation of effective preparation is challenge and also time consuming.

As treatment of psoriasis is long term process and therefore patient needs faster affect that reduces inflammation, brings relief and can have quality life. The niosomal drug delivery system encapsulating calcipotriol for treatment of psoriasis shows deeper penetration with cooling effect for faster relief in inflammation.

Objective of the research work is to formulate a noisome gel for the management of psoriasis which overcome the problems of conventional dosage forms. The focus of the research work is to enhance the penetration and absorption of calcipotriol to the affected part of skin, to have faster relief from itching and inflammation, redness and also provide cooling sensation after application of the prepared formulation, implementing enhanced encapsulation of the drug in the domain of vesicles; this reflects the novelty of the developed niosomal gel.

Material

Calcipotriol was provided as gift sample from Krishna Pharma Ltd., India. Gelling agents hydroxyl propyl methyl cellulose (HPMC) and carbopol 940 were procured from TKM pharmaceuticals, India. Other solvents and the excipients were obtained from analytical reagent quality standards from authorised vendors.

Preparation of Calcipotriol loaded Niosomes with different surfactants

The mixture of vesicles forming ingredients like Surfactant and cholesterol were dissolved in a volatile Organic solvents chloroform and ethanol (1:2) in a Round bottom flask. The organic solvent was then Removed above the lipid transition temperature by Using rotary evaporator, leaving a thin layer of solid

Mixture deposited on the wall of the flask. The dried Surfactant film could be rehydrated with 10 ml of
Aqueous phase (ph 7.4 buffer) at 0-60 °C with gentle Agitation. This process formed typical multilamellar
NiosomeNiosomes were prepared by thin film hydration technique; surfactant and cholesterol were dissolved in 10 ml volatile organic solvent diethyl ether in a round bottom flask. [13] The organic solvent was then removed above the lipid transition temperature by using rotary evaporator, leaving a thin layer of solid mixture deposited on the wall of the flask. The dried surfactant film is rehydrated with 10 ml of aqueous phase phosphate buffer ph 7.4 at 60 °C with gentle agitation. This process formed typical multilamellar niosomes. The formulations prepared are enlisted in table 1.

Since nonionic surfactants are more stable, compatible, and less toxic than their anionic, amphoteric, or cationic counterparts, they are the preferred surface-active materials for vesicle formation. Surfactants have multiple uses, such as improving permeability, emulsification, wetting, and solubilization. Strong interfacial activity is exhibited by nonionic surfactants, which have both polar and non-polar regions. Each bilayer's curvature creates continuous membranes that create vesicles, limiting exposure to the water or hydrocarbon interface and achieving thermodynamic stability [14][15].

Surfactants have a unique chemical structure which gives them dual properties. Nonionic surfactants are the main elements of niosomes. Various nonionic surfactants with different HLB values are used for the preparation of niosomes. The formation of niosomes and the encapsulation of drugs are influenced by the structure of surfactants[15].

Cholesterol is related to different characteristics of niosomes. It influences physical properties because it affects membrane cohesion and mechanical resistance, reduces membrane flexibility, regulates drug penetration, improves encapsulation and stability, and increases the phase transition temperature. Bautista SA *et al.* 2025 showed that the surfactant cholesterol ratio can affect niosome structure, producing higher viscosity niosomes. A linear increase in carboxyfluorescein encapsulation was reported, which increased its encapsulation efficiency as the cholesterol concentration increased [16]. Ugorchi O *et al.* In the research work revealed that among the passive methods studied in the preparation of 5-fluorouracil niosomes, the thin hydration method was most robust as it showed lower particle size, spherical vesicles and good entrapment efficiency compared to all the other methods. The evaporation/sonication and the reverse phase evaporation method showed larger particle sizes and were more predisposed to instability. Niosomes produced by ether injection method did not show large entrapment efficiency so was excluded in further studies [17].Therefore thin film hydration technique is applied for the preparation of niosomes.

Total nine formulations labelled as NF1 to NF9 of calcipotriol niosomes were prepared by thin film hydration technique using different ratio of span 20, span 60 and span 80.

Table 1: Niosome formulations

Formulation Code	Surfactant	Surfactant (mg)	Cholesterol (mg)	Surfactant: Cholesterol	Drug (mg)
NF1	Span 20	5	5	1:1	100
NF2	Span 20	5	10	1:2	100
NF3	Span 20	5	15	1:3	100
NF4	Span 60	5	5	1:1	100
NF5	Span 60	5	10	1:2	100
NF6	Span 60	5	15	1:3	100
NF7	Span 80	5	5	1:1	100
NF8	Span 80	5	10	1:2	100
NF9	Span 80	5	15	1:3	100

Abbreviations in table 1: Span 20 = sorbitan monolaurate; Span 60 = sorbitan monostearate; Span 80 = sorbitan monooleate. Total volume of phosphate buffer ph 7.4 used for hydration = 10 ml. Drug = Calcipotriol (100 mg per batch).

Several parameters were investigated in order to ascertain the quality of prepared formulations of niosomes. These include measurement of vesicle size, entrapment efficiency and drug content.

Particle size analysis

The vesicle size of prepared niosomes were analysed by particle size analyzer (malvern panalytical, mastersizer 3000+, lazer diffraction**)** which employs dynamic light scattering technique. A Malvern zeta sizer was used to measure the average vesicle size of the niosomes. The niosomal solution was diluted and placed in a cuvette with an appropriate blank [18][19].

Entrapment Efficiency

By using the centrifugation method, entrapment efficiency of niosomal formulations were assessed, where the niosomal dispersions were centrifuged for 10 min at 8000 rpm. The clear supernatant from the resulting solution was diluted by phosphate buffer ph 7.4 and analyzed for the drug concentration by UV spectrophotometer (Shimadzu Scientific Instruments, UV-1900i Double Beam), the drug in supernatant is the concentration of unentrapped drug [18].

Drug content

Niosomal formulation 10 mg was added and dissolved in 100 ml phosphate buffer ph 7.4 in a volumetric flask. The solution in volumetric flask was shaken continuously with the help of a mechanical shaker for 2 hour to enhance the solubility of the drug. Then 1ml of solution was made up to 10 ml phosphate buffer ph 7.4 and was scanned under UV spectrophotometer using phosphate buffer ph 7.4 as blank [16].

From the above studies, the formulation F5 with highest entrapment efficiency was selected to formulate niosomal gel formulations with different gelling agents, coded as F1-F10. In each formulation calcipotriol niosomes 100

mg, glycerine 10 ml, methyl paraben 20 mg, propyl paraben 200 mg, propylene glycol 10 ml, distilled water up to 100 ml with different gelling agents were formulated and coded as F1-F10. In this different gelling agents such as HPMC, sodium alginate, carbapol, xanthan gum, guar gum were used, enlisted in table 2.

Preparation of calcipotriol loaded niosomal gel with different gelling agents

The formulation NF5 was selected to formulate niosomal gel formulations with different gelling agents, coded as F1-F10. In this different gelling agents such as HPMC, sodium alginate, carbapol, xanthan gum, guar gum were used, as enlisted in table 2.

In each formulation calcipotriol niosomes 100 mg, glycerine, methyl paraben, propyl paraben, propylene glycol, distilled water up to 100 ml with different gelling agents were formulated and coded as F1-F10. In this different gelling agents were used [21].

Table 2: Niosome gel formulations

Formulations	Gelling agents	Gelling agents (gm)
F1	HPMC	2
F2	HPMC	4
F3	Sodium Alginate	2
F4	Sodium Alginate	4
F5	Carbopol	2
F6	Carbopol	4
F7	Xanthan Gum	2
F8	Xanthan Gum	4
F9	Guar Gum	2
F10	Guar Gum	4

Quantities listed in table 2 represent final concentration. Each formulation prepared up to 100 ml final volume with distilled water. Gelling agent concentration shown as gm per 100 ml (i.e., 2 gm = 2% w/v). Methyl paraben 20 mg, propyl paraben 200 mg, propylene glycol 10 ml, glycerine 10 ml per 100 ml. Abbreviation HPMC= hydroxy propyl methyl cellulose

These ten gel formulations prepared were further subjected to physico-chemical characteristics like clarity, spreadability, homogeneity, viscosity and ph. On the basis of these outcomes, the best formulation was chosen for *in vitro* drug release, skin irritation test and *in vivo* anti-psoriatic activity in swiss albino mice.

Clarity, spreadability, homogeneity, viscosity, pH measurement

Formulations were visually examined for clarity and homogeneity.

The spreadability was by parallel-plate method, 1 g of the sample prepared 48 hours before the test was placed between two glass plates 20 x 20 cm. A weight (50-500 g) of 125 g is placed on top for 1 minute. Then the diameter of the sample between the plates was measured. Spreadability of semi-solid dosage forms was determined by compressing the sample under several glass plates of known mass . For example, 20 plates of known mass can be sequentially placed on a sample at 1 min intervals. [22] Spreadability is determined by the formula:

$Si = d^2 \times \pi / 4$

Where, Si–spreading area (mm^2) depending on mass, d–spreading area diameter (mm)

The viscosity of the gel was measured using a brookfield viscometer (vertex scientific and lab instruments co., VSLIC- RVDV-1) at room temperature**.** A small amount of gel was taken and then rotated at 10 rpm using T bar spindle number 96 and the values were noted**,** ph of the gel was measured at room temperature using a digital ph meter **(**spectronics India, SI-139). [23]

On the basis of the outcomes from the above study, formulation F6 was chosen for *in vitro* drug release, *in vivo* anti-psoriatic analysis.

***In vitro* drug release**

In vitro release pattern of niosomes gel was carried out by dialysis bag method. A dialysis sac was washed and soaked in distilled water. The niosome gel was pipette into a bag made up of tubing and sealed followed by placing the dialysis bag into a beaker containing 200 ml of phosphate buffer ph 7.4. The vessel was placed over magnetic stirrer with 50 rpm at 37□C ± 0.5□C. Samples were withdrawn at predetermined time intervals and replaced with the fresh medium to maintain sink condition throughout the experiment [24][25]. Samples were diluted and analyzed for drug content by using UV spectrophotometer.

Skin irritation test

The animal studies were carried out at Sagar institute of research and technology-pharmacy, Sanjeev Agrawal Global Educational University, Bhopal. Mice were taken care under ethical consideration as per the guidelines, with approval from the Institutional Animal Ethics Committee (Registration: Ref/ 07/IAEC/Pharmacy/ 2024) dated 20/04/2024, for primary dermal test.

Primary dermal irritation test was conducted on swiss albino mice weighing 25 to 30 gm. Healthy mice were acclimatize for at least 7 days. The dorsal skin (approximate 2 × 3 cm area) of mice was shaved 24 hours before treatment without damaging the skin. The animals were divided into 4 groups (3 animals per group) and study was performed to examine the potentiality of niosome gel. Group I is negative control (no psoriasis), Group II is positive control (induced, untreated), group III is blank gel, group IV is calcipotriol niosomal formulation. The calcipotriol niosomal gel (50 mg/mice) and blank gel were applied uniformly on the site twice a day and observed for irritation and any reaction for 72 hours.

The animals were specially examined for erythema and edema for 30 minutes and later on signs of erythema and edema were checked**.** If the average irritation score is, score 0 it is for no irritation, score 1-2 is for mild irritation, score 3-5 is for moderate irritation and score 6-8 is for severe irritation. Skin irritation score and average irritation score were determined [26][27].

Anti psoriatic activity

The animal studies were carried out at Sagar institute of research and technology-pharmacy, Sanjeev Agrawal Global Educational University, Bhopal. Mice were taken care under ethical consideration as per the guidelines, with

approval from the Institutional Animal Ethics Committee (Registration: Ref/ 07/IAEC/Pharmacy/ 2024) dated 20/04/2024, for anti psoriatic activity

Swiss albino mice weighing 25 to 35gm were used for study. Experiments were performed as per guidelines. They were fed with standard adequate diet and water. Mice were allowed to acclimatize to the new environment before the experiments were conducted [28].

To study the anti psoriatic activity of calcipotriol niosomal gel against imiquimod induced psoriasis, albino mice were divided into four groups with six animals in each group.

Group I was negative control which did not have psoriasis, Group II was positive control which had the induced psoriasis, Group III was treated with blank gel formulation while group IV was treated with prepared Calcipotriol gel formulation F6.

The imiquimod induced psoriasis model is used as preclinical tool that mimics human plaque psoriasis by applying inducing agent imiquimod, a TLR7/8 agonist, to the skin which develops red, thick and scaly plaques. Psoriasis was induced in all group animals except Group I by applying Imiquad cream (5% w/w Imiquimod) on the shaved dorsal skin on the back side (approximate 10 cm square area) of the animals. In study, mice were given topical treatment once daily with 62.5 mg of cream and daily dose of 3.125 mg Imiquimod. This dose has been empirically determined [29] and already demonstrated to cause reproducible and optimal skin inflammation in this model in various publications [30] The signs of erythema, thickness and scales on the back skin were seen two or three days after the application of imiquimod .

Treatment of calcipotriol niosomal gel formulation F6 was initiated by applying 50mg topically in the affected area till the end of the study and visually observed the skin condition.

The efficacy of formulations for anti-psoriatic activity was assessed employing PASI score. PASI score is a tool used to measure the severity and extent of psoriasis, PASI is psoriasis area and severity index. [30]. The severity of psoriasis was decided based on erythema, scaling and skin thickening. PASI score was assigned as 0 for absence of any psoriatic signs, 1 for very slight signs or mild, 2 for moderate indications, 3 for signs that are severe and finally 4 for very severe or clearly distinguishable.

After 24 hours of treatment completion, animals were sacrificed and shaved back skin sample of mice was collected, and stored in natural phosphate buffer and 10% formalin solution. Samples were sliced transversely, and embedded with paraffin for microscopic evaluation. Skin sections of 5 mm thickness were taken by using a microtome. Samples were stained with hematoxylin and eosin, observed under light microscope (100x olymp, olympus). Histopathological changes (hyperkeratosis, orthokeratosis and parakeratosis) in the tissues were assessed. At least 25 randomly selected tissue sections from each group were studied [30].

All the three parameters along with the cumulative score showed the gradual increase in the observed physical factors with the maximum score at day 11 as compared to the control group.

Stability Evaluation Study

Stability evaluation studies were done for the optimized niosomal gel formulation F6. The best niosomal formulation F6 was subjected to stability testing, during which changes in physical attributes were tracked as a function of temperature. Accelerated stability studies evaluating vesicle size, entrapment efficiency, pH, viscosity, and drug release after storage were analyzed according to ICH recommendations.

The niosome gel formulation F6 samples were stored in tightly closed glass vials at 4 °C, long term condition (30 ± 2 °C, 75 ± 5% relative humidity) and accelerated condition (40 ± 2 °C, 75 ± 5% RH). Physical appearance was assessed, and formulations were analyzed with respect to vesicle size, entrapment efficiency, ph, viscosity and drug release at time intervals of 0, 1, 3 and 6 months[18].

Particle size analysis

Vesicle size plays an important role in the permeation of niosomes through the skin as per literature. Details of calcipotriol niosomes particle sizes are described in table 3**,** which shows that vesicle shaped with span 20 is smaller than span 80 and span 60 shaped vesicle. . It was evident that due to increase in the concentration of cholesterol the vesicle is increasing due to increasing the hydrophobicity in the formulation. Among them batch NF5 shows vesicle size of 279.7 nm followed by NF6 111.2 nm and 100.3 nm.The polydispersity index of measures the size distribution of the vesicular system. The polydispersity index of NF5 is 0.168 with zeta potential -33.9, indicates good formulation homogeneity and sufficient electrostatic repulsion to prevent vesicle aggregation

The vesicle size of prepared niosomes are smaller as compared to that reported in previous study by Gupta A *et al*. In which the vesicle size of tretinoin-saturated and Benzyl peroxide saturated vesicles was 616 nm. In dynamic laser light scattering analysis, drug-containing noisomes, dispersed in gel was analyzed for estimating the size distribution throughout the gel, which was showing that 616 nm average diameter of noisome present in the niosomal gel [19].

Table 3: Niosome vesicle size, polydispersity index and zeta potential

Formulation Code	Particle size (nm)	Polydispersity Index	Zeta Potential (mv)
NF1	67.8	0.114	-25.3
NF2	69.2	0.121	-31.4
NF3	72.6	0.129	-27.1
NF4	100.3	0.209	-29.0
NF5	279.7	0.168	-33.9
NF6	111.2	0.143	-31.3
NF7	98.6	0.120	-32.7
NF8	98.2	0.112	-29.7
NF9	97.4	0.137	-31.4

Data represented in table 3 as mean ±SD.Values are mean of three measurement in. Polydispersity index values ranged from 0.10 to 0.35

Percent drug content

All the batches of niosomes prepared were evaluated for yield of drug content, results are

given in Table 4. Higher vesicle size showed higher drug content. Niosomes formulated with span 20 gave drug content 83.1±1.1 to 91.8±2.6 %; span 60 gave drug content of 90.3±1.6 to 93.9 ±1.5 % and span 80 gave 93.1±1.5 to 85.1±1.0 %. All the assessments were done in triplicates. Among all the nine batches, NF5 batch was found to have maximum drug content i.e. 99.2 ± 1.2 %.

Table 4: Percent drug content of Niosomes

Formulation code	**Drug content %**
NF1	83.1±1.1
NF2	89.6±1.6
NF3	91.8±2.6
NF4	90.3±1.6
NF5	99.2±1.2
NF6	93.9±1.5
NF7	93.1±1.5
NF8	89.9±1.8
NF9	85.1±1.0

Data is represented as Mean ±SD, n =3 Drug content expressed as % of theoretical amount (100 mg)

Entrapment efficiency

Among all the details, NF5 (span 60/cholesterol 1:2) showed the highest entrapment efficiency. Entrapment efficiency of all formulations represented in table 5. NF5 formulation corresponds to the higher drug content and entrapment efficiency 98.686% **shown in table 4 and table 5.**

Table 5: Entrapment efficiency

Formulation Code	**Entrapment efficiency %**
NF1	80.65
NF2	85.25
NF3	90.58
NF4	93.14
NF5	98.68
NF6	96.25
NF7	88.36
NF8	87.15
NF9	86.35

Centrifugation at 8000 rpm for 10 min at 25°C. Mean ± SD, n=3.

Clarity, spreadability, homogeneity, viscosity, ph measurement

Gel preparations were evaluated for clarity, spreadability, homogeneity, viscosity and ph. Among all the formulations the gel F5 and F6 were found to be clearest with best spreadability 3245.21 and 3219.74 mm² by parallel plate method and with excellent homogeneity.

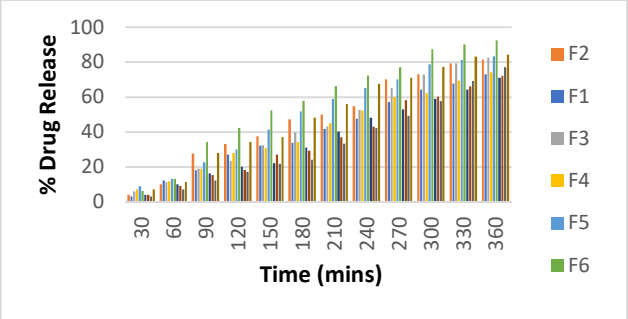
Analysing the results of all the formulations of the niosomal gels, it was seen that formulation F6 has the optimal values required for the acceptable niosomal gel preparation. Among the other nine formulations, 'F6' was more homogenous, clear as well as spreadable in nature. Also formulation F6 had higher viscosity among other preparations with the viscosity value of 8166 cps and ph 7.3 at room temperature. Therefore, formulation 'F6' has been selected for further assessment of experimental *in vivo* anti psoriatic activity.

***In vitro* drug release study**

Controlled release phenotype of calcipotriol niosomal gel will avoid the fast delivery of the drug to the exfoliated psoriatic skin, which is sensitive to accelerated absorption of drugs and can thus avoid toxicity. Calcipotriol niosomal gel batch F6 which contains carbopol showed highest release profile. Calcipotriol gel, however, demonstrated a controlled release profile (92.46±2.16)

Over the period of 48 hours. Controlled release profile of calcipotriol niosomal gel is due to slow diffusion of calcipotriol in the carbopol matrix from lipid niosomes. Graph 2 shows the percent drug release profile.

Surfactants in the niosomal bilayer act as chemical penetration enhancers. They temporarily disrupt the rigid lipid structure of the *stratum corneum*, allowing the intact vesicles to transfer through and deposit the drug into deeper skin layers.



Graph 2: Drug release profile of calcipotriol niosomal gel batches F1– F10.

Drug release of niosomal gel formulations (F1 to F10) in phosphate buffer ph 7.4 at 37°C ± 0.5°C. Data shown as mean ± SD, n=3.

Skin irritation test

The animal studies were carried out at Sagar institute of research and technology-pharmacy, Sanjeev Agrawal Global Educational University, Bhopal. Mice were taken care under ethical consideration as per the guidelines, with approval from the Institutional Animal Ethics Committee (Registration: Ref/

07/IAEC/Pharmacy/ 2024) dated 20/04/2024, skin irritation study or for primary dermal test.

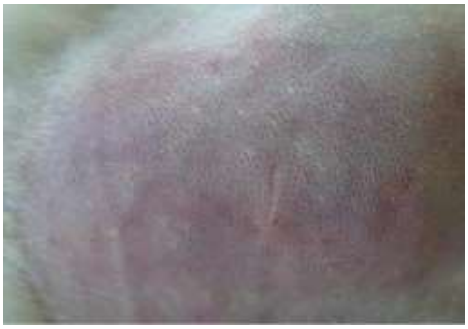
The total primary irritation score is the summation of erythema score and edema score. The average irritation score is average of summation of score at 24 hours and score at 72 hours [26][27]. Overall observations are represented in terms of average irritation score from 1 to 8.

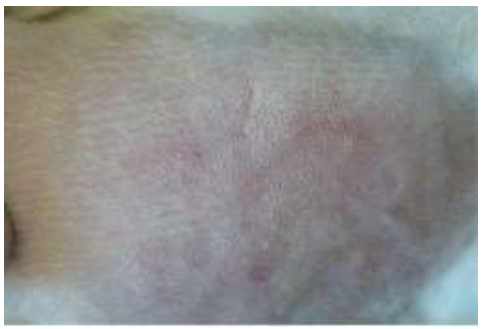
Examining the mice skin for formulations of niosomal gel, there is no evidence of irritation, erythema or eczema, no matter the time of observation. The outcome of study is shown in table 7 and figure 1.

Table 7: Skin irritation test score

Formulations	Average Irritation Score
F1	0
F2	0
F3	0
F4	0
F5	0
F6	0
F7	0
F8	0
F9	0
F10	0

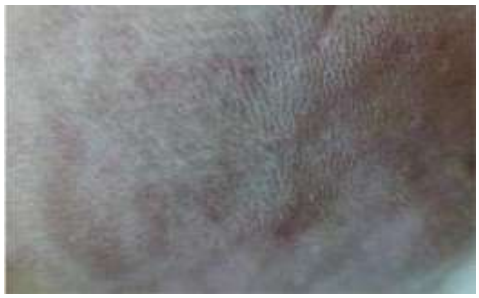
* Average irritation score in swiss albino mice. Scoring: 0 = no irritation, score 1-2 = mild irritation, score 3-5= moderate irritation, score 6-8= severe irritation. Observations made at 1, 24, 48 and 72 hours post application*





(a)

(b)



(c)

(d)

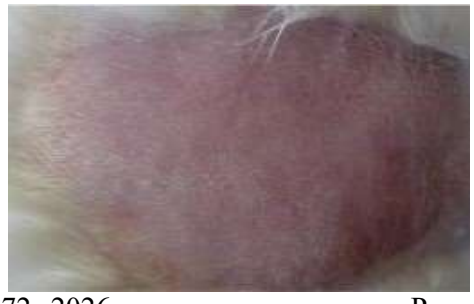
Figure 1: Skin irritation test

Representative images of swiss alboni mice skin before and after 72 hours of application of formulation F6. (a) Before application; (b) 72 hours after first application; (c) Before repeat application (day 7); (d) 72 hours after repeat application (day 10). No erythema or edema observed.

Anti psoriatic activity

For studying the anti psoriatic activity, the indications of psoriasis like scaling, erythema, and inflammation were depicted on the shaved back of mice after ten days of treatment with imiquimod formulation. During the first assessment the psoriasis area and severity index score of 1 and 2 were found. Having performed the further procedure, the outcome was quite good and the score evaluated was 0.5 and 1.6. This was significantly higher in calcipotriol loaded niosome gel F6 as compared to blank gel. However, in the positive control group it has persisted as evident in table 8.

The calcipotriol niosome gel F6 presented typical skin with extremely mild keratosis because calcipotriol had



better penetration and moderate uptake. The results of the activity is presented in figure 2.

Table 8: PASI Score of anti psoriatic activity of formulation 'F6' Niosomal Gel

Experimental Groups	Erythema		Scaling		Thickness		Average PASI score	
	Day 1	Day 11	Day 1	Day 11	Day 1	Day 11	Day 1	Day 11
I	0	0	0	0	0	0	0	0
II	1	1	2	2	3	3	2	2
III	1	1	3	2	2	2	2	1.6
IV	1	0	2	0	3	1	2	0.5

PASI score = (Erythema + Scaling + Thickness)/3. Day -1 = baseline (day before induction), Day -11 likely indicates Day 11 (10 days after induction + 1 day post treatment). Group I = negative control (no psoriasis), Group II = positive control (induced, untreated), Group III = blank gel, Group IV = Calcipotriol niosomal gel F6. Mean ± SD, n=3 Abbreviation PASI = psoriasis area and severity index.*

The analysis showed that in group IV that received the treatment with formulation F6 niosomal gel of calcipotriol has got the least mean PASI score among all the treatment groups. In relation to this when it is compared with that of negative group it is identified nearest to the value after treatment was 0.5.

Hence, it can be concluded that, calcipotriol niosome gel has the effect of the incremental release when applied topically and found to be more potent in management of psoriasis.

Comparing the outcomes of the present study with those of previous research performed by Vivek Avasatthi and his group in 2016 [31]. They synthesised methotrexate nanogel, which is the methotrexate loaded nanostructured lipid carrier. To assess the therapeutic efficacies of the methotrexate nanogel, they employed the same imiquimod induced psoriasis model for treatment of psoriasis. They concluded that highly significant reduction in the whitening of the skin in mice or in medical term a significant reduction in the PASI score as well as the restoration of the normal skin of the mice, However, the methotrexate gel in the long term experiment, showed signs such as hyper and parakeratosis at the end of the experiment [32].

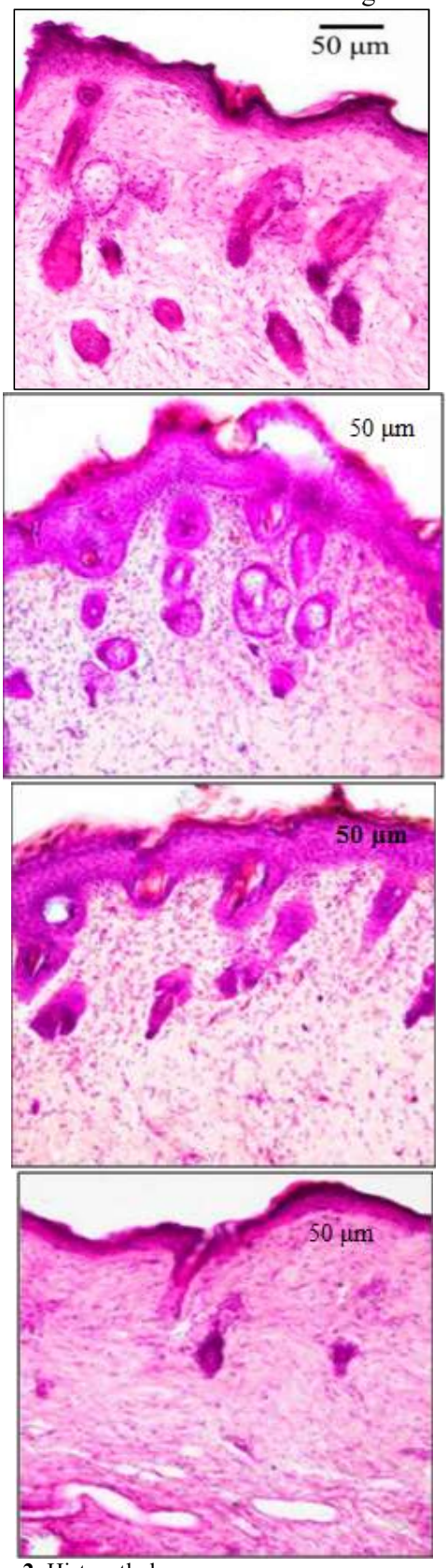


Figure 2: Histopathology

*H&E staining, original magnification 200×. Scale bar = 50 µm. (a) Positive control (imiquimod only): marked hyperkeratosis, acanthosis, and inflammatory infiltrate. (b) Negative control (normal skin): normal epidermis and dermis. (c) Blank gel: mild residual hyperkeratosis. (d)

Calcipotriol niosomal gel F6: near-normal skin architecture.*

Stability Evaluation Study

The stability evaluation study determined slight increase in vesicle from 279.7 nm to 285.9 nm at 6 month, due vesicle aggregation and decrease in entrapment efficiency due to drug leakage. The ph valueshows negligible change from 7.3 to 7.2. Slight increase in viscosity was observed from 8166 to 8171 cps which was acceptable. Percent drug release declined from 89.31 in one month to 85.21 in sixth month. The prepared formulation may be considered stable with acceptable change in physical parameters.

Table 9: Stability Evaluation Study

Physical parameter	**Day 0**	**1 Month**	**3 Months**	**6 Months**
Vesicle size (nm)	279.7	281.3	283.4	285.9
Entrapment efficiency %	98.68	96.91	95.23	94.5
pH	7.3	7.3	7.2	7.2
Viscosity (cps)	8166	8167	8170	8171
Drug release %	92.46	89.31	86.32	85.21

Stability Evaluation Study as per ICH guidelines**,** Mean ± SD, n=3

Conclusion

Niosomal gel, has been studied for the percutaneous administration for penetrating the skin barrier and for skin retention. A critical consideration according to the present study is the use of calcipotriol niosomal gel in the management of psoriasis. Vesicles were prepared by thin film hydration technique using span 20, span 60 and span 80 as surfactant and encapsulated drug calcipotriol, giving high entrapment efficiency. Calcipotriol has a role in the reduction of scars by having an inhibitory effect on the humoral immune response. The paper shows that calcipotriol treats scaly patches and prevents the impacts caused by imiquimod. Characterizations of niosomal gel formulation were done for parameters like particle size, drug content and *in vitro* release study. All formulation fell in between the established and acceptable range for these parameters. All formulation tends to have confirmatory physical and chemical properties as neutral ph, appreciable viscosity. Finally in the anti-psoriatic analysis, of formulation F6 the average PASI score was found to be about 0.5, which is an acceptable value indicating the effectiveness of the prepared niosomal gel formulation of calcipotriol. The stability evaluation study marked acceptable changes in physical parameters, so the prepared fornmulation can be considered stable.

Calcipotriol niosome gels enhances the psoriasis treatments by combining the controlled release of niosomes with the prolonged skin residence of a gel matrix. They increase drug deposition in the epidermis and also remarkably reducing the local irritation and systemic exposure typically associated with conventional creams and solutions.

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AUTHORS CONTRIBUTIONS

All authors have contributed equally**.**

AI USE STATEMENT

"No artificial intelligence (AI) or AI-assisted tools were used in the writing, data analysis, or creation of figures for this manuscript."

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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