Advanced Materials Science and Technology Vol 5 Issue 2 2023
DOI: 10.37155/2717–526X–0502–2

ORIGINAL RESEARCH ARTICLE

Open Access

Omniscient Pte.Ltd.

Tailored Non-ionic Surfactant Vesicles of Cyclosporine for the Treatment of Psoriasis: Formulation, *Ex-Vivo* and *In-Vivo* Investigation-Application of Box-Behnken Design

Peeyush Bhardwaj[1], Purnima Tripathi[2*], Sonia Pandey[3], Deepti Chaurasia[4] and Purushottam Ramchandra Patil[5]

[1]Department of Pharmacy, Bundelkhand University, Jhansi, U.P. 284128, India.
[2]B.S. Anangpuria Institute of Pharmacy, Alampur, Ballabgarh - Sohna Major District Road, Faridabad, 121004, Haryana, India.
[3]Faculty of Pharmaceutical Sciences, Rama University, Mandhana, Kanpur, U.P. 209217, India.
[4]Nkbr College of Pharmacy And Research Centre, Meerut, U.P. 245206, India.
[5]Government College of Pharmacy, Osmanpura, Aurangabad, Maharashtra, India.

***Correspondence to:** Purnima Tripathi, B.S. Anangpuria Institute of Pharmacy, Alampur, Ballabgarh - Sohna Major District Road, Faridabad, 121004, Haryana, India. Email: Tripathi.purnima@gmail.com

Received: August 15, 2023; **Accepted:** November 20, 2023; **Published Online:** November 30, 2023

Citation: Bhardwaj P, Tripathi P, Pandey S, Chaurasia D and Ramchandra Patil P. Tailored Non-ionic Surfactant Vesicles of Cyclosporine for the Treatment of Psoriasis: Formulation, *Ex-Vivo* and *In-Vivo* Investigation-Application of Box-Behnken Design. *Advanced Materials Science and Technology*, 2023;5(2):0520492. https://doi.org/10.37155/2717-526X-0502-2

Abstract: Psoriasis is an autoimmune skin disease characterized by hyperproliferation of keratinocytes. Topical delivery of drugs is mostly favored for the treatment of mild psoriatic conditions. But permeation of drugs across psoriatic skin is too complex. Niosomes are the non-ionic surfactant vesicles, reported to enhance dermal drug delivery. In the present work, cyclosporine niosomes were, formulated, optimized, and evaluated *in-vitro* to boost the dermal penetration of cyclosporine for the better management of psoriasis. Niosomes were developed using the thin film hydration method. Formulated niosomes were characterized and optimized for their percent encapsulation efficiency, size, and polydispersity index using Box-Behnken design. Optimized formulation was developed using cholesterol and span 60 (1:2.2), 30 minutes of hydration time, and 30 mg of cyclosporine. Niosomes' size, polydispersity index, and percent encapsulation efficiency were in the scale of 180.5 ± 11.16 nm, 0.156, and 93.2% ± 2.5%, respectively. The *ex-vivo* studies were carried out using excised goat skin. In the *ex-vivo* permeation experiments, though the percent drug permeated was low but the quantity

of drug permeated across the skin from the niosomes was significantly greater than from suspension. Skin deposition studies revealed deeper and more significant accumulation of cyclosporine niosomes than the free cyclosporine in the epidermis. The *in-vivo* experiments were carried out using imiquimod induced psoriatic mice, where both the histopathology and psoriasis area severity index displayed significant recovery in the skin condition of mice treated with niosomes of cyclosporine, in comparison with the dispersion of the drug. The results indicate that the non-ionic surfactant vesicles of cyclosporine can be employed for the enhanced management of psoriasis and reduction of side effects linked with the systemic delivery of cyclosporine.
Keywords: Cyclosporine; Niosomes; Dermal delivery, Box-Behnken design; Psoriasis; Optimization

1. Introduction

Psoriasis is an auto-immune skin disorder, characterized by hyperproliferation of keratinocytes secondary to an activated immune system[1]. Identified by the presence of well-demarcated erythematous plaques with silvery scales. The prevalence rate of psoriasis in India is 0.7%[2]. Clinically there are many phenotypes of psoriasis but the most common is psoriasis vulgaris[3]. Both genetic as well as environmental factors have a key role in the development of psoriasis[4]. Interleukin 17/interleukin 23 plays an important role in the pathogenesis of psoriasis,. It is recurrent and could not be cured. It can occur in any part of the body but the knees, elbows, scalp, and trunk are generally more affected[1]. Assessment of the severity is measured by Psoriasis Area and Severity Index (PASI)[5,6]. Psoriasis not only affects the physical health but the mental and social status of the patient is also affected. Treatment of psoriasis includes both topical and systemic therapies depending on the severity. When psoriasis affects less than 20% of body area topical therapy with emollients and keratolytics is given. Corticosteroids and calcipotriene ointments are also used. Phototherapy with ultraviolet A light and B light is also used in mild cases. If topical treatment is not effective than systemic therapy is used which is highly effective but highly toxic as well. These includes psoralens with Ultraviolet light, methotrexate and calcineurin inhibitors like cyclosporine.

Cyclosporine is a cyclic undecapeptide, obtained from the fungi, Tolypocladium inflatum. It is lipophilic with a molecular weight of 1202 Dalton[7-9]. It is successfully used in organ transplants to prevent rejections because of its immunosuppressive properties. Being an immunosuppressant it is also used in many other autoimmune disorders like psoriasis, where it is recommended for systemic administration in severe cases[10]. It prevents the clinical expression of psoriasis by blocking the calcineurin-dependent factor important for the transcription of interleukin-2 (IL-2). IL-2 is essential for the proliferation of activated T cells and the production of other cytokines. Thus IL-2 dependent immune responses are suppressed[5]. But long-term treatment of cyclosporine is linked with serious side effects like nephrotoxicity, hypertension, hyperlipidaemia, *etc*.[6] These disorders can be avoided by site-specific dermal delivery, especially in the case of skin disorders. The protective nature of the stratum corneum (SC) and the high molecular weight of cyclosporine make penetration difficult across the skin[11].

In the past many years, dermal delivery of cyclosporine has attracted much research and a lot of work has been done[4-7]. Some research involved physical methods like iontophoresis[12], electrophoresis, and sonophoresis, whereas some used penetration enhancers[13,14] to improve the permeation. Physical methods may disrupt the barrier properties of the skin and penetration enhancers' causes' irritation[11]. Tacrolimus and pimecrolimus, are also calcineurin inhibitors, having high lipophilicity but are successfully used topically for atopic dermatitis. Topically as ointment both tacrolimus and pimecrolimus are used in facial and intertriginous psoriasis but not effective in plaque psoriasis[1,8]. Novel delivery systems like nanoparticles[11], solid-lipid nanoparticles[10,15], nanostructured lipid carriers[15], microneedles[16], liposomes[17], microemulsion[18], amphiphilic gel[19] and micellar nanocarriers[9] were prepared to improve the dermal delivery of cyclosporine. But to date, no dermal formulation is available in the market. In this study, cyclosporine-loaded niosomes were prepared. Niosomes are a type of vesicles, made up of non-ionic surfactant and cholesterol[20]. Niosomes are non-

toxic and biodegradable and can be used for sustained or controlled deliveries. These can be used to deliver both water-soluble and non-soluble drugs[20]. For the management of psoriasis, the researchers in the past have successfully used niosomes to deliver several drugs like- methotrexate[21], acitretin[22], diacerein[23], and tretinoin[10] topically. There are some drugs recommended by dermatologists but not approved by US FDA for the treatment of psoriasis. These includes some drugs like sulfasalazine and hydroxyurea which are moderately efficative and less toxicity whereas drugs like azathiopurine and tacrolimus are moderately effective and highly toxic[24]. In the present work, an attempt was made to deliver cyclosporine topically using niosomes for the management of psoriasis.

2. Methods

2.1 Materials

Cyclosporine was received as a gift sample from Concord Biotech Limited, Gujarat, India. All solvents and chemicals used were of analytical grade.

2.2 Experimental Design

For designing Design Expert Software (DES) (VERSION 13, MINNEAPOLIS) was used. To optimize the niosome formulation Box-Behnken design (BBD) was applied. For this work we developed 15 formulations. The independent and dependent variables selected for the study were - the ratio of cholesterol to span 60, hydration time, and amount of drug; and size, percentage entrapment efficiency (%EE), and polydispersity index (PDI), respectively. All the independent variables and their responses are shown in **Table 1**.

Table 1. Independent variables and responses of BBD

Independent Variables Levels (-1, 0, +1)	X_1 Cholesterol: Span 60 1:2, 1:1, 2:1	X_2 Hydration Time (minutes) 30, 45, 60	X_3 Amount of Drug (mg) 10, 20, 30
Dependent Variables	Y_1 Size (nm)	Y_2 PDI	Y_3 %EE of Cyclosporine

2.3 Development of Cyclosporine Niosomes

The thin film hydration (TFH) method has been opted to formulate cyclosporine-loaded niosomes using varying ratios of cholesterol and span 60[11,12,25,26]. For the preparation of niosomes, cholesterol, span 60, and cyclosporine were uniformly blended in 10 mL of solvent (chloroform and methanol in a 1:1 ratio). Details of cholesterol, span 60, and cyclosporine used in the preparation are given in **Table 2**. A rotary vacuum evaporator was employed for the removal of solvent, which leads to the formation of a film on the walls of the round bottom flask. After film formation, a buffer solution was added to hydrate the film and for complete hydration it was placed on a magnetic stirrer at a temperature of 60 ^{o}C. Finally the suspension was vortexed and probe sonicated for 5 minutes and 10 minutes, respectively. The final developed formulation were kept in a refrigerator till further use.

Table 2. Composition and responses obtained for cyclosporine niosomes using BBD

Run	X_1	X_2	X_3	Y_1	Y_2	Y_3
	Cholesterol: Span 60 Ratio (Ratio)	Hydration Time (minutes)	Amount of Drug (mg)	Size (nm ± SD)	% Entrapment Efficiency (% ± SD)	PDI (Value ± SD)
1	1:1	45	20	62.27 ± 1.98	97.17 ± 2.05	0.199 ± 0.061
2	1:2	45	30	169.9 ± 1.07	95.15 ± 2.10	0.33 ± 0.0910
3	1:2	45	10	135.5 ± 2.01	88.14 ± 1.87	0.51 ± 0.110
4	1:1	45	20	61.96 ± 0.91	97.39 ± 0.49	0.119 ± 0.04
5	1:1	60	10	77.26 ± 0.66	91.08 ± 1.62	0.212 ± 0.06
6	2:1	30	20	112.1 ± 1.56	83.58 ± 1.65	0.403 ± 0.036
7	2:1	45	10	107.6 ± 1.21	80.53 ± 1.18	0.359 ± 0.053
8	1:2	60	20	104 ± 1.73	95.15 ± 0.51	0.487 ± 0.076
9	1:2	30	20	193.7 ± 0.23	88.58 ± 1.31	0.587 ± 0.098
10	2:1	60	20	70.96 ± 1.33	91.56 ± 0.72	0.303 ± 0.012
11	1:1	60	30	97.67 ± 0.74	98.19 ± 0.94	0.145 ± 0.023

Continuation Table:

Run	X_1	X_2	X_3	Y_1	Y_2	Y_3
	Cholesterol: Span 60 Ratio (Ratio)	Hydration Time (minutes)	Amount of Drug (mg)	Size (nm ± SD)	% Entrapment Efficiency (% ± SD)	PDI (Value ± SD)
12	1:1	30	10	159.1 ± 0.59	82.64 ± 1.28	0.501 ± 0.102
13	1:1	45	20	67.26 ± 1.69	96.87 ± 1.36	0.209 ± 0.054
14	2:1	45	30	95.31 ± 1.91	91.8 ± 1.71	0.113 ± 0.012
15	1:1	30	30	179.1 ± 1.84	96.06 ± 1.03	0.101 ± 0.024

2.4 Characterization of Cyclosporine Niosomes

2.4.1 Size, PDI, and zeta potential

Zetasizer (Malvern Instrument, 3000, UK) was utilized to determine size, PDI and zeta potential by dynamic light scattering technique[13]. The determination, was carried out by placing the suspension of niosomes in a glass cuvette and measured at a temperature of 25 ^{o}C and scattering angle 90o.

2.4.2 Percentage entrapment efficiency

For the estimation of cyclosporine concentration HPLC (Systronics, SYS LC -138)) was employed. For the study the isocratic mobile phase, flow rate and wavelength selected was - acetonitrile : water (75:25), 1.2 mL/min, and 210 nm, respectively.

To determine the %EE, niosomes were centrifuged in a refrigerated centrifuge at 4 ^{o}C and 20,000 rpm for 1 hour (Heraeus, Biofuge Stratos Centrifuge) to separate the niosomes and the free cyclosporine. The HPLC method explained in the previous paragraph was employed to determine the concentration of the free drug in the supernatant. The calculation was carried out using the below mentioned formula. This process was repeated three times to completely remove the free drug[14,15].

$$\%EE = \frac{A_0 - A}{A_0} \times 100$$

Where A_0 represents the total amount of drug used in the formulation and A is the amount of drug available in the supernatant.

2.4.3 Optimization of prepared niosomes

BBD was used to optimize the niosomes. Constraints were applied to get the highest %EE and niosome size and minimum PDI. The formula suggested by the software was used to prepare optimized niosomes and then, this optimized formulation was studied to find if the optimal formulation factors and predicted responses obtained by the software were valid.

2.4.4 Morphological characterization

The morphological characters like shape and size of the optimized niosomes were observed using transmission electron microscopy (TEM, Philips, Holland). For this study a drop of niosome dispersion was directly placed on the copper grid and then it was left overnight for complete removal of moisture. Eventually, the samples were analysed using TEM.

2.4.5 FTIR spectroscopy - interaction study

Interactions between cyclosporine and formulation additives were studied by employing FTIR Spectrometer (Perkin Elmer) in the range 4000 cm^{-1} to 550 cm^{-1}. FTIR Spectrum of cyclosporine API, cholesterol, span 60, and optimized cyclosporine niosomes were drawn by reflection technique, accessory UATR (resolution 4 cm^{-1}, gain 1, range 4000 cm^{-1} to 400 cm^{-1}, 20 scans).

2.4.6 Powder X-ray diffraction (P-XRD) study

P-XRD studies were carried out using an X-ray diffractometer (Rigaku, Smart Lab) on cyclosporine API and lyophilized optimized cyclosporine niosomes. P-XRD analysis was performed at a diffraction angle of 2θ and in the scale of 2.0^{o} to 70^{o} The studies were carried out to know the crystalline properties of cyclosporine (API) and cyclosporine niosomes[9].

2.4.7 Ex-vivo permeation experiments on excised goat skin

Ex-vivo permeation experiment was performed on a Vertical Franz diffusion cell with diffusion area of 1 cm^2. For this experiment, freshly cut goat skin was procured from the slaughter house. Hairs were completely removed from the posterior surface and adjacent fat and subcutaneous tissues were also detached. The prepared skin was put into the phosphate buffer solution for 30 minutes before the commencement of the experiment.

Afterwards the prepared goat skin was kept on the

diffusion cell in a manner such that the SC faces the donor and the dermis faces the receiver compartment. The receiver compartment of the diffusion assembly was filled with 10 mL phosphate buffer (pH 5.5) and temperature was maintained to 37±0.5 ^{o}C with continuous stirring (100 rpm)[14,27]. A suspension containing optimized niomosal formulation of cyclosporine and a plain dispersion of cyclosporine (drug equivalent to 5 mg) were kept over the skin surfaces, on two different assemblies. After a fixed time interval of 0, 0.5, 1, 2, 3, 4, 5, 6, 7, and 8 hours, 0.5 mL of sample was collected and replaced with the equivalent amount of dissolution media. Collected samples were filtered using a 0.45 µm syringe filter and then analyzed by HPLC (λ_{max} = 210 nm). Cumulative amount of drug permeated across the skin and flux was determined[16].

2.4.8 Skin deposition experiments

After the successful completion of above experiment, the skin was washed with phosphate buffer to remove remainings of the cyclosporine, if any. Afterwards the skin is dried. To determine the cyclosporine concentration in the SC the tape stripping method was employed. Initially, the skin was stripped for 15 times using adhesive tape. The first piece was discarded and remaining pieces were dipped in 10 mL of methanol and stirred for 30 minutes on a magnetic stirrer. After stirring, methanolic content was filtered using a 0.45 µm syringe filter and drug concentration was determined by HPLC. The leftover skin was utilized to detrmine the drug concentration in the epidermis and dermis (lopes 2006). The skin was cut into tiny pieces and placed in methanol for 30 minutes on a magnetic stirrer. Finally, the methanolic content were filtered using a syringe filter of 0.45 µm pore size and estimation by HPLC[17,27].

2.5 In-vivo Studies in Imiquimod Induced Psoriatic Mice

In-vivo studies were conducted in order to determine the comparative efficacy of cyclosporine-laden niosomes with a dispersion of cyclosporine API. The *in-vivo* studies were conducted according to the earlier reported methods[28]. Before the study, hairs were removed from the posterior surface using hair removing gels. For the present study, mice were divided into 4 groups, each group containing 4 mice. Group I; negative control i.e. neither psoriasis was induced nor any treatment was given, group II; positive control i.e. consists of mice in which psoriasis was induced (Psoriasis Induced, PI) but no treatment was provided, group III; i.e. PI and received treatment with dispersion of cyclosporine API, group VI; PI and received treatment with cyclosporine niosomes. Swiss albino mice were induced with psoriasis by rubbing 5% w/w imiquimod (dose-62.5 mg/day/mouse) cream on the posterior surface for seven days. The mice were carefully monitored visually for induction of psoriasis. Once the psoriasis, was successfully induced a suspension of cyclosporine API and suspension of optimized niosomes was applied to group III and group IV of mice, respectively, for next seven days and PASI was measured. PASI evaluation was based on erythema, scaling and thickness and recorded on a 0-4 scale where 0 represents no symptoms at all, 1 represents mild symptoms, 2 represents moderate symptoms, 3 severe and 4 very severe. Once the work was successfully concluded, the mice were sacrificed and dissected skin was placed in 10% formalin solution. Histopathological studies were performed using haematoxylin dye. The stained slides were examined for the characteristic pathological features of psoriasis: acanthosis (increase in epidermal thickness); hyperkeratosis (thickening of SC); granular infiltrates along with edema.

2.6 Stability Studies

The stability studies were peroformed on the optimized cyclosporine-loaded niosomes for a period of 3 months and afterwards the formulation was assessed for the size, PDI, and %EE. The niosomes were stored at two different temperatures -4±1 ^{o}C (refrigeration) and 25±2 ^{o}C for a period of 3 months in clear glass vials. For the first time the parameters were measured on the day of formulation and then after 1st, 2nd, and 3rd months of storage[22].

3. Results and Discussion

In the beginning of this research the most suitable method, non-ionic surfactant, and duration of sonication for the preparation of cyclosporine niosomes were determined. TFH was found to be the most suitable method for the development of formulations. The formulations were sonicated for different lengths of time. The most suitable sonication period was discovered to be 10 minutes because longer sonication

time produced smaller niosomes and also lowered EE. Longer sonication time disrupts the vesicular structure, resulting in the leakage and less encapsulation of drug. Span 60 was selected for the formulation of niosomes because of its HLB (4.7), saturated alkyl chain, and high gel transition temperature. These factors result in the good entrapment efficiency[20,26].

3.1 Preparation of Cyclosporine Niosomes

DES was used to apply BBD, for the development of final formulations. All the parameters used in developing formulations are shown in **Table 1**. The impact of factors on responses are shown with the help of 3D response surface plots (**Figures 1 to 3)**. The different formulations of niosomes so developed were then optimized to get the highest %EE, maximum size for niosomes, and minimum PDI. The size, PDI, and zeta potential of the developed niosomes were measured using DLS approach, with a zetasizer and were found to be in the range of 61.96-193.7 nm, 0.101-0.587, and -20.7-31.6 mV, respectively (**Table 2**).

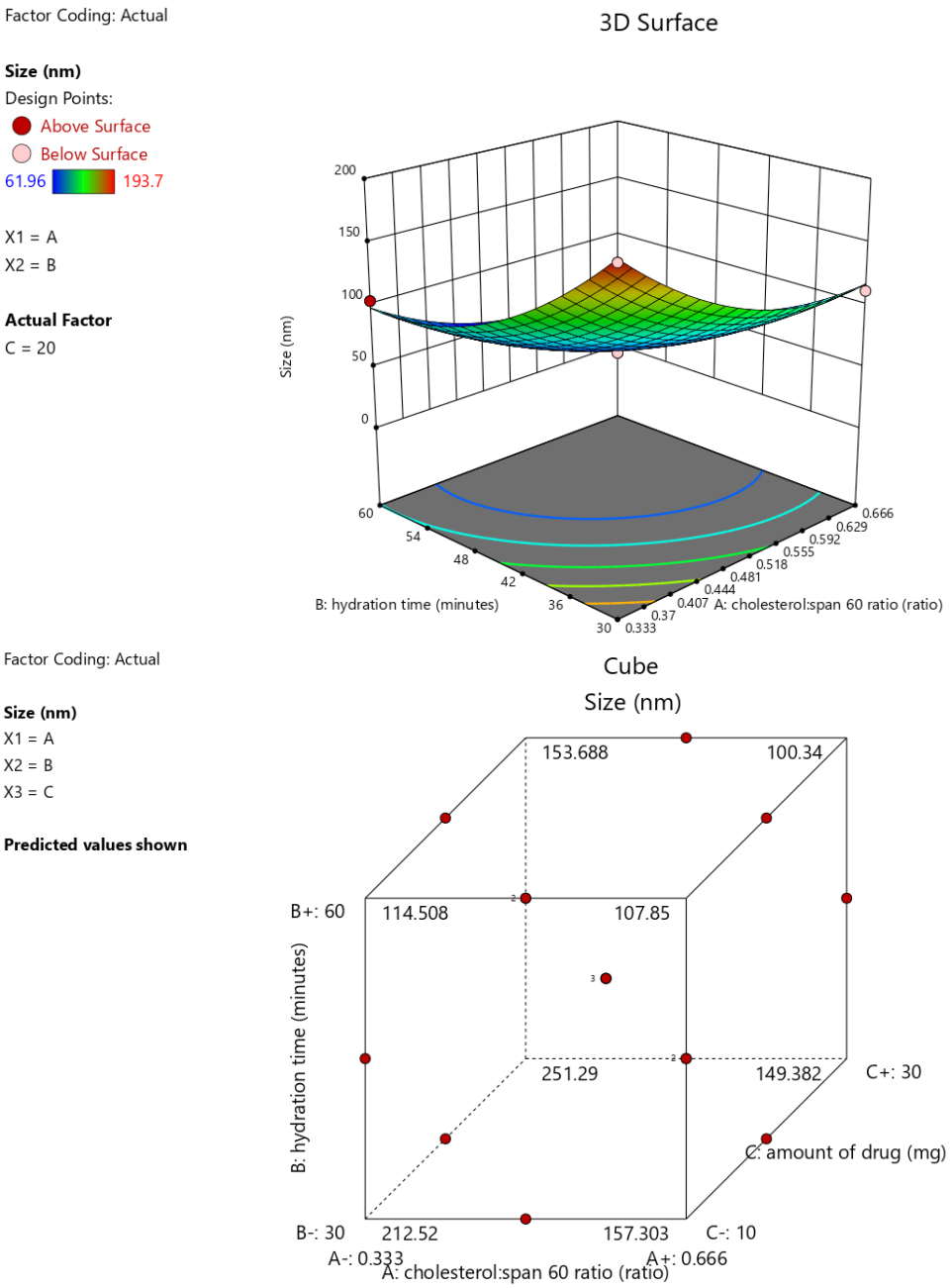


Figure 1. 3D response contour plots and a cubic plot displaying the effect of factors on niosome size

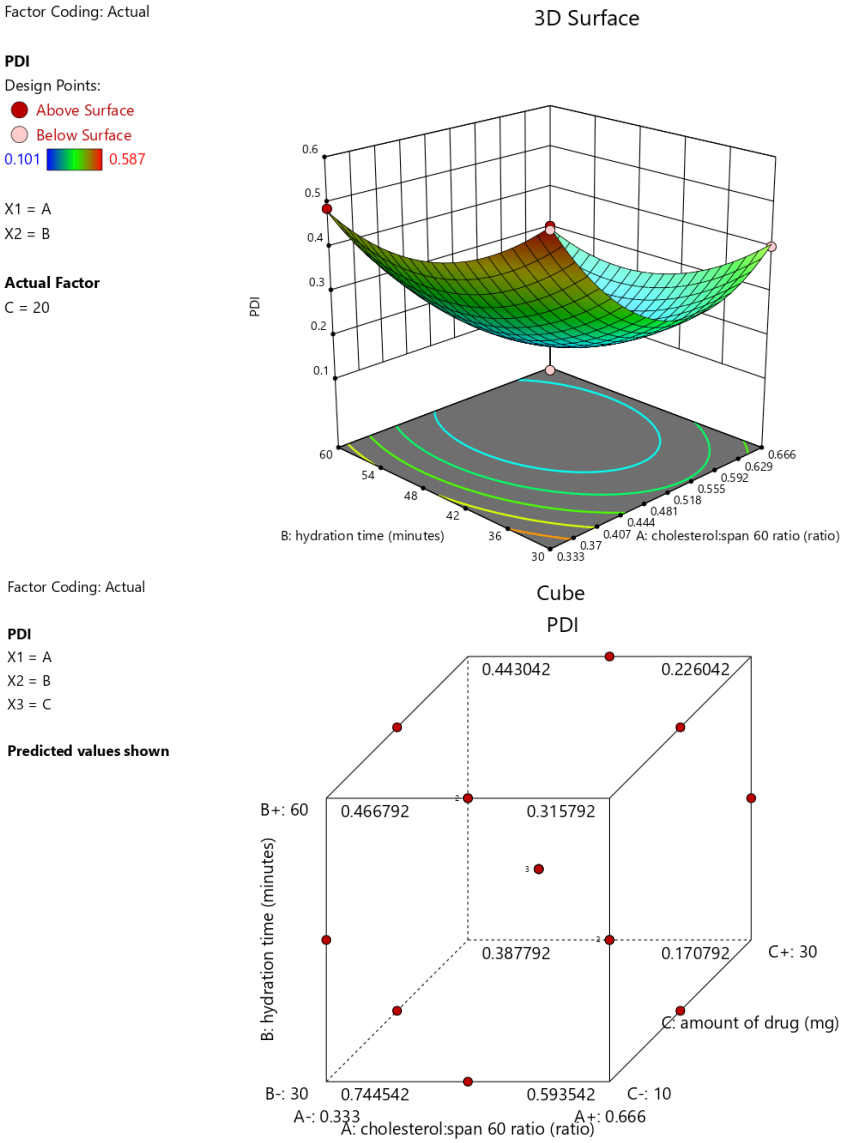
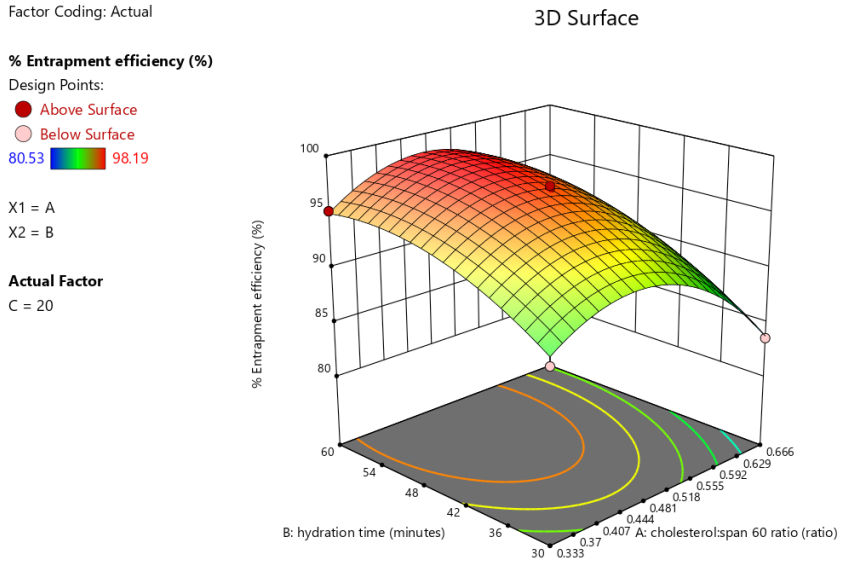


Figure 2. 3D response contour plots and a cubic plot displaying the effect of factors on PDI



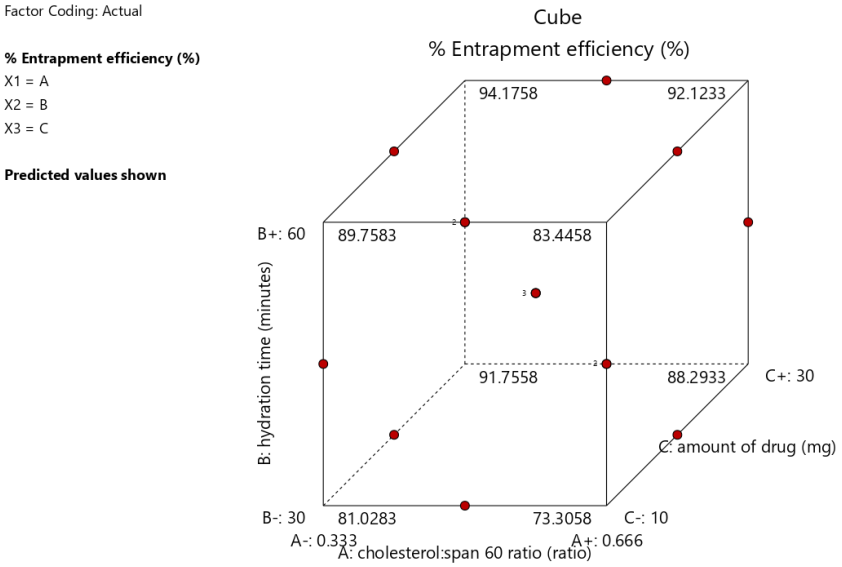


Figure 3. 3D response contour plots and a cubic plot displaying the effect of factors on %EE

3.2 Characterization of Cyclosporine Niosomes

3.2.1 Impact of factors on the size of niosome

To study the impact of factors on the niosome size, the result derived from the DLS technique (shown in **Table 2**) were applied using DES. From the Analysis of variance (ANOVA) test, it was found that quadratic model was significant (*P*-value < 0.0001) and lack of fit was not significant (*P*-value = 0.124), for the response particle size. The final equation obtained in terms of coded value is given below:

$$\text{Size}(Y_1) = +63.83 - 27.14X_1 - 36.76X_2 + 7.82X_3 + 12.14X_1X_2 - 11.67X_1X_3 + 0.1025X_2X_3 + 27.58X_1^2 + 28.78X_2^2 + 35.67X_3^2$$

A significant impact of factor X_1 (cholesterol: span 60) on the niosome size was observed (*P*-value < 0.0001). With an increase in the concentration of surfactant, the size also increased. In the previous studies, similar observations are reported[21]. In another way we can say that with an increase in concentration of cholesterol the size decreased[29]. This enhancement in the size of niosomes with increasing span 60 concentration may be because of the increasing entrapment efficiency. As we have already mentioned that low HLB of span 60 (4.7), its saturated alkyl chain and high gel transition temperature favors high entrapment efficiency.

Effect of factor X_2 (hydration time) on the size was significant (*P*-value < 0.0001). An increase in the duration of hydration results in the decreased size of niosomes. The results are similar to the earlier studies[30]. This may be a result of swelling of the lipoidal bilayer which leads to the disrupted vesicular structure[18].

Factor X_3 (amount of drug) also showed a significant (*P*-value = 0.0189) impact on the size of niosome. An increase in the amount of drug results in the increased size of niosomes. This could be due to the availability of more amount of drugs in the core, which causes the extension of the bilayer structure.

3.2.2 Impact of factors on the PDI of niosomes

The ANOVA test was applied on the DLS data of PDI, which indicates the quadratic model as significant (*P*-value = 0.0004) and lack of fit not significant (*P*-value = 0.9744). The final equation for PDI in terms of coded value is given below:

$$\text{PDI}(Y_2) = +0.1757 - 0.0920X_1 - 0.0556X_2 + 0.1116X_3 + X_1X_2 - 0.0165X_1X_3 + 0.0832X_2X_3 + 0.1788X_1^2 + 0.0905X_2^2 - 0.0265X_3^2$$

The PDI was significantly (*P*-value < 0.05) affected by each of the three factors X_1, X_2, and X_3. A negative coefficient for the factor X_1 (cholesterol: span 60 ratio) indicates that as the amount of cholesterol increases PDI decreases. In the earlier articles it is reported that a PDI value of less than 0.3 indicates a monodisperse

system[31,32].

A negative coefficient for the factor X_2 (hydration time) suggests that with an improvement in the hydration time PDI decreases. Similar results were also observed in the previous studies[32,33]. As discussed above, with increase in hydration time size also decreases, the small size vesicles make a more uniform dispersion, hence reduced PDI.

The third factor produces a synergistic effect on the PDI as already reported in the previous studies[34]. As the amount of drug increases PDI also increases. This may be because increased amount of drug results in the drug leakage, extension of the membrane, and hence non-uniformity in the size[35].

3.2.3 Impact of factors on %EE

During optimization the constraints were applied to the maximum %EE of cyclosporine. The % cyclosporine entrapped in the optimized niosomes was found to be 93.8%. The influence of formulation factors on responses was studied using DES. The test of ANOVA shows quadratic model was significant (*P*-value < 0.0001) and lack of fit was not significant (*P*-value = 0.0586). The final equation in terms of coded value was as shown below:

$$\%EE(Y_3) = +97.14 - 2.44X_1 + 3.14X_2 + 4.85X_3 + 0.3525X_1X_2 + 1.06X_1X_3 - 1.58X_2X_3 - 5.26X_1^2 - 2.17X_2^2 - 2.98X_3^2$$

With an increase in the cholesterol: span 60 ratio (factor X_1) the %EE of cyclosporine decreases (*P*-value = 0.0004). This is because higher lipid concentration leads to a saturated environment where the formation of a vesicle is prevented[30]. Since span 60 has low HLB and a smaller critical packing parameter, it needs a little quantity of cholesterol to form the lamellar structure[36].

The effect of factor X_2 (hydration time) was also significant (*P*-value = 0.0001) on the %EE of cyclosporine. With an increase in hydration time, %EE also increases because cyclosporine gets more time to enter in the bilayer structure of the niosomes.

A positive coefficient for factor X_3 (amount of drug) indicates a significant (*P*-value < 0.0001) influence on the %EE. With an increase in the amount of drug, an increase in %EE might be due to the presence of saturated conditions, which results in an increased amount of cyclosporine in the niosomes.

3.3 Optimization of Formulation Using BBD

In comparison with the full factorial design the number of experiments required in BBD are less. Signal to noise ratio is measured by adequate precision. Its value must be more than 4 to navigate the design space. For each of the 3 observed responses, the value obtained was above 4. The predicted R^2 value is an indicator of model's appropriateness in predicting a response value[21]. For all the three responses the difference between the predicted and adjusted R2 was less than 0.2, suggesting the two values are in reasonable agreement with each other. The optimized niosomes were prepared by using the formula, obtained by putting limits, on the size, PDI, and %EE. The desirability of the formula was 0.904. To prepare the optimized niosomes, a 0.456 ratio of cholesterol and span 60, 30 mg of cyclosporine, and hydration time of 30 minutes was used. The difference in the values of predicted and observed responses shows that the optimization process was valid. The regression summary statistics, for responses Y_1, Y_2, and Y_3 are given in **Table 3**.

Table 3. Regression summary statistics for responses Y_1, Y_2, and Y_3

Response	Model	Adequate Precision	R^2	Adjusted R^2	Predicted R^2	SD	%CV	P-value
Y_1	Quadratic	25.05	0.9924	0.9788	0.8879	6.47	5.73	< 0.0001
Y_2	Quadratic	18.46	0.9857	0.9601	0.9509	0.0326	10.7	= 0.0004
Y_3	Quadratic	26.33	0.9926	0.9794	0.8864	0.8308	0.9071	< 0.0001

3.4 TEM Analysis

The morphological characteristics of the niosomes were anlysed using TEM. Niosomes appear spherical and unaggregated in TEM pictures (**Figure 4**). The size of niosomes observed by TEM is similar to the results obtained by DLS analysis.

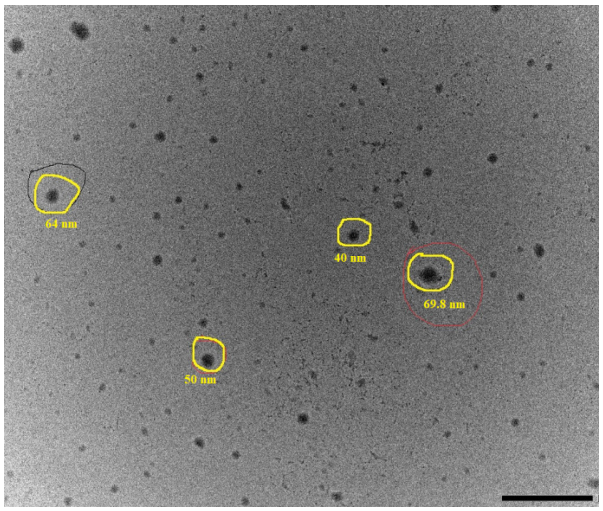


Figure 4. Showing TEM image of optimized cyclosporine loaded niosomes

3.5 FTIR Spectroscopy and P-XRD Analysis

FTIR studies were performed for the identification and compatibility analysis of cyclosporine API, cholesterol, span 60 and cyclosporine niosomes, respectively (**Figure 5**). The FTIR spectra of cyclosporine niosomes confirms the presence of cyclosporine in the niosomes since characteristic peaks were present at the same intensity. In the spectra of cholesterol two characteristic peaks were seen in the range of 2800 cm^{-1} to 3500 cm^{-1}, which corresponds to the stretching vibration of the methyl group for the C-H bond and vibrations for cyclic hydrocarbons. FTIR spectra of cyclosporine shows characteristic peaks at 2875.51 cm^{-1} (C-C stretching)[9], 1622 cm^{-1} (C=O) stretching vibration, and 3313.37 cm^{-1} (NH stretching)[10]. The low noise ratio, also confirm the purity of the niosomes.

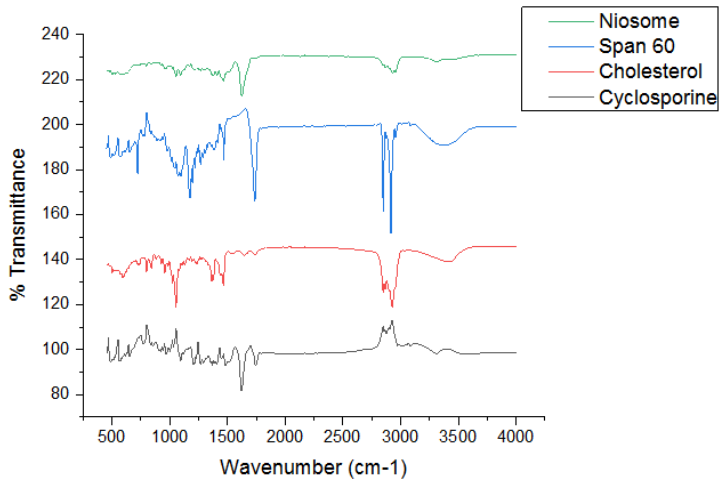


Figure 5. FTIR spectra of cholesterol, Span 60, cyclosporine, and optimized cyclosporine loaded niosomes

The absence of sharp peaks of cyclosporine in the P-XRD graph of cyclosporine-laden niosomes might be due to the formation of niosomal structure, entrapment of drug inside this structure[36], and transformation of cyclosporine from crystalline to the amorphous state (**Figure 6**)[22].

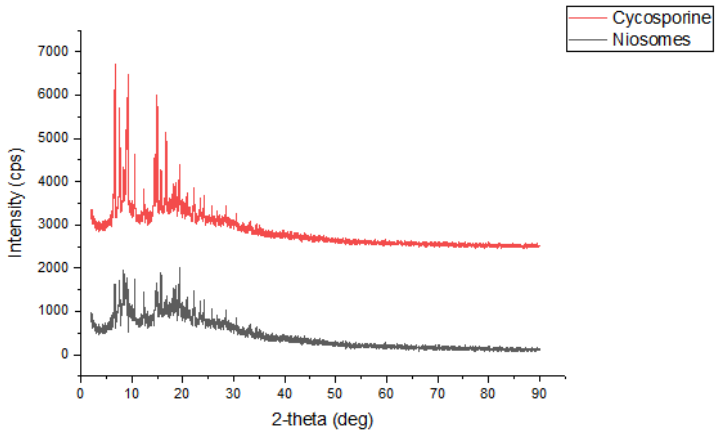


Figure 6. P-XRD of cyclosporine and optimized cyclosporine loaded niosomes

3.6 *Ex-vivo* Permeation Experiment

The amount of cyclosporine permeated across the excised goat skin was significantly higher for the cyclosporine niosomes (13.19%) than for control drug dispersion of cyclosporine (6.21%) for the duration of the experiment. The better permeation results obtained from the niosomes, may be due to the availability of non-ionic surfactant and it's also observed in the earlier studies that the structure of niosomes also aids in the permeation[25]. The percent cyclosporine permeated across the skin from niosomes was found to be very less but it is clearly evident from the comparative permeation data of niosomes and dispersion that niosomes were able to improve the permeation through the skin. Results of permeation are shown in **Figure 7**. Permeation of cyclosporine from the dispersion of API was very less because it's a lipophilic drug with high molecular weight. We can relate the overall poor permeation of cyclosporine through the skin with the size of niosomes. Since, as the size of vesicle increases the permeation into and across the skin decreases. Vesicles having size larger than 600 nm usually remain on the SC, less than 300 nm can reach to inner layers in a limited amount whereas vesicles with size below 70 nm can significantly accumulate in the inner tissues and can permeate through the skin[37,38].

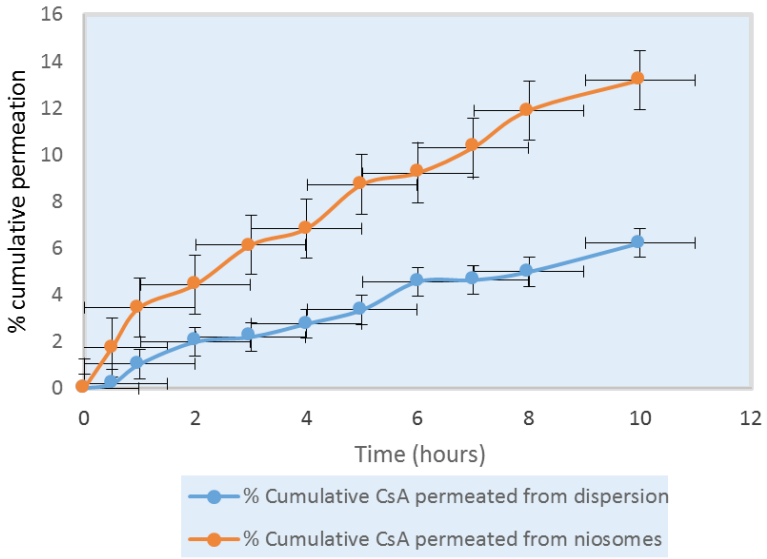


Figure 7. Permeation of cyclosporine across the skin from dispersion and niosomes

The permeation rate (J) was determined using the permeation data. Cyclosporine's permeation rates from dispersion and niosomes were determined to be 23.4±1.5 g/h and 65.95±4.9 g/h, respectively. This indicates that niosomes were able to improve permeation by around three times.

3.7 Skin Deposition Experiments

The findings of the skin deposition experiment for optimized cyclosporine niosome and suspension of cyclosporine are displayed in **Figure 8**. In the present experiment the amount of cyclosporine available in the SC and accumulated in viable dermis i.e. epidermis + dermis (E+D), along with the drug remaining over the skin was measured. It could be clearly seen in the **Figure 8**, that in the case of cyclosporine suspension, most of the cyclosporine was remaining over the skin. And the percentage of cyclosporine accumulated in the SC and E+D was also very less, only 3.164% and 7.35%, respectively. But the percentage of cyclosporine accumulated in SC and E+D for niosomal cyclosporine was found to be 15.79% and 35.84%, respectively. Accumulation of cyclosporine by niosomes was significantly more than the plain suspension of the cyclosporine API.

The amount of cyclosporine deposited in the skin's layers was substantially higher than that of the permeated amount. In the earlier research, it was observed that niosomes generally enhance the permeation of drugs in the epidermal areas but not in the systemic circulation and deeper skin tissues[21,39]. The percent of drug deposited from the niosomes in the SC and VED was found to be significantly greater

than from the dispersion. Niosomes effectively diffuse across the skin after getting adsorbed to the skin's surface, causing considerable drug deposition in the skin.

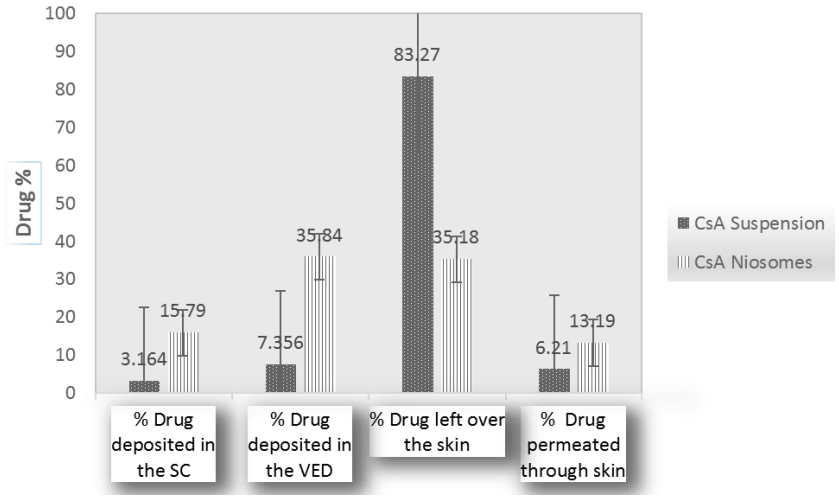
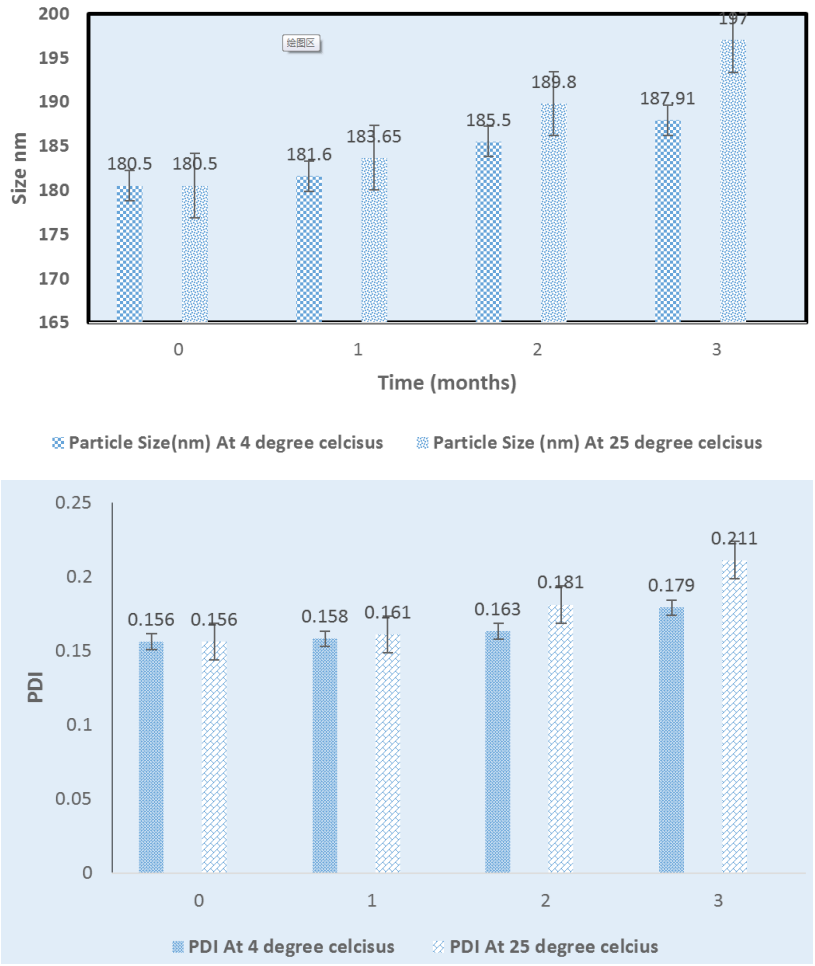


Figure 8. Skin deposition study of drug solution (control) and cyclosporine niosomes

3.8 Stability Studies

Results obtained from stability studies are shown in **Figure 9**. The data obtained from the study shows that niosomes are more stable at 4 °C as compared to 25 °C. Niosmes formed aggregates on ageing but those were found to be dispersible.



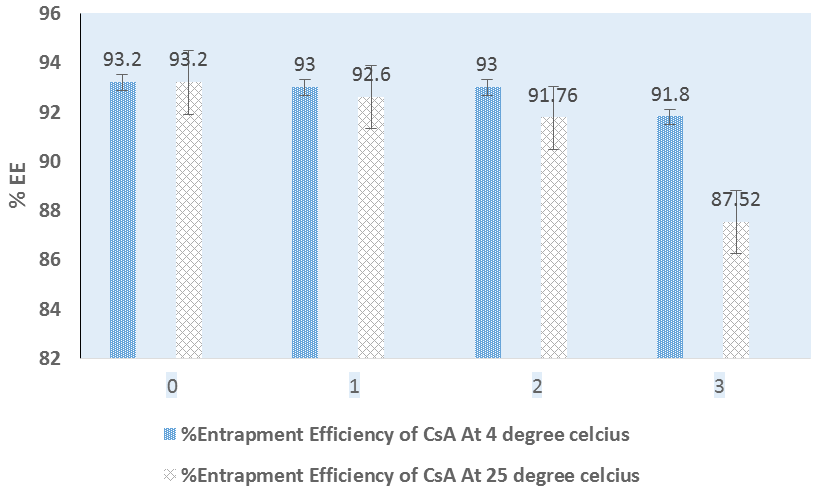


Figure 9. Stability data of optimized niosomes at 4 °C and 25 °C

3.9 *In-vivo* Studies in Imiquimod Induced Anti-psoriatic Mice

The imiquimod-induced antipsoriatic plaque model, a very well-known, rapid, and reliable method for the preclinical studies in psoriasis, was utilized for in-vivo activity assessment[19]. The changes brought about by imiquimod treatment resembled those of psoriatic skin. The antipsoriatic effect was assessed by PASI and histopathology. For the entire period of study, group 1's PASI score was zero i.e. none. On the seventh day, all other groups revealed substantial variations from group 1. Between the seventh and fourteenth days, the PASI score for group 2 stayed in the 3-4 range. Between the seventh and fourteenth days, group 3's PASI score revealed considerable resembles with group 2 since it remained close to 3. Between the seventh and fourteenth days, group 4 has shown remarkable modifications in the PASI. The PASI was approximately 4 on the seventh day, gradually falling to less than 1 on the fourteenth day, an indication of full recovery.

Figure 10 displays images of skin types including normal, induced psoriatic, and skin that has been treated with various formulations. **Figure 10A** depicts the epidermis, dermis, and hair follicles of healthy, normal mouse skin. The skin in **Figure 10B** exhibits clear signs of psoriasis like keratinocyte hyperproliferation and an epidermal thickness. The effects of various formulations on psoriatic skin are depicted in **Figure 10C and 10D. Figure 10C** did not show any appreciable improvement in the stratum corneum and keratin layers of the skin. In **Figure 10D**, the group treated with cyclosporine niosomes displayed improvement in the restructured keratin layer (a).

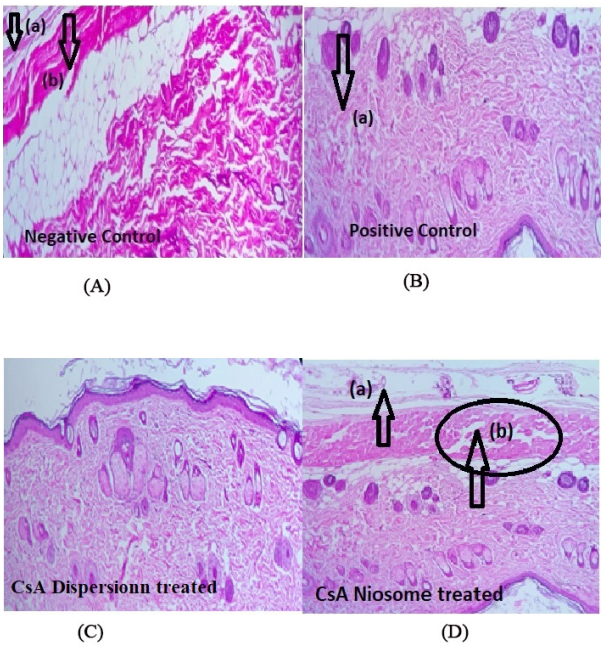


Figure 10. Histopathology of mice (A) Normal (Negative control)l mice skin showing the (a) layers stratum corneum followed by dense collagen tissues in dermis (b); (B) Imiquimod induced psoriaisis (positive control) untreated showing Hyperkeratosis with marked dermal hyperplasia; (C) Cyclosporine dispersion treated psoriasis skin showing a slight reduction in epidermal and dermal thickness; (D) Niosomes containing cyclosporine treated psoriasis skin showing the marked improvement and restructuring of stratum corneum and keratin layer

Cyclosporine-containing niosomes produced the best outcomes It was able to resolve some significant

aspects of psoriasis like the keratin and stratum corneum remodelling. Due to its huge molecular weight and extremely lipophilic character, cyclosporine was unable to permeate the skin when administered as a simple suspension and could not, therefore, have any appreciable antipsoriatic effects. The better results obtained for the niosomal cyclosporine were due to the permeation capability, small size of niosomes and presence of surfactant in the basic structure.

4. Conclusion

In the present research, cholesterol and span 60 were used to develop niosomes laden with cyclosporine for topical administration in psoriasis. To get the highest %EE and suitable particle size, the developed niosomes were characterized and optimized by employing BBD. The optimized niosomes were evaluated further using FTIR, TEM, and XRD approaches, and it proved to be suitable for dermal delivery. *Ex-vivo* permeation was performed using excised goat skin, and it was found that the drug's ability to pass through the skin using niosomes was significantly improved. From the skin deposition studies, it was found that the cyclosporine accumulated in the SC and E+D for niosomal formulation was much more than its suspension. Further, the niosomes turned out to be stable after three months of storage stability tests at 40 ^{o}C and room temperature. In the *in-vivo* studies, the best results in subsiding psoriasis like features was produced by the group administered with cyclosporine-loaded niosomes. Hence, the prepared niosomes laden with cyclosporine are effective for its dermal delivery.

Author's Contributions

Conceptualization: Bhardwaj P and Tripathi P
Methodology: Pandey S and Chaurassia D
Software: Pandey S and Tripathi P
Formal analysis and investigation: Tripathi P
Resources: Chaurasia D and Ramchandra Patil P
Writing-original draft preparation: Tripathi P and Pandey S
Supervision and project administration: Bhardwaj P

Funding Sources

This research work did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ethics Statement

Not applicable.

Consent for publication

Not applicable.

Availability of Supporting Data

The data presented in this study are available in the article.

Conflict of Interest

Declaration of interest: none.

References

[1] Tripathi P and Bhardwaj P. Psoriasis: an autoimmune disorder. *Journal of Drug Delivery and Therapeutics*, 2020;10(5):316-324. https://doi.org/10.22270/jddt.v10i5.4327

[2] Christophers E. Psoriasis-epidemiology and clinical spectrum. *Clinical and Experimental Dermatology*, 2001;26(4):314-320. https://doi.org/10.1046/j.1365-2230.2001.00832.x

[3] Nestle FO, Kaplan DH and Barker J. Mechanisms of disease: psoriasis. *New England Journal of Medicine*, 2009;361(5):496-509.

[4] Wolf M and Shnyra A. Autoimmune mechanisms of psoriasis: pathogenic role of the IL-23/IL-17 axis. J Autoimmune Disord, 2018;4(1):5. https://doi.org/10.21767/2471-8513.100057

[5] Greaves MW and Weinstein GD. Treatment of psoriasis. *New England Journal of Medicine*, 1995;332(9):581-589. https://doi.org/10.1056/NEJM199503023320907

[6] Pandey S, Tripathi P, Gupta A, *et al*. A comprehensive review on possibilities of treating psoriasis using dermal cyclosporine. *Drug Delivery and Translational Research*, 2022;12:1541-1555. https://doi.org/10.1007/s13346-021-01059-5

[7] Fradin MS, Ellis CN and Voorhees JJ. Efficacy of cyclosporin A in psoriasis: a summary of the United States' experience. *British Journal of Dermatology*, 1990;122(s36):21-25. https://doi.org/10.1111/j.1365-2133.1990.tb02878.x

[8] Tedesco D and Haragsim L. Cyclosporine: a

review. *Journal of Transplantation*, 2012;2012. https://doi.org/10.1155/2012/230386

[9] Guada M, Sebastián V, Irusta S, *et al*. Lipid nanoparticles for cyclosporine A administration: development, characterization, and in vitro evaluation of their immunosuppression activity. *International Journal of Nanomedicine*, 2015;6541-6553. https://doi.org/10.2147/IJN.S90849

[10] Essaghraoui A, Belfkira A, Hamdaoui B, *et al*. Improved dermal delivery of cyclosporine a loaded in solid lipid nanoparticles. *Nanomaterials*, 2019;9(9):1204. https://doi.org/10.3390/nano9091204

[11] Jain S, Mittal A and Jain AK. Enhanced topical delivery of cyclosporin-A using PLGA nanoparticles as carrier. *Current Nanoscience*, 2011;7(4):524-530. https://doi.org/10.2174/157341311796196835

[12] Boinpally RR, Zhou SL, Devraj G, *et al*. Iontophoresis of lecithin vesicles of cyclosporin A. *International Journal of Pharmaceutics*, 2004;274(1-2):185-190. https://doi.org/10.1016/j.ijpharm.2004.01.016

[13] Liu H, Li S, Wang Y, *et al*. Effect of vehicles and enhancers on the topical delivery of cyclosporin A. *International Journal of Pharmaceutics*, 2006;311(1-2):182-186. https://doi.org/10.1016/j.ijpharm.2005.12.029

[14] Lopes LB, Collett JH and Bentley MVLB. Topical delivery of cyclosporin A: an in vitro study using monoolein as a penetration enhancer. *European Journal of Pharmaceutics and Biopharmaceutics*, 2005;60(1):25-30. https://doi.org/10.1016/j.ejpb.2004.12.003

[15] Arora R, Katiyar SS, Kushwah V, *et al*. Solid lipid nanoparticles and nanostructured lipid carrier-based nanotherapeutics in treatment of psoriasis: a comparative study. *Expert Opinion on Drug Delivery*, 2017;14(2):165-177. https://doi.org/10.1080/17425247.2017.1264386

[16] Jeong HR, Kim JY, Kim SN, *et al*. Local dermal delivery of cyclosporin A, a hydrophobic and high molecular weight drug, using dissolving microneedles. *European Journal of Pharmaceutics and Biopharmaceutics*, 2018;127:237-243. https://doi.org/10.1016/j.ejpb.2018.02.014

[17] Walunj M, Doppalapudi S, Bulbake U, *et al*. Preparation, characterization, and in vivo evaluation of cyclosporine cationic liposomes for the treatment of psoriasis. *Journal of Liposome Research*, 2020;30(1):68-79. https://doi.org/10.1080/08982104.2019.1593449

[18] Benigni M, Pescina S, Grimaudo MA, *et al*. Development of microemulsions of suitable viscosity for cyclosporine skin delivery. *International Journal of Pharmaceutics*, 2018;545(1-2):197-205. https://doi.org/10.1016/j.ijpharm.2018.04.049

[19] Prasad V, Kumar N and Mishra PR. Amphiphilic gels as a potential carrier for topical drug delivery. *Drug Delivery*, 2007;14(2):75-85. https://doi.org/10.1080/10717540600642431

[20] Bhardwaj P, Tripathi P, Gupta R, *et al*. Niosomes: a review on niosomal research in the last decade. *Journal of Drug Delivery Science and Technology*, 2020;56:101581. https://doi.org/10.1016/j.jddst.2020.101581

[21] Abdelbary AA and AbouGhaly MHH. Design and optimization of topical methotrexate loaded niosomes for enhanced management of psoriasis: application of Box-Behnken design, in-vitro evaluation and in-vivo skin deposition study. *International Journal of Pharmaceutics*, 2015;485(1-2):235-243. https://doi.org/10.1016/j.ijpharm.2015.03.020

[22] Abu Hashim II, Abo El-Magd NF, El-Sheakh AR, *et al*. Pivotal role of Acitretin nanovesicular gel for effective treatment of psoriasis: ex vivo–in vivo evaluation study. *International Journal of Nanomedicine*, 2018;13:1059-1079. https://doi.org/10.2147/IJN.S156412

[23] Moghddam SRM, Ahad A, Aqil M, *et al*. Formulation and optimization of niosomes for topical diacerein delivery using 3-factor, 3-level Box-Behnken design for the management of psoriasis. *Materials Science and Engineering: C*, 2016;69:789-797. https://doi.org/10.1016/j.msec.2016.07.043

[24] Zhu B, Jing M, Yu Q, *et al*. Treatments in psoriasis: from standard pharmacotherapy to nanotechnology therapy. *Advances in Dermatology and Allergology/Postępy Dermatologii i Alergologii*, 2022;39(3):460-471. https://doi.org/10.5114/ada.2021.108445

[25] Pandey SS, Shah KM, Maulvi FA, *et al*. Topical

delivery of cyclosporine loaded tailored niosomal nanocarriers for improved skin penetration and deposition in psoriasis: Optimization, ex vivo and animal studies. *Journal of Drug Delivery Science and Technology*, 2021;63:102441. https://doi.org/10.1016/j.jddst.2021.102441

[26] Agarwal R, Katare OP and Vyas SP. Preparation and in vitro evaluation of liposomal/niosomal delivery systems for antipsoriatic drug dithranol. *International Journal of Pharmaceutics*, 2001;228(1-2):43-52. https://doi.org/10.1016/S0378-5173(01)00810-9

[27] Lopes LB, Lopes JLC, Oliveira DCR, *et al*. Liquid crystalline phases of monoolein and water for topical delivery of cyclosporin A: characterization and study of in vitro and in vivo delivery. *European Journal of Pharmaceutics and Biopharmaceutics*, 2006;63(2):146-155. https://doi.org/10.1016/j.ejpb.2006.02.003

[28] Ghate VM, Kodoth AK, Shah A, *et al*. Colloidal nanostructured lipid carriers of pentoxifylline produced by microwave irradiation ameliorates imiquimod-induced psoriasis in mice. *Colloids and Surfaces B: Biointerfaces*, 2019;181:389-399. https://doi.org/10.1016/j.colsurfb.2019.05.074

[29] Radhi AA. Benazepril hydrochloride loaded niosomal formulation for oral delivery: Formulation and characterization. *International Journal of Applied Pharmaceutics*, 2018;10:66-70. http://dx.doi.org/10.22159/ijap.2018v10i5.27564

[30] Yeo LK, Chaw CS and Elkordy AA. The effects of hydration parameters and co-surfactants on methylene blue-loaded niosomes prepared by the thin film hydration method. *Pharmaceuticals*, 2019;12(2):46. https://doi.org/10.3390/ph12020046

[31] Hao Y, Zhao F, Li N, *et al*. Studies on a high encapsulation of colchicine by a niosome system. *International Journal of Pharmaceutics*, 2002;244(1-2):73-80. https://doi.org/10.1016/S0378-5173(02)00301-0

[32] Aboul-Einien MH, Kandil SM, Abdou EM, *et al*. Ascorbic acid derivative-loaded modified aspasomes: formulation, in vitro, ex vivo and clinical evaluation for melasma treatment. *Journal of Liposome Research*, 2020;30(1):54-67. https://doi.org/10.1080/08982104.2019.1585448

[33] Priya S, Koland M and Suchetha KN. Formulation and characterization of ropinirole hydrochloride loaded solid lipid nanoparticles. *International Journal of Pharmacy and Pharmaceutical Sciences*, 2015;7(9):85-89.

[34] Zainol S, Basri M, Basri HB, *et al*. Formulation optimization of a palm-based nanoemulsion system containing levodopa. *International Journal of Molecular Sciences*, 2012;13(10):13049-13064. https://doi.org/10.3390/ijms131013049

[35] Pagar K and Vavia P. Rivastigmine-loaded L-lactide-depsipeptide polymeric nanoparticles: decisive formulation variable optimization. *Scientia Pharmaceutica*, 2013;81(3):865-888. https://doi.org/10.3797/scipharm.1211-20

[36] Manosroi A, Wongtrakul P, Manosroi J, *et al*. Characterization of vesicles prepared with various non-ionic surfactants mixed with cholesterol. *Colloids and Surfaces B: Biointerfaces*, 2003;30(1-2):129-138. https://doi.org/10.1016/S0927-7765(03)00080-8

[37] Aghajani A, Kazemi T, Enayatifard R, *et al*. Investigating the skin penetration and wound healing properties of niosomal pentoxifylline cream. *European Journal of Pharmaceutical Sciences*, 2020;151:105434. https://doi.org/10.1016/j.ejps.2020.105434

[38] Verma DD, Verma S, Blume G, *et al*. Particle size of liposomes influences dermal delivery of substances into skin. *International Journal of Pharmaceutics*, 2003;258(1-2):141-151. https://doi.org/10.1016/S0378-5173(03)00183-2

[39] Shahiwala A and Misra A. Studies in topical application of niosomally entrapped nimesulide. *Journal of Pharmacy & Pharmaceutical Sciences*, 2002;5(3):220-225.