International Journal of Science and Research (IJSR)
ISSN: 2319-7064
Impact Factor 2025: 7.089

Development and Assessment of Nanoparticles: A Clarithromycin Niosomal Gel for Atopic Dermatitis

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Abstract: ***Atopic dermatitis (AD), also called atopic eczema, is a persistent inflammatory skin condition characterized by eczematous dermatitis, irritated, itchy skin, breakdown of the epidermal barrier, activation of t-cells, and imbalance in the microbiome. AD affects 2.1 to 4.1% of adults globally, posing challenges due to its relapsing nature. Clarithromycin was used to treat the AD but failed due to low solubility and permeability on the target site. The present study aims to develop and evaluate the niosomal gel containing clarithromycin is used for the cure of atopic dermatitis. The niosomal suspension was optimized by using a design expert and the optimized formulation was characterized with the help of drug excipients compatibility, morphology, particle size, zeta potential, entrapment efficiency, drug loading, and in-vitro drug release which was found to be suitable for incorporation into the Carbopol-based gel to formulate CLR-N-Gel 0.1% w/w. The entrapment efficiency, drug loading, and cumulative drug release of niosomal suspension were found to be 86.17%, 23.16%, and 82.39% respectively. The suitable niosomal suspension was incorporated in carbopol-based gel and characterization has been done like physical appearance, pH, viscosity, spreadability, drug content, and in-vitro drug release of prepared CLR-N-Gel. The drug content of CLR-N-Gel was found to be 96 ± 2.16 %, cumulative drug release was found to be 80.74 %, and cumulative drug permeability was found to be 77.20%. The in-vivo anti-dermatitis efficacy and histopathology were carried out to check the efficacy of prepared CLR-N-Gel 0.1% w/w and found that the developed CLR-N-Gel 0.1% w/w gel showed fast recovery and better therapeutic efficacy with low concentration better skin permeation and prolonged drug release.***

Keywords: Atopic dermatitis, clarithromycin, Optimization, nanoparticles, niosomal gel

1. Introduction

1.1 Atopic dermatitis

Atopic dermatitis (AD), also called atopic eczema, is a chronic inflammatory skin condition characterised by itchy, irritated skin, eczematous dermatitis, and a weakened epidermal barrier. The condition primarily affects children, with prevalence rates ranging from 15% to 30%, while adult cases are estimated to be between 2% and 10%. Type 2 helper T (Th2) lymphocytes drive AD pathogenesis by releasing cytokines and increasing IgE production and skin inflammation. Furthermore, Th17 and Th22 cells contribute to the immune response. AD is frequently associated with other allergic conditions, including food allergies, asthma, and allergic rhinitis. Itching and scratching can exacerbate the condition, resulting in a cycle of skin damage (1)(2).

A cure for atopic dermatitis (AD) has yet to be discovered. The primary goal of treatment is to manage symptoms through primary skin care, long-term use of emollients, topical corticosteroids, and antibiotics for moderate cases. Itching, also known as pruritus, is a common symptom that can cause significant discomfort and disrupt sleep (3)(4). AD can also cause various types of dermatitis, such as topical dermatitis, contact dermatitis, allergic contact dermatitis, irritant contact dermatitis, neurodermatitis, and dyshidrotic dermatitis. This dermatitis shares characteristics such as skin inflammation, irritation, rashes, and the formation of fluid-filled blisters containing lipid pus. Although treatment can help with these symptoms, a cure for Alzheimer's disease remains elusive (5)(6).

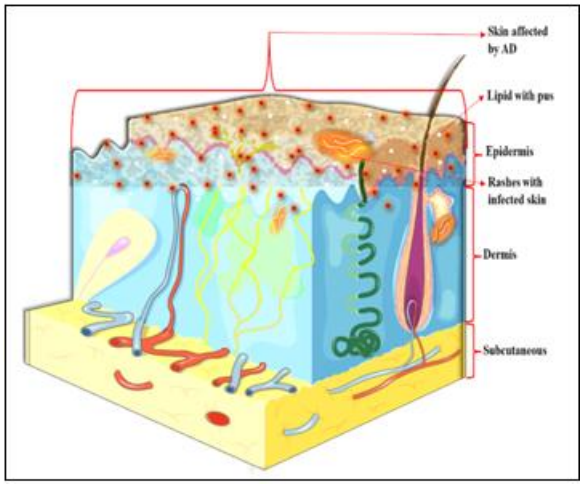

Figure 1: Atomic dermatitis of Epidermis layer of skin

1.2 Clarithromycin

Clarithromycin is a broad-spectrum, second-generation macrolide antibiotic used to treat various infections, including Mycobacterium avium complex (MAC) infections in people with HIV. It works by inhibiting protein synthesis, specifically by binding to the 50S ribosomal subunit and blocking the translocation of mRNA. This results in bacteriostatic activity but can be bactericidal at high concentrations (7). Clarithromycin is often used to treat skin infections and other bacterial conditions. However, its oral bioavailability is only about 55% due to poor aqueous solubility. The drug has a terminal half-life of 3–4 hours, requiring regular dosing. Encapsulating clarithromycin in a vesicular system may improve its bioavailability and extend its circulation time for controlled release (8).

1.3 Niosomes

Niosomes are advanced drug delivery systems composed of non-ionic surfactants, such as dialkyl polyglycerol or dialkyl polyglycerol ethers, mixed with cholesterol and hydrated in water. These nanometer-sized vesicles have a bilayer structure with hydrophilic ends on the surface and inside and hydrophobic chains facing inward. Niosomes are especially effective at targeting the reticuloendothelial system, making them useful for administering vaccines and medications (9). They increase the permeability and bioavailability of weakly water-soluble drugs, resulting in significant improvements in topical drug delivery. Because of their small size, niosomes maintain localised drug activity, increase residence time in the stratum corneum, and reduce epithelial penetration (10). They help to prevent drug degradation and side effects while increasing bioavailability and targeting specific areas of pathology. Furthermore, surfactants in niosomes act as penetration enhancers by dissolving mucus layers and disrupting functional complexes, with the vesicle's properties influencing its effectiveness (11)(12).

2. Materials and Methods

2.1 Materials

Clarithromycin, Carbopol-940, Span-60, Cholesterol, Propylparaben, Propyl methylene, PEG 400, 1-Chloro-2,4-dintrobenzene, Sodium bromide, Sodium chloride, Potassium dihydrogen phosphate, Sodium hydroxide, Chloroform, Ethanol, Methanol, Acetone.

2.2 Animals

The 150 to 200 grams of 24 female albino rats are needed.

2.3 Methods

2.3.1 Optimization of CLR-Niosomes:

Design expert:
To optimise niosome formulation, key parameters such as the drug: cholesterol: surfactant ratio and rotation speed were considered. The concentrations of span-60 (25-35 mg), cholesterol (8-12 mg), and rotation speed (80-120 rpm) were chosen as critical material attributes (CMA). A Box-Behnken design (BBD) with a full 23 factorial design was used to investigate the effect of these variables on the critical quality attributes (CQAs) of niosomes. Seventeen runs were performed to create optimised batches, with particle size, zeta potential, and drug entrapment efficiency identified as the three main responses, which were analysed using ANOVA. The interactions between these formulation parameters were investigated in order to optimise the output. Based on the results, the design expert predicted the best formulation, which was confirmed by the response analysis. The optimised niosome formulation was characterised using these critical parameters to ensure the desired quality attributes (13)(14)(15).

2.3.2 Preparation of CLR-Niosomes:

The niosomal formulation was prepared using the thin film hydration method, employing cholesterol as the lipid and Span-60 as the surfactant. The surfactant, cholesterol, and drug were dissolved in 10 ml of chloroform, which was then transferred to a 100 ml round-bottom flask. The solvent was evaporated using a rotating vacuum evaporator under reduced pressure at 60±2°C until a thin lipid film formed. This film was then hydrated with 10 ml of phosphate buffer saline (pH 7.4) and further hydrated for 2.5 hours at 60±2°C with optimal rotation speed. The resulting niosomal suspension contained both free and entrapped drugs of various sizes and zeta potentials. To ensure proper formulation, the mixture underwent 15 minutes of bath sonication in a Rivotek bath sonicator, followed by an additional 15 minutes of probe sonication with a Hydro 200 µm probe sonicator. This process helped refine the niosomal suspension for further analysis (16)(17).

2.3.3 Characterization of CLR-Niosomes:

Particle size and distribution:
The particle size of the niosomal suspension formulation was analyzed using a Lite-Sizers-500 particle size analyzer. The average particle size was measured (18).

Zeta potential of CLR-Niosomal suspension:
The surface charge of the niosomal formulation was quantified for zeta potential analysis by using lite sizer-500 (18).

Drug content CLR-niosomal suspension:
The drug content of CLR-N was analyzed in a UV-visible spectrophotometer UV-1800) at the wavelength 207 nm.

Drug entrapment efficiency and drug loading:
The entrapment efficiency (% EE) and drug loading (DL) of CLR-N were determined using the centrifugation method. A known quantity of CLR-N suspension was dissolved in phosphate buffer (pH 5.5) with 10% methanol and then filtered using a 0.45 µm membrane filter. The filtered suspension was centrifuged, and the drug content was quantified spectrophotometrically using a Shimadzu-1800 at a wavelength of 207 nm. This method allowed for the accurate determination of the entrapment efficiency and drug loading in the CLR-N formulation. The drug entrapment and drug loading were calculated by using the following formula (19)(20).

$$\%\,EE = \frac{W_{total\ drug} - W_{free\ drug}}{W_{total\ drug}} \times 100$$

And,

$$\%\,DL = \frac{Concentration\ of\ drug}{Drug + Polymer} \times 100$$

Where, $W_{total\ drug}$= Weight of total drug present in the niosomes, $W_{free\ drug}$= Weight of free drug present in the niosomes

Stability study:
The stability of the optimized CLR-N formulation was evaluated under two different storage conditions: 2°C to 8°C and 25°C (room temperature), away from sunlight, over a 90-day period. During this time, the mean particle size and zeta potential of the CLR-N suspension were regularly monitored to assess any changes in the formulation's stability. This study helped determine the stability and potential degradation of the CLR-N formulation under varying environmental conditions (18).

In vitro drug release study:
The in-vitro drug release study for CLR-N was conducted using the dialysis membrane method and a Franz diffusion cell, which was thermoregulated at 37 ± 2°C. A 5 ml sample was withdrawn at specified time intervals, and an equal volume of phosphate buffer (pH 5.5) was added to the receiver compartment to maintain sink conditions. The collected samples were analyzed using a UV-visible spectrophotometer (UV-1800) at a wavelength of 207 nm. The study was performed over a 24-hour period to assess the drug release profile of CLR-N (21).

Release kinetic study:
The in-vitro release data of CLR-N were analyzed using various kinetic models to understand the drug release process and its kinetics. The models applied included zero-order, first-order, Higuchi model, and Korsmeyer-Peppas model. These models helped evaluate the release profile of the formulation and identify the best-fit model that accurately described the drug release behavior over time (21).

2.3.4 Preparation of CLR-N-Gel:
The Carbopol-based gel was prepared and optimized by evaluating its viscosity and appearance. Among the five different gel concentrations (G1 to G5), the G5 concentration was selected for the final formulation as it exhibited the desired viscosity. This concentration of the Carbopol-based gel was confirmed to be the most suitable for the intended application based on its optimal viscosity characteristics.

Table 1: Composition of CLR-N-Gel containing different concentrations of carbopol-940.

S. No.	Carbopol-940	Methylparaben	Propylene glycol	Ethanol	CLR-N	Triethanolamine	Water
G1	0.5 %	0.01 %	15 %	10 ml	q.s.	q.s.	q.s.
G2	1 %	0.01 %	15 %	10 ml	q.s.	q.s.	q.s.
G3	1.25 %	0.01 %	15 %	10 ml	q.s.	q.s.	q.s.
G4	1.50 %	0.01 %	15 %	10 ml	q.s.	q.s.	q.s.
G5	1.75 %	0.01 %	15 %	10 ml	q.s.	q.s.	q.s.

To prepare CLR-N-Gel (0.1% w/w), Carbopol-940 and methylparaben were weighed and dispersed in distilled water, then stirred continuously for a specific time until a uniform gel-like mass formed. The mixture was allowed to rest for 3 hours to ensure the proper swelling of the polymer. Afterward, the CLR-N suspension was added, and the mixture was stirred continuously. The pH was adjusted to 5.8 by adding Triethanolamine. Finally, the required amount of polyethylene glycol was incorporated into the formulation, and the gel was allowed to rest for 6 hours to complete the preparation process (20).

2.3.5 Evaluation of CLR-N-Gel:
Physical appearance and pH:
The physical appearance of drug-loaded gel was white with a uniform texture, and the pH was 5.7 ± 2 (19).

Spreadability:
To evaluate the spreadability of CLR-N-Gel, 0.5 gm of the gel was placed in a 1 cm diameter circle on a glass slab, and another clean glass slab was placed on top to cover the gel. The setup was allowed to rest for 4-5 minutes. The increase in diameter due to the spreading of the gel was then measured using a specific formula to assess its spreadability (20):

$$S = M \times \frac{l}{t}$$

Where M= mass tied to the upper plate, L= length of the plate, t = time taken.

Rheology study of CLR-n-Gel:
The viscosity of CLR-N-Gel was measured using a Brookfield viscometer (Ametek Brookfield India). The spindle number (R3-64) was set to a rotational speed of 50 rpm, and the measurements were conducted at a temperature of 25 ± 2°C. The shear rate was varied within the range of 5 to 50 s-1 to assess the gel's viscosity under different conditions (20).

Drug content of CLR-N-Gel:
The drug content of CLR-N-Gel was determined by weighing 1 gram of the gel and dissolving it in 100 ml of methanol in a volumetric flask. The solution was then analyzed using a UV-visible spectrophotometer (UV-1800) at a wavelength of 207 nm to quantify the drug content (19).

In vitro drug release study of CLR-N-Gel:
The in-vitro drug release study for CLR-N-Gel was conducted using the dialysis membrane method. CLR-N-Gel was enclosed in a dialysis membrane, secured with thread, and placed into a Franz diffusion cell, which was thermoregulated at 37 ± 0.5°C. At specified time intervals, 5 ml of the sample was withdrawn and replaced with an equal volume of receiver solution to maintain sink conditions. The collected samples were diluted with methanol and analyzed using a UV-visible spectrophotometer (UV-1800) at a wavelength of 207 nm. The study was conducted over a 24-hour period to assess the drug release profile (19).

2.3.6 Ex-Vivo drug permeation studies;

Processing of the pig ear skin:
A freshly excised pig ear was carefully cleaned under running water to remove any debris or excess tissue. The skin layer was then gently dried and tapped. The cleaned ear was immersed in a freshly prepared 2M sodium bromide solution and left at room temperature for 72 hours. After this incubation period, the epidermal layer was separated, rinsed thoroughly with distilled water, and preserved in a solution containing 0.9% NaCl and 1% formaldehyde. The preserved tissue was stored at 4°C until it was required for further study (19)(22).

Ex-Vivo permeation study using pig epidermis:
An Ex-Vivo permeation study was conducted using a modified Franz diffusion cell (Zenith Glassware Pvt. Ltd.) with an effective permeation area of 2.54 cm². The processed pig epidermis was mounted in the donor compartment, with the epidermal side facing the receptor compartment, which

contained a pH 5.8 buffer solution maintained at 37 ± 0.5°C. The donor compartment was filled with 1 g of CLR-N-Gel formulation and covered to prevent contamination. The receptor chamber was stirred at 150 rpm using a magnetic stirrer for 24 hours. At specified intervals, 5 ml of the sample was withdrawn from the receptor compartment, and an equal volume of buffer was added to maintain sink conditions. The amount of drug permeated through the epidermis was quantified spectrophotometrically at a wavelength of 207 nm (23).

Ex-Vivo drug permeation kinetic studies:

The Ex-vitro release data were analyzed to evaluate the release kinetics of the formulation using four different models: zero-order, first-order, Higuchi model, and Korsmeyer-Peppas model. The obtained values from these models were used to determine the release mechanism governing the drug release process for the prepared formulation (22).

Analysis of Transmission Electron Microscopy (TEM):

Transmission electron microscopy (JEM-200 CX, JEOL, Tokyo, Japan) at 80KV was used to characterize the prepared niosomal gel formulation for its shape after being stained, and a TEM micrograph was taken at an appropriate magnification (21).

2.3.7 In-Vivo evaluation prepared CLR-N-Gel:

Skin irritation study:

The skin irritation study was conducted in accordance with OECD 404 guidelines. The animals were randomly divided into three groups, and the dorsal areas were shaved using a trimmer. In the standard group, 0.8% (v/v) formaldehyde was applied as the skin irritant, while the prepared gel (approximately 6 cm^2) was applied to the test group. The animals were observed for signs of irritation, and the responses were scored at 60 minutes, as well as at 24, 48, and 72 hours post-application. The severity of irritation was graded and recorded using the Draize scoring method (22)(23).

Table 2: Skin irritation study based on the Draize scoring method.

S. No.	Score	Translation
1	0	No erythema and edema.
2	1	Very inappreciable erythema or edema.
3	2	Small edema with raised skin at the edges of the area.
4	3	Moderate to severe erythema or edema.
5	4	Severe erythema or edema.

In-Vivo Efficacy on DNCB-induced Atomic Dermatitis:

The study involved randomly dividing animals into four groups, with six animals in each group. A 4x4 cm^2 area of the rat's dorsal skin was shaved, and atopic dermatitis was induced by applying a 2% w/v solution of 2,4-dinitrochlorobenzene (DNCB) to the skin every three days for nine days. Symptoms of atopic dermatitis were monitored throughout the dosing interval. The control group was treated with 150 µl of saline water, while Group II received a 20 µl topical application of 1% w/w clarithromycin (standard formulation). Group III was treated with 20 µl of the prepared 0.1% w/w CLR-N-Gel formulation every 24 hours for six days. The severity of skin lesions was evaluated using a scale (0=none, 1=mild, 3=severe) on days 3, 5, 8, 11, and 15. After the study, the animals were sacrificed, and dorsal skin samples were fixed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E) and toluidine blue to assess skin lesions and mast cell count under a microscope (22).

Table 3: In-vivo assessment of developed niosomal formulation and standard formulation on atopic dermatitis-induced rat animal model:

Group		No. of Animals	Test compound to be Administered	Route of administered
I-	Normal	6	-	-
II-	Negative	6	2 % DNCB	Topical
III-	Standard	6	2 % DNCB + Clarithromycin ointment	Topical
IV-	Test	6	2 % DNCB + CLR-N-Gel	Topical

3. Results

3.1 Formulation, Evaluation, and Optimization of Clarithromycin-Loaded Niosomal Suspension

3.1.1 Preparation of CLR-loaded niosomal Suspension

The Niosomal suspension was prepared by the film hydration method by using a rotary vacuum evaporator (Cole-Parmer India Pvt. Ltd.).

3.1.2 Design of expert

The experiment was analyzed using Design-Expert software (Version 10.0.3) with a Box-Behnken design to evaluate 17 formulations. The software generated polynomial equations that described the main effects and interaction effects of the independent factors on each dependent variable. The results of the analysis, as shown in the table, provided insights into how the various factors influenced the outcomes of the formulations.

Table 4: Selected factors and observed responses from design experts.

Std.	Run	Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3
		A: Span-60	B: Cholesterol	C: rpm	P. Size	Z. P.	E. E.
		Mg.	Mg.	rpm	nm.	Mv.	%
14	1	30	10	100	190.6	-21.3	88.69
11	2	30	8	120	205.6	-20.9	82.04
1	3	25	8	100	202.8	-21.1	64.07
2	4	35	8	100	207.3	-20.8	68.05
7	5	25	10	120	203.1	-20.9	62.73

15	6	30	10	100	192.3	-21.2	88.69
4	7	35	12	100	203	-21.4	69.3
8	8	35	10	120	201.9	-21.5	76.72
17	9	30	10	100	197.7	-20.7	87.36
10	10	30	12	80	195	-21.6	84.7
5	11	25	10	80	207.4	-21.1	65.39
16	12	30	10	100	193.4	-20.9	87.36
6	13	35	10	80	201.9	-21.5	66.73
12	14	30	12	120	196.3	-21.6	83.37
3	15	25	12	100	196.6	-21.1	64.07
9	16	30	8	80	203.8	-21.1	69.38
13	17	30	10	100	194.1	-21.7	88.68

The particle size of the niosomal formulation was found to be in the range of 190.06 nm to 207.4 nm and zeta potential was found to be in the range of -20.8 to -21.7.

Table 5: Statistical analysis result of P. size, Z. potential, and E. efficiency

Particle size (F1)			Zeta Potential		E. Efficiency	
Source	Sum. of Squares	p-value Prob > F	Sum. of Squares	p-value Prob > F	Sum. of Squares	p-value Prob > F
Model	379.87	0.0032	0.41	0.0339	1498.01	<0.0001
A-Span-60	2.21	0.6239	-	75.28	75.28	0.0653
B-Cholesterol	102.24	0.0063	0.41	0.0339	40.05	0.1647
C-rpm	0.18	0.8879				
A^2	156.80	0.0016			1228.81	<0.0001
B^2	30.75	0.0882			108.95	0.0311
C^2	62.49	0.0226				
Residual	86.16		1.12		219.44	
Lack of Fit	58.37	0.3882	0.52	0.9390	217.33	0.0009
Pure Error	27.79		0.59		2.11	
Cor Total	466.03		1.52		1717.45	

3.1.3 Effect on Particle Size

The following equation illustrates the influence of various factors on the response variable, particle size (R1), as generated from the analysis:

P. Size = +193.62 + 0.53*A - 3.57*B – 0.15*C + 0.47*AB + 1.08*AC – 0.12BC + 6.10*A2 + 2.70*B 2 + 3.85*C2 ……. (eq. 6.1)

From Tables 4 and 5, the "prob > F" value was determined to be 0.0032, which is less than 0.0500, indicating that the Quadratic model is statistically significant. A "prob > F" value greater than 0.1000 would suggest that the model is not significant. In this analysis, the terms B, A^2, and C^2 were identified as significant model terms.

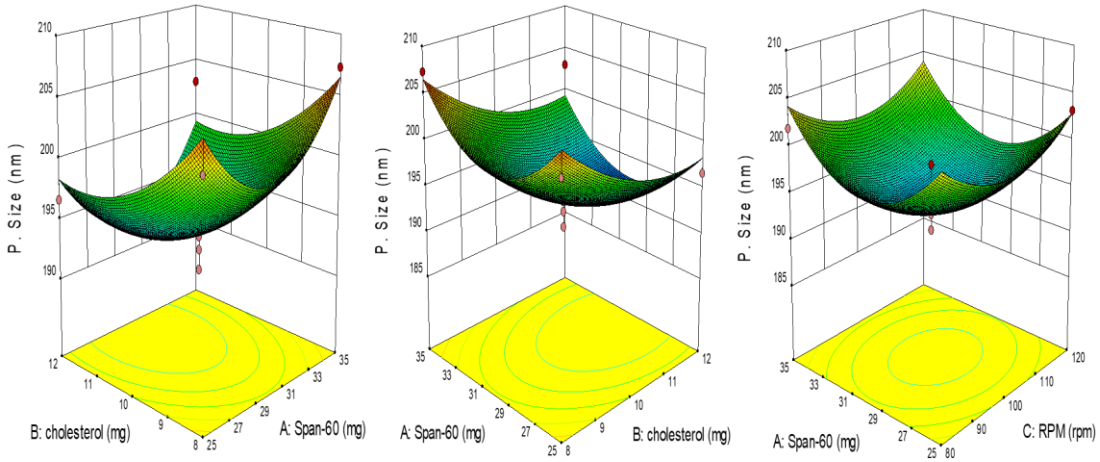

Figure 2: 3D-Analysis of particle size.

Effect on Zeta Potential

The following equation illustrates the influence of various factors on the response variable, particle size (R1), as determined from the analysis:

E.E.= +86.73 + 3.07*A + 2.24*B – 17.06*A^2 – 5.08*B^2

The determination of the value of "Prob > F" was found to be <0.0001 which was less than 0.0500 indicating the modified model was significant.

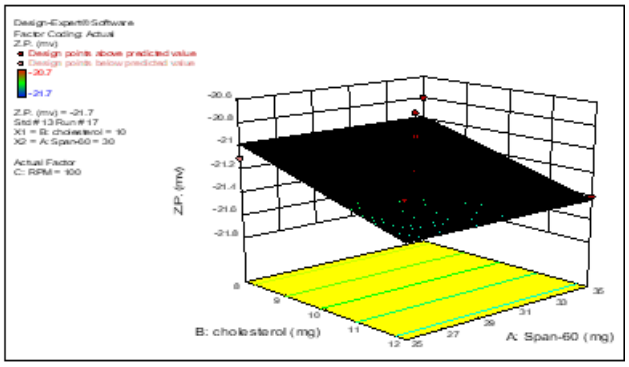

Figure 3: 3D-Analysis of Zeta Potential.

The optimized predicted solution, after analysis, was evaluated using Design Expert and found to be statistically significant, as presented in Tables 4 and 5.

Effect of Entrapment Efficiency

The following equation demonstrates the influence of various factors on the response variable, particle size (R1), as derived from the analysis:

$$E.E.= +86.73 + 3.07*A + 2.24*B - 17.06*A^2 - 5.08*B^2$$

The "Prob > F" value was determined to be less than 0.0001, which is lower than 0.0500, indicating that the modified model is statistically significant. However, the significant lack of fit, as shown in Tables 4 and 5, suggests that the model may not be adequate for predicting a range of experimental variables. Figure 4 highlights the crucial role of cholesterol (CHO) and Span-60 in the drug's entrapment efficiency, demonstrating that increasing the amounts of CHO and Span-60 leads to a decrease in entrapment efficiency. The figure further suggests that a medium level of surfactant and phospholipid is optimal for the formulation.

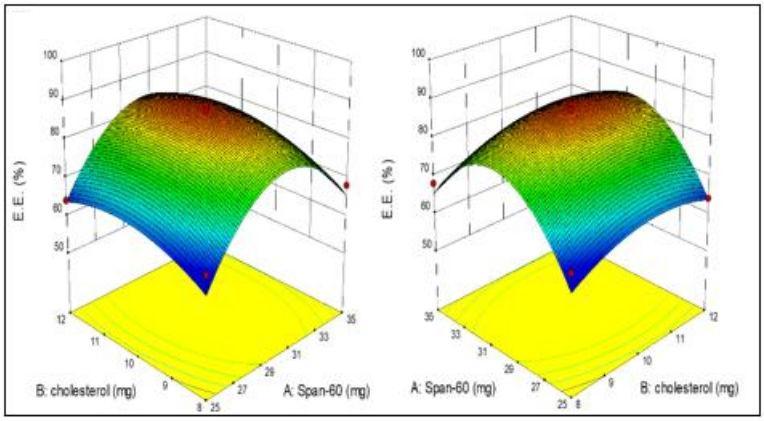

Figure 6: 3D-Analysis of Entrapment Efficiency.

Table 6: The optimized solution from a design expert.

Solution							
Number	Span-6	Cholesterol	rpm	P. Size	Z.P.	E.E.	Desirability
F1	30.219	11.641	100.374	192.539	-21.385	85.251	0.807

3.1.4 Post-analysis solution and confirmation and Characterization of CLR-Niosomal suspension

The post-analysis of the optimized solution was conducted following the preparation of the optimized CLR-niosomal suspension. The mean values of the responses were calculated, and the post-prediction and confirmation results are presented in Table 7.

Table 7: Confirmation and characterization of the optimized niosomes.

Response	Predicted Mean	predicted Median[1]	95 % PI low	Data Mean	95 % PI high
P. Size	193.62	193.62	186.46	196.14	200.78
Z.P.	21.2	21.2	-20.60	-21.20	-21.80
E.E.	86.7321	86.7321	76.71	86.17	96.76

The particle size of the final optimized CLR-niosomal formulation was within the predicted range of the prediction interval. Similarly, the zeta potential of the CLR-Niosomes was negative, as anticipated by the design expert, and aligned with the predicted range for the optimized formulation. The entrapment efficiency also fell within the prediction interval, confirming the optimization of the formulation. Specifically, the particle size and zeta potential of the optimized niosomal suspension were found to be 196.14 nm and -21.2 mV, respectively, indicating a successfully optimized formulation.

3.1.5 Particle size of optimized niosomal suspension

The particle size of the suspension was determined using a Lite-Sizer analyzer, and the measured value was found to fall within the desirable range of the predicted value.

3.1.6 Zeta Potential of Optimized Niosomal Suspension

The zeta potential of the suspension was measured using a LiteSizer analyzer and was found to fall within the desirable range of the predicted value.

3.1.7 Drug content of optimized niosomal suspension

The drug content of the suspension was found to be 12.92 mg (86.17 %).

3.1.8 Drug Entrapment Efficiency and Drug Loading

The drug entrapment efficiency and drug loading of formulation F1 were determined to be 86.17% and 23.16%, respectively.

3.2 Stability study

The stability of the CLR-Niosomal suspension was evaluated by storing it at 4°C and room temperature (25°C) for 90 days. At 4°C, there was no significant increase in particle size, indicating stable conditions. However, at room temperature, the system's increased kinetic energy accelerated particle collisions, resulting in a slight increase in nanoparticle aggregation, as shown in Table 8.

Table 8: Stability of optimized niosomes at 4° C and room temperature.

S. No.	Storage time (Days)	Particle size (nm)	
		At 4 °C	At 25 °C (Room Temp.)
1	0	190.30	190.30
2	14	194.97	199.05
3	28	196.83	217.09
4	60	227.58	248.39
5	90	225.01	251.39

3.2.1 In-vitro drug release study of niosomal suspension

The in vitro drug release of the formulation was investigated using a Franz diffusion cell with a dialysis membrane over 24 hours. The drug release was measured by absorbance using a Shimadzu-1800 spectrophotometer, and the release was found to be 82.392% after 24 hours.

3.2.2 In-Vitro Drug Release Kinetic Study

The kinetic drug release mechanism of the formulation was analyzed using various kinetic models, including zero order, first order, Higuchi plot, Peppas plot, and Hixon Crowell. The R^2 values for each model were 0.7906, 0.8557, 0.9717, 0.9905, and 0.6487, respectively. The R^2 value of the Korsmeyer-Peppas plot was closest to 1, indicating that the prepared suspension followed the Korsmeyer-Peppas release model.

3.3 Formulation and Evaluation of Clarithromycin-Loaded Niosomal Gel

3.3.1 Preparation of CLR-N-Gel

The optimized clarithromycin niosomal suspension (F1), which was previously optimized using a Box-Behnken design through Design Expert based on particle size, zeta potential, and entrapment efficiency, was selected for the preparation of a 0.1% w/w CLR-N-Gel. This gel was prepared using a Carbopol-based gel formulation (24).

3.3.2 Evaluation of CLR-N-Gel

3.3.2.1 Physical appearance and pH of the CLR-N-Gel

The physical appearance of the Carbopol-based gel formulations at various concentrations 0.5%, 1.0%, 1.5%, and 1.75%) was observed to be transparent to off-white in color, with a smooth texture and a homogeneous consistency. The pH of the CLR-N-Gel was determined to be 5.83 ± 0.41.

3.3.2.2 Spreadability of CLR-N-Gel

The Spreadability of the CLR-N-Gel was found to be 14.39 ± 6.08 g-cm/sec.

3.3.2.3 Viscosity of CLR-N-Gel

The viscosity of CLR-N-Gel was found to be 3860 centipoises.

3.3.2.4 Drug content of CLR-N-Gel

The drug content of the prepared CLR-N-Gel 0.1% w/w gel was found to be 96 ± 2 %.

3.3.2.5 In-Vitro Drug Release Study of CLR-N-Gel

The in vitro drug release of the formulation was evaluated using a Franz diffusion cell with a dialysis membrane over a 24-hour period. The release was quantified by measuring absorbance using a Shimadzu-1800 spectrophotometer. The results indicated that the drug release from the formulation was 80.74%.

3.3.2.6 In-Vitro Drug Release Kinetic Study of CLR-N-Gel

The kinetic release mechanism of the gel was analyzed using various kinetic models. The R2 values obtained for the zero-order, first-order, Higuchi, Peppas, and Hixon-Crowell models were 0.7803, 0.8331, 0.9101, 0.993, and 0.6578, respectively. The R2 value for the Peppas plot was closest to 1, indicating that the release mechanism of the prepared formulation closely follows the Korsmeyer-Peppas model.

3.3.2.7 Ex-Vivo Drug Permeation Study

The ex-vivo permeation study was conducted using a Franz diffusion cell, and the results indicated that the prepared CLR-N-Gel demonstrated enhanced permeation through excised pig ear skin, with sustained retention within the skin layers for an extended period. Additionally, the gel exhibited a lower initial drug release burst compared to the nanoparticles. The drug permeation through the pig ear skin was found to be 77.20% over a 24-hour period.

3.3.2.8 Ex-Vivo Drug Permeation Kinetic Study

The kinetic drug permeation mechanism of the gel was analyzed using various models. The R2 values for the zero-order, first-order, Higuchi plot, Peppas plot, and Hixon Crowell were found to be 0.6944, 0.6342, 0.9328, 0.9849, and 0.461, respectively. The R2 value of the Peppas plot was closest to 1, indicating that the prepared formulation follows the Korsmeyer-Peppas model for drug release.

3.3.2.9 In-Vivo Efficacy on DNCB-Induced Atopic Dermatitis

3.3.2.9.1 Skin irritation study

The dermal irritation study results, presented in Table 6.14, showed no visible signs of skin irritation, such as edema or erythema, or inflammation throughout the study period when compared to the standard control. The skin irritation scores, based on the Draize scoring method, indicated no significant irritation.

Table 9: Skin irritation score in rabbits.

Sl. No.	Control group	Standard Group	Test Group
1	0	0	0
2	0	0	0
3	0	2	0
4	0	2	0
5	0	2	0

3.3.2.9.2 In-Vivo Animal Study of Atopic Dermatitis

The in-vivo anti-dermatitis study was conducted over a 15-day period, with the animals randomly divided into four groups, each consisting of six female Wistar albino rats.

DNCB was topically applied every two days to the pre-shaved areas of groups II, III, and IV, using the minimum effective dose that did not cause toxicity. The application continued until atopic dermatitis-like symptoms became visibly apparent on the rats' skin. After the induction period, the skin lesions were assessed visually. During the treatment phase, group III received the standard marketed Clarithromycin formulation (Crixan Gel 1% w/w), while group IV was treated with the developed CLR-N Gel (0.1% w/w). The results demonstrated that the CLR-N Gel (0.1% w/w) exhibited faster and more significant recovery compared to the marketed formulation, with the added benefit of requiring a lower dose.

Histopathological analysis was performed on the skin samples from the control, standard, and test groups with atopic dermatitis using Hematoxylin and Eosin (H&E) staining. The positive control group exhibited no abnormal changes in the skin structure, while the negative control group displayed irregularities in the epidermal and subcutaneous tissues, including changes in thickness and structural integrity. Figure 7 illustrates the histopathological condition of the atopic dermatitis skin, comparing the responses of the positive control, negative control, marketed standard, and test formulation.

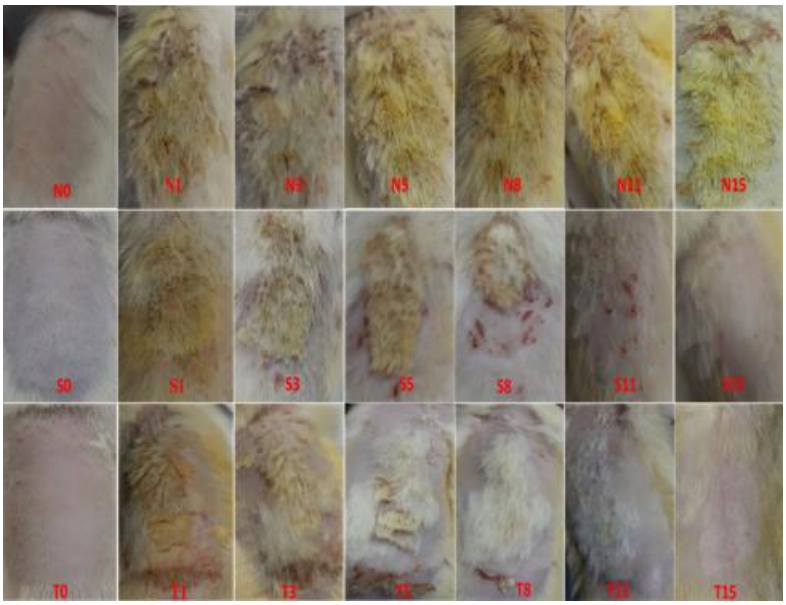

Figure 7: In-vivo evaluation of DNCB induced atopic dermatitis, (N0-N15): Negative control, (S0-S15): Standard cured, (T0-T15): Test and treated respectively.

4. Conclusion

Atopic dermatitis (AD) is a chronic inflammatory skin condition characterized by eczematous dermatitis, irritated and itchy skin, disruption of the epidermal barrier, activation of T-cells, and microbial imbalance. Clarithromycin, a commonly used treatment for AD, faces limitations due to its poor solubility and permeability at the target site. To overcome these challenges, nanocarrier-based delivery systems were developed, but they still struggled with issues such as rapid drug release, nanoparticle rupture before reaching the target site, low drug loading capacity, poor efficacy at the cell membrane, and instability during storage.

The primary objective of this study was to formulate and evaluate a clarithromycin-loaded niosomal gel (CLR-N-Gel) to improve solubility, permeability, bioavailability, and therapeutic efficacy for AD management. Clarithromycin was chosen as the model drug, and surfactant (Span-60) along with phospholipid (Cholesterol) were utilized for formulating the niosomal suspension via the thin-film hydration method. The niosomal suspension was optimized using Design Expert software, and the optimized formulation was characterized for drug-excipient compatibility, morphology, particle size, zeta potential, entrapment efficiency, drug loading, and in-vitro drug release.

The results showed the optimized formulation was suitable for incorporation into a Carbopol-based gel, leading to the development of CLR-N-Gel 0.1% w/w. The entrapment efficiency, drug loading, and cumulative drug release of the niosomal suspension were found to be 86.17%, 23.16%, and 82.39%, respectively. The stability study of the niosomal suspension, conducted over 90 days, demonstrated its stability. The suitable niosomal suspension was then incorporated into the Carbopol-based gel, which was further characterized for physical appearance, pH, viscosity, spreadability, drug content, and in-vitro drug release.

Ex-vivo skin permeation studies of CLR-N-Gel were performed using a Franz diffusion cell on pig ear skin. The drug content of CLR-N-Gel was 96 ± 2.16%, with a cumulative drug release of 80.74% and an ex-vivo cumulative drug permeability of 77.20% over 24 hours. In-vivo anti-dermatitis efficacy was evaluated by comparing CLR-N-Gel 0.1% w/w with the standard marketed clarithromycin gel (1% w/w). AD was induced by the topical application of 2% w/v 2,4-dinitrochlorobenzene, followed by treatment with either the standard or test formulations for 15 days. The results indicated that CLR-N-Gel 0.1% w/w exhibited faster recovery and superior therapeutic efficacy at a lower concentration compared to the standard clarithromycin gel, demonstrating maximum reduction of AD-like symptoms. This was attributed to better skin permeation and prolonged

drug release. Histopathological analysis was performed to examine the changes in the dermatitis skin, supporting the enhanced efficacy of CLR-N-Gel. Based on the findings, the study concluded that CLR-N-Gel offers an effective drug delivery system for managing AD. It enhances the solubility and skin penetration of clarithromycin by controlling the permeation rate, resulting in higher therapeutic effects. CLR-N-Gel can, therefore, be considered a promising alternative for the treatment of atopic dermatitis.

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