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DUAL DRUG-LOADED NIOSOMAL GEL OF LICOFELONE AND SPIRAMYCIN: FORMULATION AND EVALUATION

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ABSTRACT

The present study focused on the development and evaluation of a dual drug-loaded niosomal gel for the topical delivery of Licofelone and Spiramycin. The optimized formulation (F5) exhibited a particle size of 136.7 ± 8.37 nm, a low polydispersity index (PDI) of 0.316 ± 0.92, and a zeta potential of –20.9 mV, indicating uniformity and good colloidal stability. Entrapment efficiency was found to be 70.8 ± 0.55%, attributed to the optimal balance of surfactant and cholesterol concentration. Transmission electron microscopy (TEM) confirmed spherical vesicle morphology with no aggregation. In vitro drug release studies showed a sustained release pattern, achieving 94.19% cumulative release over 24 hours, compared to 26.25% release from the plain gel. Rheological characterization revealed a viscosity of 11,924 ± 1.051 cp and spreadability of 3.6 ± 0.256 cm, allowing for effective topical application and retention. Pharmacodynamic evaluation using the carrageenan-induced paw edema model demonstrated 95.7% inhibition of edema at 24 h when the formulation was applied 12 hours after induction, surpassing the marketed diclofenac gel, which achieved 93.5% inhibition. Stability studies over 90 days indicated excellent stability at 2–8 °C, with particle size changing minimally from 137.5 ± 0.436 nm to 139.9 ± 0.963 nm, while room temperature led to greater instability. Skin irritation studies confirmed no erythema or allergic reaction. Overall, the dual drug-loaded niosomal gel exhibited sustained release, enhanced anti-inflammatory activity, favorable rheological properties, excellent stability, and dermal safety, positioning it as a promising future platform for effective and patient-compliant topical therapy in inflammatory and infectious skin disorders.

KEYWORDS: Niosomal gel, Dual drug delivery, Sustained release, Anti-inflammatory, Stability, Topical therapy.

INTRODUCTION

Topical drug delivery systems offer targeted therapeutic effects and minimize systemic side effects, making them suitable for treating inflammatory and infectious skin conditions.[1-3] Recent advances in topical formulations have improved drug penetration, patient compliance, and bioavailability, particularly through innovative designs such as gels and vesicular carriers.[4-7]

Niosomes, formed from non-ionic surfactants and cholesterol, have emerged as robust drug carriers for topical and transdermal delivery due to their stability, cost-effectiveness, and ability to encapsulate diverse drugs.[8-12] These vesicular systems enhance drug localization, control release profiles, and facilitate penetration through the skin barrier, which is otherwise a major limitation for topical therapy.

Combining anti-inflammatory and antimicrobial agents is a rational strategy for treating multi-factorial skin disorders. Licofelone, a dual COX/5-LOX inhibitor, has demonstrated potent anti-inflammatory and analgesic effects with reduced gastrointestinal toxicity versus traditional NSAIDs.[13-17] Spiramycin, a macrolide antibiotic, is clinically established for its efficacy against gram-positive bacteria and protozoa, reducing infection risk in dermatological applications. Synergistic pharmacological activity is anticipated when both agents are delivered concurrently in a single vesicular gel formulation.

Niosomal gels, incorporating optimized niosomes into a topical semisolid base, offer both improved drug spreadability and sustained release, leading to enhanced therapeutic outcomes and patient adherence.[8, 18-20]

Preparation via thin-film hydration ensures reproducibility and customization of vesicle size, surface charge, and entrapment efficiency. To maximize safety and drug efficacy, formulations must be extensively characterized for physicochemical properties, in vitro release, stability, rheological behavior, and in vivo pharmacodynamics.[9, 21-24] Skin compatibility and irritation studies are essential to confirm clinical translation potential.

This study aimed to develop a dual drug-loaded niosomal gel containing Licofelone and Spiramycin, evaluate its physicochemical and pharmacodynamic characteristics, and establish its suitability for topical anti-inflammatory and antibacterial therapy.

MATERIALS AND METHODS

Materials

Licofelone and Spiramycin were obtained from Hi Media (India). Surfactants (Span-40, Span-60) and other analytical grade chemicals were purchased from Sigma-Aldrich (India). All reagents were of analytical grade, and double-distilled water was used throughout the experiments.

Preparation of Dual Drug-Loaded Niosomal Gel

Niosomes were prepared by thin-film hydration. Licofelone, Spiramycin, cholesterol, and surfactants (Span-40/Span-60/Tween-60) were dissolved in chloroform and evaporated under reduced pressure at 55–65 °C using a rotary evaporator to obtain a thin lipid film. The film was hydrated with PBS (pH 7.4) and sonicated to yield a uniform dispersion. Optimized niosomes were incorporated into a gel base by mechanical stirring for 60 min.[25]

Characterization

Particle size and morphology: Transmission electron microscopy (TEM) was used for morphology, while size, zeta potential, and polydispersity index (PDI) were measured using a Zetasizer Nano ZS (Malvern Instruments, UK).

Entrapment efficiency (EE): Unentrapped drug was separated by centrifugation (14,000 rpm, 10 min) and quantified by UV–Vis spectrophotometry at 278 nm. EE (%) was calculated as:

$$\text{EE (\%)} = \frac{Cinitial - Cfree}{Cinitial} \text{X } 100$$

In vitro release: Drug release was studied across the egg membrane using PBS (pH 6.8) as the receptor medium. Samples were withdrawn at fixed intervals and analyzed spectrophotometrically.

Viscosity: Measured at 25 ± 0.5 °C using a Brookfield DV-III Ultra rheometer with spindle CPE61.

Stability: Formulations were stored at 0 °C, 2–8 °C, and 25 °C for up to three months and evaluated for particle size, drug content, and physical appearance.[26]

Pharmacodynamic Study

Anti-inflammatory activity was evaluated in Wistar rats (150–250 g) using the carrageenan-induced paw edema model. Animals were divided into groups receiving oral curcumin (110 mg/kg), marketed diclofenac gel, or test gel. Paw edema was induced with 0.1 ml carrageenan (1%) and measured up to 24 h. Percent inhibition was calculated as:

$$\text{\% Inhibition} = \frac{Tc - Tt}{Tc} \text{X } 100$$

Data were analyzed by one-way ANOVA, with $p < 0.05$ considered significant.[27]

Skin Irritation Study

The Draize test was conducted on rats divided into three groups: untreated control, marketed diclofenac gel, and test gel. Formulations were applied to shaved dors[28] al skin and observed for erythema, redness, or allergic reactions over 24 h.[29, 30]

RESULTS AND DISCUSSION

Physical Characterization of Niosomal Gel

The physicochemical properties of the prepared dual drug-loaded niosomal gel formulations (F1–F6) are summarized in Table 1. The particle size of the formulations ranged from 84.93 ± 11.7 nm (F1) to 318 ± 15.3 nm (F4). Smaller vesicles were observed in F1 and F2, while larger vesicles were obtained in F4 and F6, indicating that surfactant concentration and cholesterol ratio influenced vesicle diameter. Formulations F3 and F5 demonstrated intermediate particle sizes of 157.7 ± 10.2 nm and 136.7 ± 8.37 nm, respectively, which are within the nanometric range reported to enhance dermal penetration and sustain release (Table 1).

Table 1: Physical characterization of prepared niosomal gel formulation.

Code	Particle size(nm)	PDI	EE %	Zeta potential
F1	84.93±11.7	0.853±0.113	29.2±0.26	-17.6
F2	102.3±6.65	0.719±0.413	47.9±0.99	-15.4
F3	157.7±10.2	0.440±0.313	62.5±0.86	-19.4
F4	318±15.3	0.518±0.815	56.4±1.6	-19.5
F5	136.7±8.37	0.316±0.92	70.8±0.55	-20.9
F6	218.8±19.3	0.518±0.52	69±2.2	-17.7

Note: All data articulated as mean ± S.D.; n = 3, all the experiments performed triplicate

The polydispersity index (PDI) values varied between 0.316 ± 0.92 and 0.853 ± 0.113. F5 exhibited the lowest PDI, indicating a more homogeneous size distribution, while F1 displayed the highest heterogeneity. Generally, PDI values below 0.5 are considered acceptable for monodisperse nanoparticle systems, suggesting that F3 and F5 met the criteria for uniformity and stability.

Entrapment efficiency (EE) ranged from 29.2 ± 0.26% in F1 to 70.8 ± 0.55% in F5. The higher EE values of F5 and F6 could be attributed to an optimized balance of surfactant and cholesterol, which stabilizes the bilayer and enhances drug encapsulation. Lower EE in F1 may be due to insufficient surfactant concentration, resulting in poor bilayer entrapment. These findings are consistent with earlier reports that optimal cholesterol levels improve vesicle rigidity while maintaining adequate flexibility for efficient drug incorporation.

The zeta potential values of all formulations were negative, ranging from –15.4 to –20.9 mV, indicating moderate surface charge and colloidal stability. Negative zeta potential is typical for niosomes due to the non-ionic surfactants and cholesterol in their bilayer structure. F5 exhibited the most negative value (–20.9 mV), supporting its superior stability among the tested formulations.

Overall, based on particle size, PDI, EE, and zeta potential, formulation F5 demonstrated the most desirable properties and was selected as the optimized formulation for further evaluation.

Morphological Analysis

Transmission electron microscopy (TEM) images (Figure 1) confirmed the nanoscale spherical morphology of the prepared vesicles.

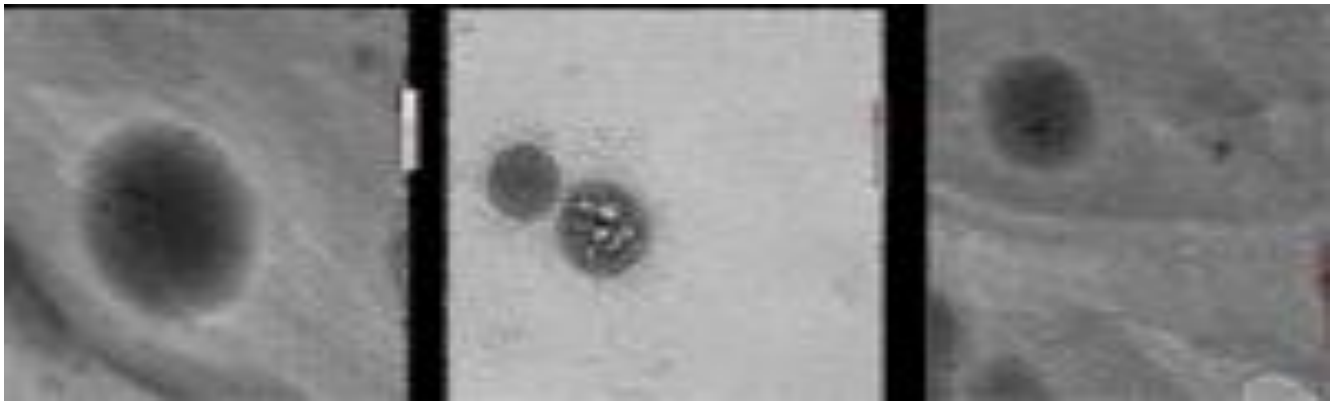

Figure 1: TEM images of the prepared dual drug-loaded niosomal gel.

The niosomes exhibited smooth surfaces with distinct boundaries and showed no signs of aggregation, indicating good dispersion stability. The particle size observed by TEM correlated well with the dynamic light scattering results, further validating the reliability of the preparation method. Similar spherical morphologies have been reported in other niosomal formulations, confirming that the thin-film hydration technique was effective for vesicle preparation.

***In Vitro* Drug Release**

The cumulative drug release profiles of the formulations are shown in Figure 2. The dual drug-loaded niosomal gel exhibited a sustained release pattern, achieving approximately 94.19% cumulative release over 24 h, compared with only 26.25% release from the plain gel. The slower release from niosomes can be attributed to the lipid bilayer structure, which provides controlled drug diffusion. The release rate was also superior to that of carbopol-based gels, which displayed comparatively lower release. These findings support the potential of niosomal gels to prolong drug release, thereby reducing dosing frequency and improving therapeutic outcomes.

Rheological Properties

The viscosity and spreadability of the selected formulations (F3, F5, and F6) are presented in Figure 2. Viscosity increased with surfactant concentration, with F3 showing the lowest viscosity (6214 ± 0.244 cp), F5 showing intermediate viscosity (11,924 ± 1.051 cp), and F6 exhibiting the highest viscosity (23,955 ± 0.965 cp). Spreadability was inversely related to viscosity, with F3 spreading most easily (4 ± 0.252 cm), followed by F5 (3.6 ± 0.256 cm) and F6 (2.6 ± 0.4 cm).

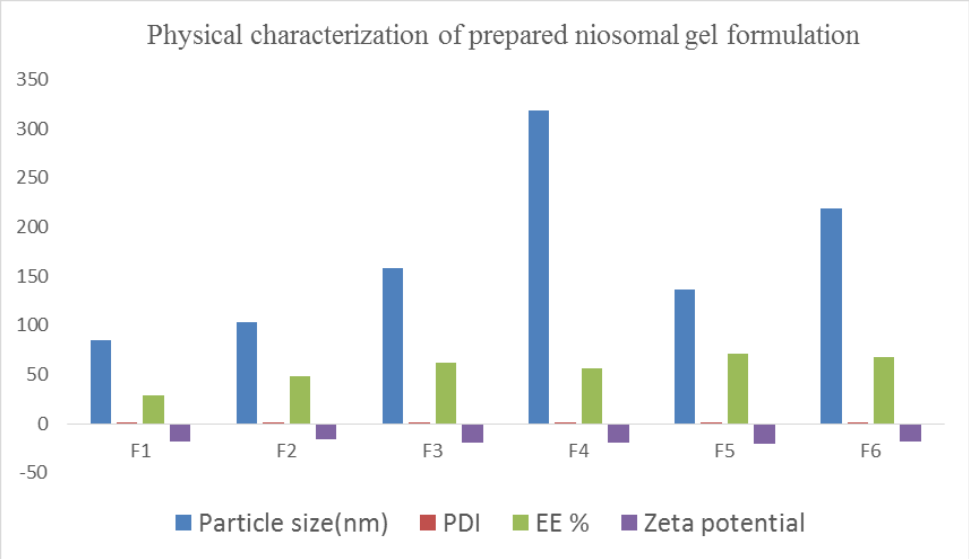

Figure 2: Comparative data of optimization of niosomal gel formulation variables and their effect on particle size and entrapment efficiency.

From a pharmaceutical perspective, F5 achieved an optimal balance between viscosity and spreadability. Its moderate viscosity ensured prolonged retention at the application site, while its spreadability allowed easy application, both of which are crucial for patient compliance (Table 2).

Table 2: Physical characterization of prepared niosomal gel formulation. Characterization of niosomal gel, Viscosity determined on different compositions of niosomal gel.

Characterization of niosomal gel		
Formulation Code	**Viscosity (cp)**	**Spreadibility (cm)**
F3	6214±0.244	4±0.252
F5	11924±1.051	3.6±0.256
F6	23955±0.965	2. 6±0.4

Note: All data articulated as mean ± S.D.; n = 3, all the experiments performed triplicate

Pharmacodynamic Evaluation

The anti-inflammatory activity of the formulations was assessed using carrageenan-induced paw edema in rats (Table 3). The oral curcumin group showed an initial high inhibition, which rapidly declined to 27.02% at 24 h, indicating a short duration of effect. The marketed diclofenac gel demonstrated consistent activity, with 62.21% inhibition at 2 h and 56.23% at 24 h.

The test niosomal gel formulation showed 56.69% inhibition at 2 h, peaking at 73.82% at 3 h, which was higher than the marketed gel (64.99% at 3 h). However, its activity decreased to 36.53% at 24 h after a single application. When applied 12 h after carrageenan injection, the test formulation exhibited strong and prolonged anti-inflammatory activity, achieving 95.7% inhibition at 24 h compared with 93.5% for the marketed gel (Table 3).

Table 3: Effect of different formulations on carrageenan-induced paw oedema.

Group	Treatment	% paw edema inhibition by different groups						
		0 h	1 h	2 h	**3 h**	4 h	5 h	24 h
1	Control	--	--	--	--	--	--	--
2	Oral	100±0.167	66.08±0.167	51.08±0.167	**56.17±0.2**	56.92±0.169	42.73±0.167	27.02±0.1856
3	Standard	100±0.167	68.28±0.167	62.21±0.1453	**64.99±0.1998**	70.58±0.1856	70.17±0.4	56.23±0.2604
4	Test	100±0.0897	58.78±0.2879	56.69±0.0623	**73.82±0.2001**	57.78±0.161	58.97±0.2035	36.53±0.078
5	Standard (12h after)	100±0.1667	71.21±0.1767	64.66±0.2112	**70.66±0.1888**	71.78±0.167	74.33±0.146	93.5±0.167
6	Test (12h after)	100±0.0897	72.43±0.1667	64.22±0.2132	**69.12±0.1668**	70.35±0.214	76.55±0.1846	95.7±0.167

Note: All data articulated as mean ± S.D.; n = 3, all the experiments performed triplicate

The enhanced efficacy of the niosomal gel may be attributed to the synergistic effects of Licofelone and Spiramycin, combined with the nanosized carrier system that improved drug penetration and sustained release. These findings are in line with previous reports where niosomal formulations enhanced anti-inflammatory efficacy compared with conventional gels (Figure 3).

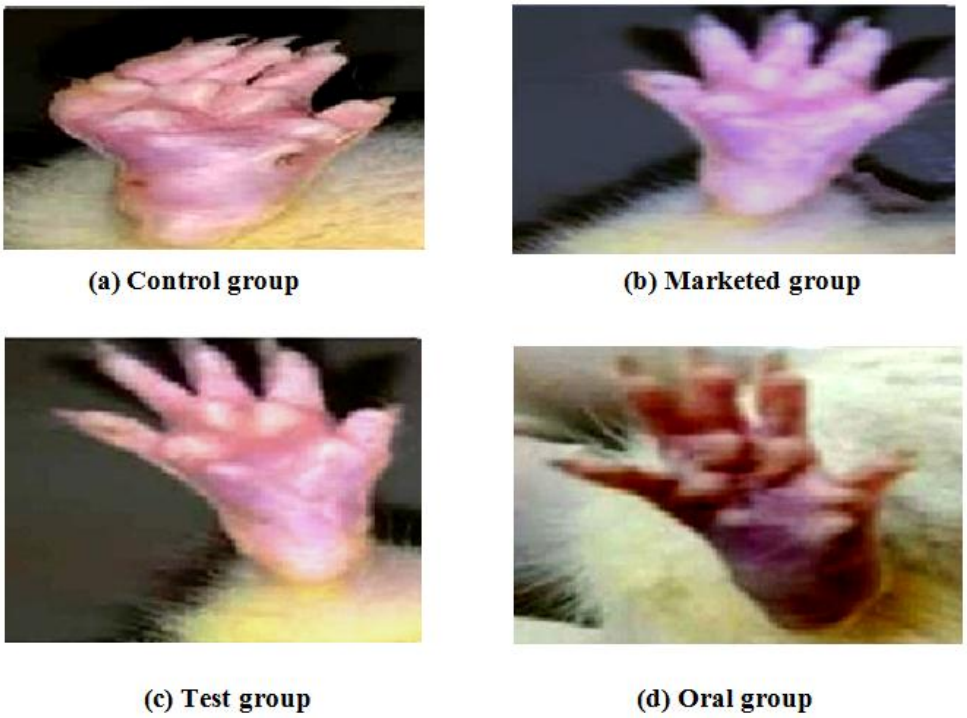

Figure 3: Inflammation inhibition in the rat paw in different groups a, b, c, and d.

Skin Irritation Studies

The Draize test confirmed the dermal safety of the developed formulations (Figure Z). The control and marketed gel groups exhibited normal skin with no signs of irritation. The test formulation group also showed no erythema, redness, or allergic reactions, indicating good dermal tolerance. In contrast, the oral group showed mild skin irritation, likely due to systemic inflammatory responses rather than formulation-related effects. The absence of irritation in the test group confirms that the dual drug-loaded niosomal gel is safe for topical application (Table 4).

Table 4: Stability studies of niosomal gel based on their particle size.

Days for observation	Stability studies based on the change in particle size		
	0 ˚c temperature	4-8 ˚C temperature	Room temperature
Day -0	137.4±0.436	137.5±0.435	137.5±0.436
Day -15	137.6±0.424	136.3±0.343	139±0.135
Day -30	137.5±0.388	138.8±0.227	138±0.777
Day -45	136±0.222	138.9±0.766	138.6±0.555
Day -60	136.2±0.326	137.3±0.822	142.9±0.129
Day -75	136.2±0.564	138.2±0.792	148±0.199
Day -90	136.5±0.768	139.9±0.963	151±0.876

Note: All data are articulated as mean ± S.D.; n = 3, all the experiments were performed in triplicate.

Stability Studies

The stability of the optimized niosomal formulation was evaluated by monitoring changes in particle size under different storage conditions (0 °C, 4–8 °C, and 25 °C) for up to 90 days (Table 4). At 0 °C and refrigerated conditions, particle size remained relatively stable throughout the study period, with only minor fluctuations observed (137.4 ± 0.436 nm at Day 0 and 136.5 ± 0.768 nm at Day 90 for 0 °C). In contrast, samples stored at room temperature exhibited a gradual increase in particle size, rising from 137.5 ± 0.436 nm at Day 0 to 151 ± 0.876 nm at Day 90.

These findings suggest that the formulation remains stable under refrigerated and freezing conditions but shows reduced stability at ambient temperature. The increase in particle size at room temperature may be due to vesicle aggregation or structural rearrangement of the bilayer. Similar trends have been reported in other niosomal systems, where refrigeration was recommended to preserve vesicle integrity. Overall, the stability study confirmed that the optimized formulation is best stored at 2–8 °C to maintain its physicochemical characteristics and therapeutic efficacy.

CONCLUSION

The optimized dual drug-loaded niosomal gel formulation (F5) demonstrated promising physicochemical, pharmacological, and stability characteristics that highlight its potential as a future

advanced topical drug delivery system. The particle size was measured at 136.7 ± 8.37 nm, with a low polydispersity index (PDI) of 0.316 ± 0.92, indicating a uniform and stable nanoscale dispersion. Entrapment efficiency was high at 70.8 ± 0.55%**,** while the zeta potential of –20.9 mV suggested good colloidal stability. In vitro drug release studies revealed a sustained release profile, achieving 94.19% cumulative release over 24 hours, significantly surpassing conventional plain gels. Pharmacodynamic evaluation showed superior anti-inflammatory activity, with 95.7% paw edema inhibition at 24 hours, compared to 93.5% inhibition for the marketed formulation when applied 12 hours post-induction. Rheological properties revealed a balanced viscosity of 11,924 ± 1.051 cp and spreadability of 3.6 ± 0.256 cm, providing optimal application and retention on the skin. Stability studies confirmed minimal change in particle size from 137.5 ± 0.436 nm to 139.9 ± 0.963 nm over 90 days at 2–8 °C, supporting long-term stability under refrigerated conditions. No skin irritation was observed, confirming dermal safety. These results indicate that the developed niosomal gel holds significant promise as a future platform for controlled and targeted topical therapy in inflammatory and infectious skin disorders, offering sustained drug release, enhanced therapeutic efficacy, and improved patient compliance, subject to further *in vivo* and clinical validation.

CONFLICT OF INTEREST

The authors have no conflicts of interest regarding this investigation**.**

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