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FORMULATION AND EVALUATION OF CLINDAMYCIN PHOSPHATE AND METHYLGLYOXAL CO-LOADED TRANSFERSOMAL GEL FOR TOPICAL ANTIMICROBIAL DELIVERY

Mohammed Mafaz K.[1]*, Saranya P.[1], Lakshmipathi G.[1], Anusiya C.[1], Vimal P.[1], Mr. V. Parthasarathi[2]

[1]Department of Pharmaceutics, G.P Pharmacy College, Mandalavadi, Jolarpet, Tamil Nadu, India.
[2]Associate Professor, Department of Pharmaceutics, GP Pharmacy College, Jolarpet.

***Corresponding Author: Mohammed Mafaz K.**

Department of Pharmaceutics, G.P Pharmacy College, Mandalavadi, Jolarpet, Tamil Nadu, India.

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ABSTRACT

The present study developed and evaluated an egg yolk lecithin-based transfersomal gel co-loaded with clindamycin phosphate and methylglyoxal for topical antimicrobial delivery. Five transfersome formulations were prepared by thin-film hydration using Tween 80 as edge activator and citrate buffer as the hydration medium. The optimized formulation (F4) showed a particle size of 184 ± 5 nm, polydispersity index of 0.24 ± 0.01, zeta potential of −31.2 ± 0.9 mV, and entrapment efficiency of 87.9 ± 1.1% for clindamycin phosphate and 70.4 ± 1.3% for methylglyoxal. The formulation exhibited sustained release over 24 h and produced greater antimicrobial activity against Staphylococcus aureus and Cutibacterium acnes than single-drug transfersomes, free drug combination, and marketed clindamycin gel. The optimized dispersion was incorporated into an HPMC gel with acceptable pH, viscosity, spreadability, extrudability, and drug content uniformity. Short-term refrigerated stability was satisfactory. The study supports co-loaded transfersomal gel as a promising topical system for acne-associated antimicrobial therapy.

KEYWORDS: *Transfersomes; Clindamycin phosphate; Methylglyoxal; Topical delivery; Acne; Antimicrobial gel.*

1. INTRODUCTION

Topical and transdermal delivery offer an attractive route for localized treatment because they can bypass first-pass metabolism and reduce systemic adverse effects. However, the stratum corneum remains a major barrier to drug penetration, especially for molecules that require delivery into deeper skin layers or the pilosebaceous unit.[1-7]

Transfersomes are ultra-deformable vesicles designed to overcome this barrier by combining phospholipids with an edge activator such as Tween 80. Their flexibility allows them to squeeze through narrow intercellular channels under the influence of the skin's hydration gradient.[11,12,15,16] Natural lecithin-based systems are attractive because they are biocompatible, economical, and suitable for topical formulation.[13,38,49]

Clindamycin phosphate remains a widely used topical anti-acne antibiotic, but its long-term utility is limited by poor penetration at the follicular level and rising resistance among Cutibacterium acnes isolates.[18-21]

Methylglyoxal is a naturally occurring reactive dicarbonyl compound found in Manuka honey and has antimicrobial activity through protein glycation, membrane disruption, and oxidative damage.[23-25,33,37] Combining clindamycin phosphate with methylglyoxal in a single transfersomal carrier may broaden antibacterial action and reduce dependence on antibiotic monotherapy.[32,35,36]

On this basis, the present work was designed to formulate and evaluate a co-loaded transfersomal gel for topical antimicrobial delivery against acne-associated skin pathogens.

2. MATERIALS AND METHODS

2.1 Materials

Clindamycin phosphate, methylglyoxal 40% w/w solution, egg yolk lecithin, Tween 80, HPMC K100, phenoxyethanol, propylene glycol, disodium EDTA dihydrate, α-tocopherol, citric acid, sodium citrate, chloroform, methanol, and microbiological media were used as received from the stated suppliers. Distilled water was used throughout.

2.2 Pre-formulation Studies

The drug substances were examined for organoleptic properties, pH, melting point (for clindamycin phosphate), solubility in selected solvents, partition coefficient, and TLC-based identification. UV spectrophotometric and DNPH-based methods were developed and validated for clindamycin phosphate and methylglyoxal, respectively.

2.3 Preparation of Egg Yolk Lecithin

Lecithin was isolated from fresh hen's egg yolk by acetone precipitation, washing, dissolution in chloroform:methanol (2:1), filtration, evaporation, and storage at 4°C until use.

2.4 Preparation of Transfersomes

Transfersomes were prepared by thin-film hydration. Egg yolk lecithin, Tween 80, and α-tocopherol were dissolved in chloroform:methanol (2:1), the solvent was removed by rotary evaporation, and the dry lipid film was hydrated with citrate buffer containing clindamycin phosphate and methylglyoxal. The dispersion was probe-sonicated and centrifuged to remove unentrapped drug. Five formulations (F1-F5) were prepared by varying the lecithin-to-Tween 80 ratio.

2.5 Characterization of Transfersomes

Particle size and polydispersity index were measured by dynamic light scattering, zeta potential by electrophoretic light scattering, and entrapment efficiency and drug loading capacity were determined indirectly from the supernatant after centrifugation. Morphology of the optimized formulation was examined by TEM and SEM.

2.6 In Vitro Drug Release

Drug release was studied using dialysis membrane diffusion in citrate buffer pH 5.5 containing Tween 80 at 32°C under continuous stirring. Samples were withdrawn over 24 h and analyzed for both drugs. Release data were fitted to zero-order, first-order, Higuchi, and Korsmeyer-Peppas models.

2.7 Antimicrobial Activity

Antimicrobial activity was evaluated against Staphylococcus aureus ATCC 25923 and Cutibacterium acnes ATCC 6919 using agar well diffusion and broth microdilution. The co-loaded formulation was compared with single-drug transfersomes, free drug combination, blank transfersomes, and marketed clindamycin gel.

2.8 Preparation and Evaluation of Transfersome Gel

The optimized transfersomal dispersion was incorporated into an HPMC K100 gel containing phenoxyethanol, propylene glycol, disodium EDTA dihydrate, α-tocopherol, and citrate buffer. The gel was evaluated for appearance, homogeneity, pH, viscosity, spreadability, extrudability, and drug content uniformity.

2.9 Preliminary Stability Study

The optimized dispersion and gel were stored at 4°C and examined on Days 0, 7, and 14 for particle size, PDI, zeta potential, pH, drug content, and physical appearance.

2.10 Statistical Analysis

All experiments were performed in triplicate and expressed as mean ± standard deviation. Group comparisons were analyzed using one-way ANOVA with Tukey's post-hoc test, with $p < 0.05$ considered significant.

3. RESULTS AND DISCUSSION

Pre-formulation observations confirmed the identity and suitability of both active ingredients. Clindamycin phosphate appeared as a white to off-white crystalline powder and methylglyoxal as a pale yellow, pungent aqueous liquid. The measured pH, melting point, solubility profile, and TLC behavior were consistent with the expected properties of each drug.[18,23,37,45,48]

The analytical methods were linear and precise. Clindamycin phosphate showed λmax at 210 nm with a linear range of 2-20 µg/mL (R^2 = 0.9992), while methylglyoxal quantified by DNPH derivatization showed λmax at 360 nm with linearity over 10-100 µg/mL (R^2 = 0.9987). Accuracy and precision were satisfactory for both methods.[47]

FTIR analysis showed no major incompatibility between the drugs and selected excipients, supporting their use in a combined vesicular system.

Table 1: Formulation design of transfersomes (F1-F5).

Formulation	Egg yolk lecithin (mg)	Tween 80 (mg)	Lecithin: Tween 80 ratio	Drug load (mg/mL)
F1	90	10	9:1	1 + 1
F2	85	15	17:3	1 + 1
F3	80	20	4:1	1 + 1
F4	75	25	3:1	1 + 1
F5	70	30	7:3	1 + 1

The key formulation variables were the lecithin-to-Tween 80 ratio, while the drug concentrations and total lipid content were kept constant.

3.1 Vesicle size, charge, and entrapment

Particle size decreased from F1 to F4 and increased slightly in F5. F4 gave the most favorable balance of small size and narrow distribution, with 184 ± 5 nm particle size and 0.24 ± 0.01 PDI. Zeta potential became more negative with increasing Tween 80 up to F4, where the value reached −31.2 ± 0.9 mV, indicating good colloidal stability. Entrapment efficiency also peaked in F4 at 87.9 ± 1.1% for clindamycin phosphate and 70.4 ± 1.3% for methylglyoxal.[15,28,29]

Table 2: Key performance of optimized formulation F4.

Parameter	Result
Particle size	184 ± 5 nm
PDI	0.24 ± 0.01
Zeta potential	−31.2 ± 0.9 mV
EE% clindamycin phosphate	87.9 ± 1.1%
EE% methylglyoxal	70.4 ± 1.3%
Gel pH	5.32 ± 0.04
Gel viscosity	6125 ± 115 mPa·s
Spreadability	6.42 ± 0.21 cm
Extrudability	158 ± 6 g/30 sec
Clindamycin content	99.2 ± 1.1%
Methylglyoxal content	98.6 ± 1.3%

3.2 Morphology and drug release

TEM showed spherical, well-defined vesicles with a smooth surface and visible bilayer structure, while SEM confirmed that the lyophilized vesicles retained structural integrity. In vitro release studies showed sustained delivery from the transfersomal system compared with the free drug solution. After 24 h, clindamycin phosphate release from F4 was 88.4% and methylglyoxal release was 86.9%, whereas the free drug solution reached complete release much earlier. The release profile was biphasic, with an initial burst followed by slower diffusion-controlled release. Korsmeyer-Peppas provided the best fit for the release data, and F4 showed n = 0.54, indicating anomalous transport.[52,53]

3.3 Antimicrobial activity

The co-loaded formulation showed the strongest antimicrobial effect among all tested samples. F4 produced zones of inhibition of 31.6 ± 1.4 mm against Staphylococcus aureus and 22.8 ± 1.1 mm against Cutibacterium acnes. These values were higher than clindamycin phosphate transfersomes, methylglyoxal transfersomes, the free drug combination, and the marketed clindamycin 1% gel. The MIC of F4 was also lower, at 0.25 µg/mL against S. aureus and 0.50 µg/mL against C. acnes, suggesting an additive or synergistic benefit from co-delivery.[24,25,32,54,55]

3.4 Transfersome gel evaluation and stability

The optimized transfersome dispersion was successfully converted into a smooth, pale yellow, homogeneous gel with pH 5.32 ± 0.04, which is compatible with skin pH. The formulation showed good spreadability and extrudability, and drug content remained within accepted limits. During 14 days of refrigerated storage, no visible phase separation, precipitation, or major drift in particle size, zeta potential, pH, or drug content was observed, supporting short-term physical stability at 4°C.[39,57]

4. CONCLUSION

A co-loaded transfersomal gel of clindamycin phosphate and methylglyoxal was successfully developed using egg yolk lecithin and Tween 80. The optimized formulation offered small vesicle size, good colloidal stability, high entrapment efficiency, sustained dual-drug release, improved antimicrobial activity, and acceptable gel properties. The combination of a conventional antibiotic with methylglyoxal inside an ultradeformable vesicular carrier provides a rational topical approach for acne-associated antimicrobial therapy. Further ex vivo and in vivo studies are warranted to confirm skin penetration, safety, and therapeutic superiority.

Figure Legends

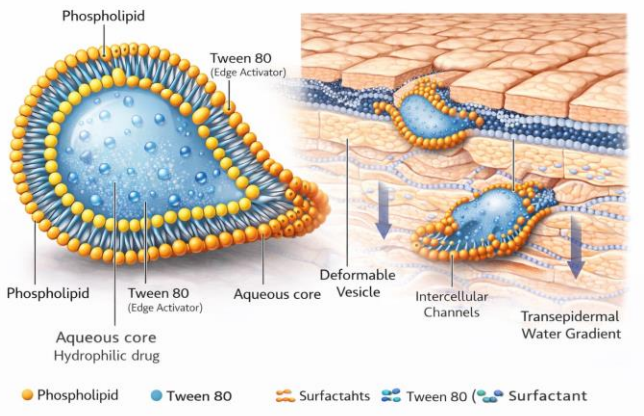


Figure 1: Transfersome structure and transepidermal penetration mechanism.

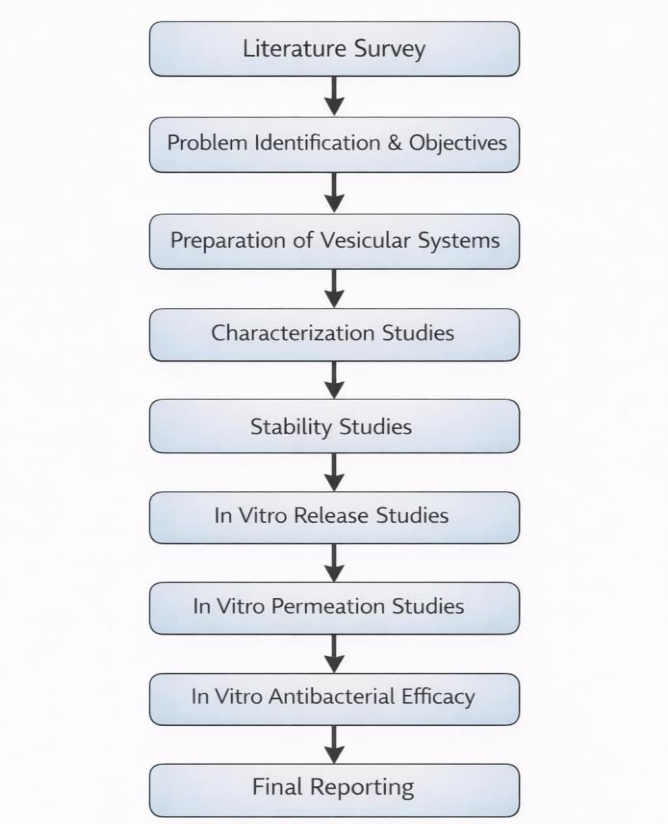
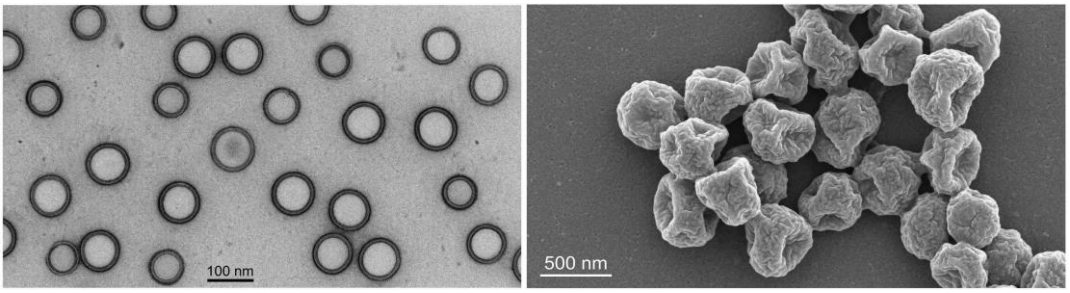


Figure 2: Flowchart of the transfersome preparation and evaluation workflow.



TEM image **SEM image**

Figure 3: TEM and SEM images of the optimized transfersomal formulation (F4).

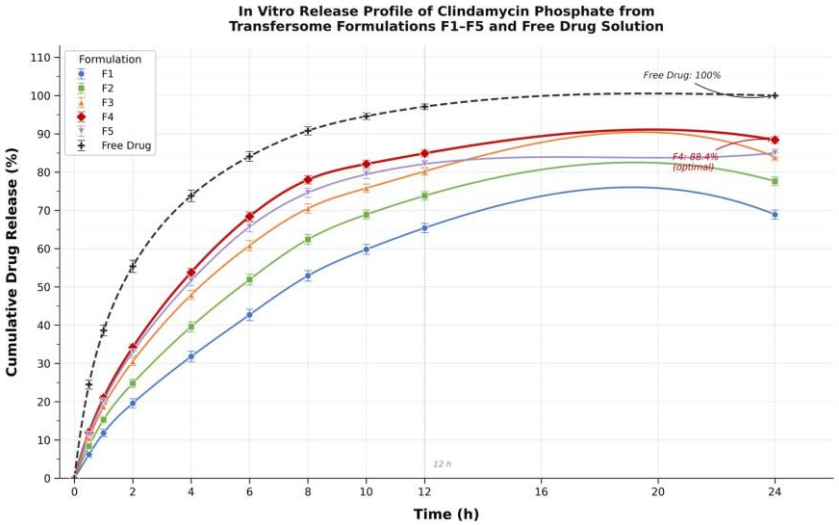


Figure 4: In vitro release profile and antimicrobial activity comparison of the optimized formulation.

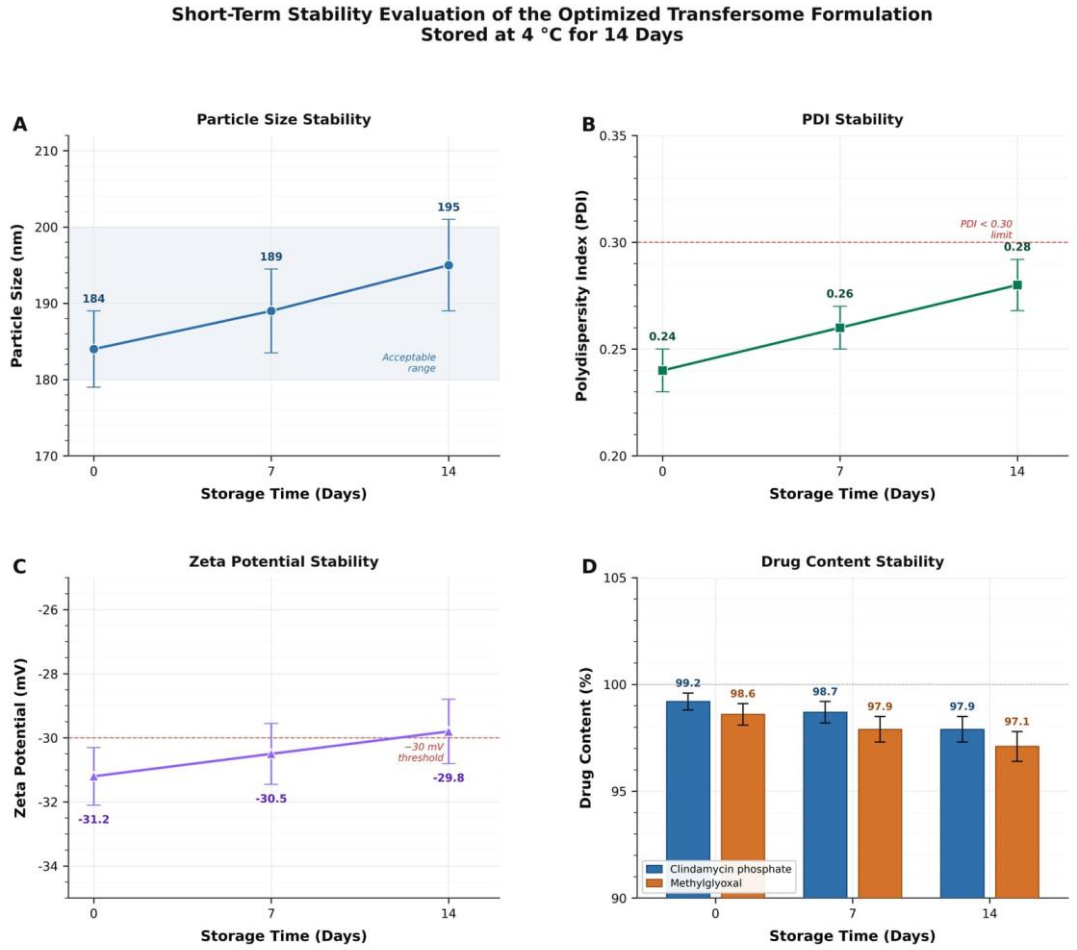


Figure 5: Short-term stability profile of the optimized transfersomal dispersion and gel.

5. ACKNOWLEDGEMENTS

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