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Design and Evaluation of Clindamycin Hydrochloride Proniosomal Gel for Topical Delivery

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ABSTRACT

The present study aimed to formulate and evaluate Clindamycin hydrochloride-loaded proniosomal gel for topical drug delivery with improved entrapment efficiency, controlled release, antimicrobial activity, and skin tolerability. The formulations were evaluated for preformulation properties, drug–excipient compatibility, entrapment efficiency, particle size, zeta potential, SEM, physicochemical properties, *in-vitro* drug release, release kinetics, antimicrobial activity, skin irritation, and stability. UV spectrophotometric analysis showed maximum absorption at 210 nm, with good linearity over 2–10 µg/ml (R^2 = 0.9992). FTIR studies indicated no significant incompatibility between Clindamycin hydrochloride and the selected excipients (Span 60, cholesterol, and soya lecithin). Among the prepared formulations, F6 was selected as the optimized formulation. It exhibited an entrapment efficiency of 86.7 ± 0.3%, particle size of 249.69 nm, PDI of 0.362, and zeta potential of 35.7 mV. SEM revealed discrete, approximately spherical vesicles. The optimized gel showed a pH of 6.45 ± 0.03, spreadability of 28.9 ± 0.05 g·cm/sec, viscosity of 8890 cps, and drug content of 85.7 ± 0.3%. *In-vitro* drug release reached 86.34% at 7 hours, following first-order kinetics (R^2 = 0.9901). The formulation demonstrated antimicrobial activity and caused no visible erythema or edema. Additionally, only minor changes in entrapment efficiency were observed during the four-week stability study.

Overall, F6 demonstrated promising physicochemical properties, controlled drug release, antimicrobial activity, and topical tolerability.

Keywords: Proniosomes; Clindamycin hydrochloride; Co-acervation phase separation; Topical gel; *In vitro* drug release; Release kinetics.

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INTRODUCTION

Keeping a drug at an effective concentration on or within the skin for a meaningful length of time is one of the harder problems in topical therapeutics, and it becomes especially important in anti-infective treatment, where under-dosing at the site can allow an infection to persist or a resistant subpopulation to emerge[1]. Bilayer-forming vesicles liposomes and, more recently, niosomes have been used to address this problem because their internal and membrane compartments can hold both water-soluble and lipid-soluble actives and because the composition of the bilayer can be tuned to slow or speed release[2]. The trade-off is that these vesicles, once dispersed in water, tend not to sit still on a shelf: they fuse, settle, leak their payload, or undergo oxidative and hydrolytic breakdown of the phospholipid components over time.

Proniosomes sidestep much of this instability by not existing as a dispersion until the point of use. In their stored form they are dry, surfactant-coated powders or granules; only on contact with water do they hydrate into niosomal vesicles[3]. That dry-state storage is the key advantage it removes most of the degradation pathways that trouble pre-formed vesicles, and several groups working with proniosomal systems have reported vesicle populations more uniform than those obtained by direct niosome preparation. Formulated further into a semisolid typically by dispersing the proniosomal powder into a carbopol gel the system becomes a proniosomal gel: something that spreads and adheres like an ordinary topical gel but converts to niosomes in situ once it picks up moisture from the skin surface[4].

The drug chosen for this work, clindamycin hydrochloride, is a lincosamide that blocks bacterial protein synthesis at the 50S ribosomal subunit and covers most Gram-positive organisms implicated in skin and soft-tissue infection[5]; where resistance does arise, it is usually traced to alteration of that ribosomal binding site rather than drug inactivation[6]. Its clinical drawbacks are well known gastrointestinal upset and a bitter aftertaste orally, first-pass hepatic loss, and, topically, a tendency to be cleared from the application site before it has done its job[7]. A carrier able to hold more drug at the site, release it gradually, and keep it pharmacologically intact would address exactly this shortfall[8].

This study set out to build and evaluate such a carrier: a clindamycin hydrochloride proniosomal gel made by co-acervation phase separation from Span 60, cholesterol and soya lecithin. Concretely, the work covered preformulation testing of the drug; FTIR-based compatibility screening against the chosen excipients; preparation and full characterization of the proniosomes and the gel derived from them entrapment efficiency, particle size, zeta potential, morphology, pH,

viscosity, spreadability, drug content; in vitro release and its kinetic modelling; and, for the best-performing batch, antibacterial testing, dermal tolerability and short-term storage stability.

MATERIALS AND METHODS

Materials:

Yarrow Chemicals (Mumbai) supplied clindamycin hydrochloride, cholesterol and soya lecithin. Span 60 and Carbopol 934 came from Oxford Lab Fine Chem LLP (Maharashtra), and ethanol from Labrot Fine Chemicals (Mumbai). Every other reagent used was of analytical grade.

Animals

The Wister rats weighing between 150-200 gm were procured from Animal house of Shree Devi college of Pharmacy, Mangalore and maintained under constant conditions (temperature 25 ± 2C, Humidity 40-60%, 12 h light/ 12 h dark cycle). During maintenance the animals received a diet of food pellet supplied from animal house and water ad libitum. These experiments were approved by the Institutional Animal Ethics Committee, Shree Devi college of pharmacy of , Mangalore (IAEC registration no.) SDCP/IAEC/24/2025)

Preformulation studies:

Appearance, colour, odour and taste were noted by simple organoleptic inspection, while melting point was read off using the open-capillary technique. For solubility, an excess of drug was equilibrated with water, pH 7.4 phosphate buffer, chloroform and ethanol in a shaking water bath (37 ± 0.5 °C, 24 h); each supernatant was then filtered, diluted as needed, and its drug content read spectrophotometrically at 210 nm against the appropriate calibration line, broadly following an approach used elsewhere for preformulation work[9].

Spectrophotometric analysis:

Buffer (pH 7.4) was made by combining 85 mL of a 119.34 g/L disodium hydrogen phosphate solution with 15 mL of a 45.31 g/L potassium dihydrogen phosphate solution, with pH adjusted afterward if needed, in line with the pharmacopoeial procedure[10]. Dissolving the drug in this buffer at 100 µg/mL and scanning between 200 and 400 nm identified λmax; from there, a five-point dilution series spanning 2–10 µg/mL was read at that wavelength to build the calibration curve used for the remainder of the study.

Drug–excipient compatibility study:

Whether clindamycin hydrochloride's chemical identity survived contact with Span 60, cholesterol and soya lecithin was checked by FTIR, using an approach adapted from earlier proniosome compatibility work[11]. Each sample — drug, excipients individually, and the finished gel — was

ground with potassium bromide, pressed into a disc, and scanned; spectra were then overlaid to look for band shifts or the disappearance of any characteristic peak.

Preparation of clindamycin hydrochloride proniosomes and gel:

Proniosomes were built up by co-acervation phase separation[12] according to the seven compositions. For each batch, Span 60, cholesterol, soya lecithin and a fixed 100 mg of clindamycin hydrochloride were combined with 5 mL of ethanol in a wide-mouthed vial and heated on a water bath (60–70 °C) until fully dissolved. Aqueous phase was then introduced and heating continued at the same temperature until a proniosomal dispersion formed. A quantity of this dispersion equal to 10 mg of proniosomes was worked into Carbopol 934 gel base, giving a finished 1% w/w clindamycin hydrochloride proniosomal gel.

Table 1: Composition of clindamycin hydrochloride proniosomal formulations (F1–F7)

Formulation	Clindamycin HCl (mg)	Span 60 (mg)	Cholesterol (mg)	Soya lecithin (mg)
F1	100	1350	321	900
F2	100	1800	200	900
F3	100	1350	179	900
F4	100	900	300	900
F5	100	900	200	900
F6	100	1986	250	900
F7	100	714	250	900

Characterization of clindamycin hydrochloride proniosomes:

To separate bound from free drug, a 100 mg portion of each proniosomal batch went into 10 mL of pH 7.4 buffer and spun down on a bench centrifuge (10,000 rpm, half an hour); whatever drug remained unencapsulated showed up in the clear supernatant on UV assay at 210 nm. From that reading, percentage entrapment efficiency was worked out as $\%EE = [1 - (Cf/Ct)] \times 100$, with Cf the free-drug concentration and Ct the total drug used per batch[13].

Particle sizing followed the usual dynamic-light-scattering route on a Malvern Zetasizer held at 25 °C, which also yielded the polydispersity index and zeta potential for each batch[14]. To look at vesicle shape directly, a small volume of the F6 dispersion was air-dried onto an aluminium stub, given a thin conductive coat of gold and carbon, and imaged under SEM.

Characterization of the proniosomal gel:

A 1 g portion of gel dispersed in 100 mL distilled water and left to equilibrate for 2 h gave the pH reading, taken in triplicate on a digital meter. Brookfield viscometry (spindle 64, 20 rpm) yielded viscosity. Spreadability was worked out by sandwiching 1 g of gel between two 14 × 5 cm glass slides under a fixed load and timing how long the slides took to separate, from which the spreadability coefficient $SC = M \times L / T$ was calculated (M: applied mass; L: slide length; T:

separation time)[15]. Drug content was obtained by dissolving 100 mg of gel in pH 7.4 PBS, diluting appropriately, and reading absorbance at 210 nm against a blank, then back-calculating the concentration from the standard curve.

***In vitro* drug diffusion study:**

Diffusion was tracked across a Franz cell fitted with a dialysis membrane, using a setup adapted from work on a related proniosomal formulation[16]. The donor side received gel equivalent to 10 mg of drug; the receptor side held pH 7.4 PBS under continuous magnetic stirring (200 rpm). Every hour for 7 h, a 2 mL aliquot was removed for assay at 210 nm and replaced with an equal volume of fresh buffer.

Drug release kinetics:

The cumulative-release profile of the best-performing batch was tested against four standard models — zero-order, first-order, Higuchi and Korsmeyer–Peppas[17] — and the model returning the highest R^2 was taken to best describe how the drug was leaving the formulation.

Antimicrobial activity:

An agar-well diffusion assay was used to gauge antibacterial performance[18]: the zone of clearing produced by the optimized gel around a well was measured and set against that produced by an untreated control well.

Skin irritation study:

Dermal tolerability of the optimized batch (F6) was checked in rats using a conventional irritation protocol[19]. A 2 cm^2 patch of skin was clipped free of hair two weeks ahead of the test to let any clipping-related irritation resolve. Formulation (0.5 g) was then held against the site under a semi-occlusive dressing for 4 h, with a second, untreated animal serving as control. Erythema and oedema were graded on a fixed scale right after dressing removal and again at 24 h, 48 h, 72 h, 7 days and 14 days.

Stability studies

Foil-wrapped, tightly sealed containers of the chosen formulation were held for four weeks under two conditions — 8 ± 2 °C and 30 ± 2 °C/65 ± 5% RH — after which entrapment efficiency was re-measured and set against the day-zero value[19].

RESULTS

Preformulation studies:

The procured clindamycin hydrochloride presented as a white to near-white, odourless, bitter, amorphous powder, melting at 141 °C — within the 141–143 °C literature window — and absorbing maximally at 210 nm, again inside the reported 206–210 nm band. It dissolved readily in

water, ethanol, methanol and pH 7.4 buffer but not in chloroform or acetone, a pattern that lines up with published data for the drug

Organoleptic Characteristics:

Organoleptic properties like general description, colour, taste and odour of drug were characterized. It was found that Clindamycin hydrochloride is a amorphous, white to pale yellow, bitter and odorless powder.

Determination of Melting Point:

The melting point of the drug was determined and found to be 141 °C, which falls within the reported range of 141–143°C. This indicates the purity of the drug.

Determination of Solubility:

Tolfenamic acid was found to be **soluble in water, ethanol, methanol,** and phosphate buffer pH 7.4, **insoluble in chloroform and acetone.**

Determination of Absorption Maximum (λ_{max}):

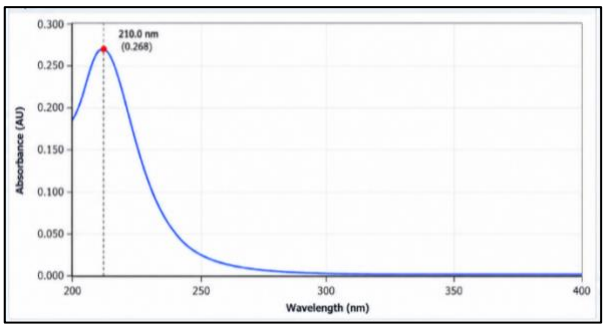


Figure 1: UV Spectra of Clindamycin hydrochloride using pH 7.4 phosphate buffer

Determined of absorption maxima was performed by using UV spectrometer by using phosphate buffer with pH 7.4 as medium. The absorption spectrum of Clindamycin hydrochloride was scanned between 200-400 nm. Maximum absorbance was found at 210 nm.

UV calibration curve:

Across 2–10 µg/ml, absorbance tracked concentration in a straight line: $y = 0.0418x - 0.0009$, $R^2 = 0.9992$ a fit tight enough to rely on for every subsequent quantification step in the study.

Table 2: Calibration data for clindamycin hydrochloride in phosphate buffer pH 7.4

Concentration (µg/ml)	Absorbance
0	0.000
2	0.083
4	0.164
6	0.243
8	0.340
10	0.420

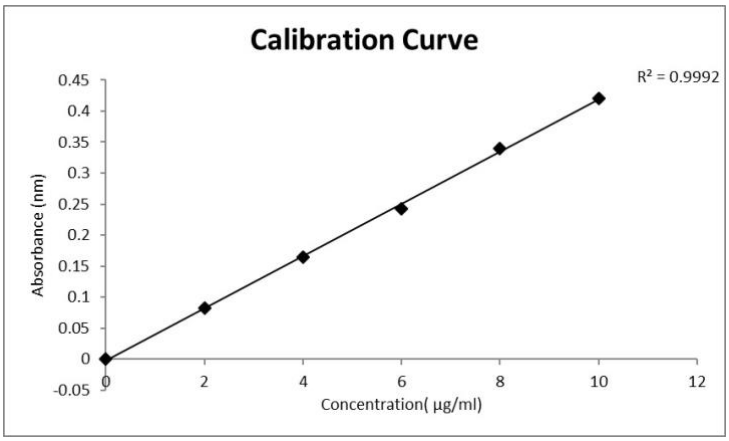


Figure 2: Standard plot of Clindamycin hydrochloride pH 7.4 phosphate buffer

The calibration data showed an increase in absorbance with increasing drug concentration from 2 to 10 µg/ml. The calibration curve demonstrated good linearity, with the regression equation y = 0.0418x − 0.0009 and an R^2 value of 0.9992. This indicates that the analytical method was suitable for quantitative estimation of clindamycin hydrochloride during the subsequent formulation and drug-release studies.

Drug–excipient compatibility (FTIR): Overlaying the spectra showed every characteristic band of clindamycin hydrochloride still present in the finished gel, shifted at most by a few wavenumbers and with nothing missing a result that argues against any substantial chemical interaction between drug and excipients.

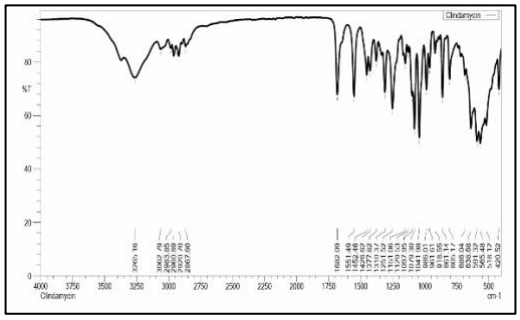


Figure 3: FTIR of Clindamycin hydrochloride

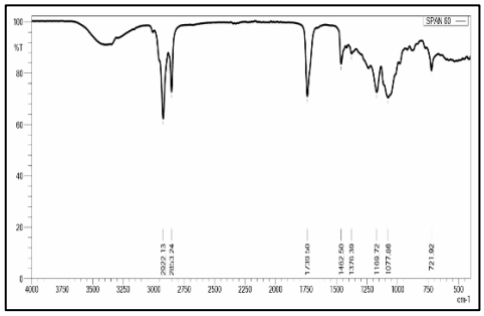


Figure.4: FTIR spectra of Span 60

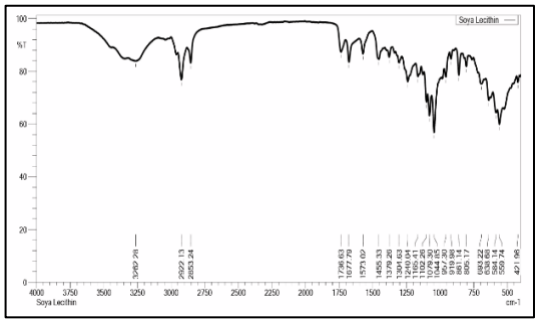


Figure 5: FTIR spectra of Cholesterol

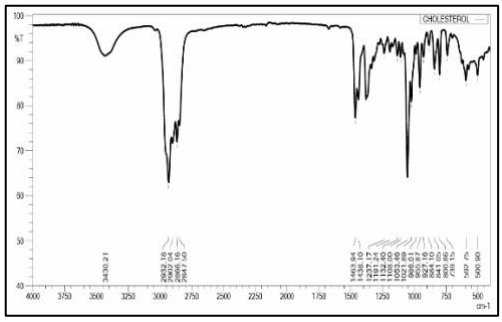


Figure 6: FTIR spectra of SoyaLecithin

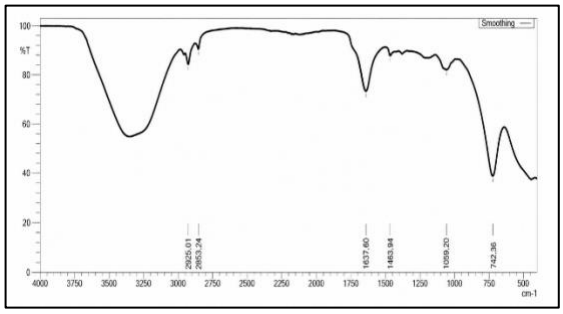


Figure 7: FTIR spectra of Clindamycin hydrochloride proniosomal gel

Across the seven batches, entrapment spanned 57.5 ± 0.35% (F2, the lowest) to 86.7 ± 0.3% (F6, the highest).

FTIR spectroscopy was carried out to investigate the compatibility of clindamycin hydrochloride with the selected excipients, namely Span 60, cholesterol and soya lecithin. The characteristic functional groups of the drug and excipients were observed in their respective spectra. In the final formulation, the important characteristic peaks were retained with only minor shifts in their frequencies. The final formulation showed peaks corresponding to alkane C–H at approximately 2925.01 cm^{-1}, alkene C=C at 1637.60 cm^{-1}, O–H at 3300 cm^{-1}, ether C–O–C at 1089.20 cm^{-1}, ester –COO– at 1737 cm^{-1}, C–Cl at 742.26 cm^{-1}, and phosphate P–O at 1089.20 cm^{-1}.

The absence of major disappearance of the characteristic functional-group peaks suggests that there was no significant chemical incompatibility between clindamycin hydrochloride and the selected excipients. The observed minor shifts may be attributed to interactions within the formulation environment rather than formation of new chemical bonds. Therefore, the FTIR findings support the compatibility of clindamycin hydrochloride with Span 60, cholesterol and soya lecithin and indicate their suitability for preparation of the proniosomal gel.

Characterization of Clindamycin Hydrochloride Loaded Proniosome

Entrapment efficiency (EE):

Table 3: Entrapment efficiency of clindamycin hydrochloride proniosomes

Formulation	Entrapment efficiency (%)
F1	63.5 ± 0.35
F2	57.5 ± 0.35
F3	73.5 ± 0.3
F4	62.5 ± 0.35
F5	78.6 ± 0.3
F6	86.7 ± 0.3
F7	66.2 ± 0.35

The entrapment efficiency of the prepared proniosomal formulations ranged from 57.5± 0.35% to 86.7±0.3%. Among the formulations, F6 exhibited the highest entrapment efficiency of

86.7±0.3%, followed by F5 with 78.6± 0.3%. F2 showed the lowest entrapment efficiency of 57.5 ± 0.35%.

The relatively high entrapment efficiency observed for F6 indicates efficient incorporation of clindamycin hydrochloride into the proniosomal system. The differences in entrapment efficiency among the formulations may be associated with variations in the composition of the proniosomal system and the ability of the lipid/surfactant components to accommodate the drug. Higher entrapment is desirable because it can provide a greater amount of drug available within the vesicular system and may contribute to improved drug retention and controlled release.

Vesicle size, zeta potential and morphology:

F6's particle-size run put the average vesicle diameter at 249.69 nm (PDI 0.362), with a zeta potential of 35.7 mV numbers that fit a nanoscale population carrying enough charge to stay dispersed rather than clump. The SEM images told the same story: individual, roughly round vesicles rather than fused or irregular clusters.

pH, spreadability, viscosity and drug content of the gel:

Gel pH sat between 6.08 ± 0.03 and 6.45 ± 0.03 and spreadability between 20.79 ± 0.1 and 28.9 ± 0.05 g·cm/s (Table 7), F6 topping both. Viscosity ranged 6320–8890 cP (Table 8) and drug content 57.5 ± 0.35–85.7 ± 0.3% (Table 9), with F6 again leading on both counts.

Table 4: pH, spreadability Viscosity (cP) and Drug content (%) of clindamycin hydrochloride proniosomal gel

Formulation	pH	Spreadability (g·cm/s)	Viscosity (cP)	Drug content (%)
F1	6.08 ± 0.03	23.6 ± 0.2	7380	61.5 ± 0.35
F2	6.38 ± 0.035	22.27 ± 0.2	7640	57.5 ± 0.35
F3	6.18 ± 0.03	20.79 ± 0.1	6502	73.5 ± 0.3
F4	6.29 ± 0.03	22.67 ± 0.3	7980	65.5 ± 0.35
F5	6.31 ± 0.035	20.91 ± 0.6	8720	78.6 ± 0.3
F6	6.45 ± 0.03	28.9 ± 0.05	8890	85.7 ± 0.3
F7	6.21 ± 0.03	23.6 ± 0.2	6320	63.2 ± 0.35

***In vitro* drug release and kinetics:**

Every batch released its drug gradually over the 7 h window (Table 10), F6 pulling ahead of the rest with 86.34% released against 60.5–78.4% for the others. Testing F6's release profile against the four kinetic models (Table 11) put first-order ahead of the field (R^2 = 0.9901), with Korsmeyer–Peppas close behind (0.9861), then zero-order (0.9727) and Higuchi (0.9594) a ranking that points to a concentration-dependent, diffusion-driven release mechanism.

Table 5: In vitro cumulative drug release (% CDR) of clindamycin hydrochloride proniosomal gel

Time (h)	F1	F2	F3	F4	F5	F6	F7
0	0	0	0	0	0	0	0
1	8.5	10.17	11.45	12.46	7.5	15.95	10.5
2	17.2	19.5	20.45	25.64	15.5	33.18	20.17
3	26.46	29.5	27.5	38.64	24.46	48.69	30.5
4	35.5	40.16	37.5	49.8	33.56	62.56	41.5
5	44.5	50.5	47.47	58.47	42.65	72.8	51.5
6	52.5	59.21	55.5	69.54	49.51	80.14	60.5
7	60.5	68.17	64.5	78.4	59.56	86.34	71.16

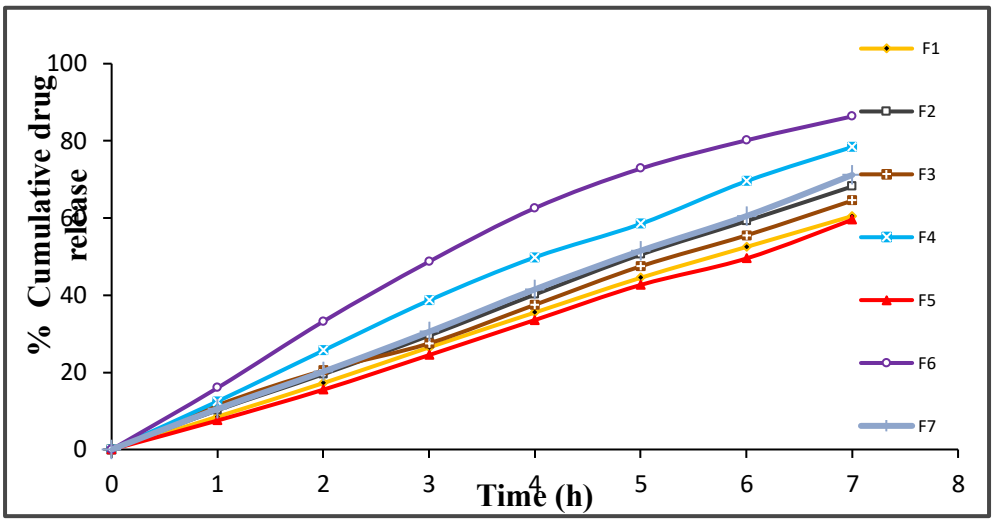


Figure 8: *In vitro* drug release profile for clindamycin hydrochloride formulation

The *in-vitro* drug-release study demonstrated a gradual increase in cumulative drug release with increasing time for all formulations. At 7 hours, the cumulative drug release was 15.95 % to 86.34 % for F6. Among all formulations, F6 exhibited the highest cumulative drug release of 86.34% at 7 hours. The progressive release observed from F6 indicates that the proniosomal system was capable of providing sustained drug release while allowing a substantial amount of clindamycin hydrochloride to become available over the study period.

The improved release profile of F6 may be related to its optimized vesicular characteristics and formulation composition. The proniosomal structure can act as a reservoir for the drug, thereby controlling its diffusion from the formulation. The gradual release pattern is desirable for topical delivery becausee it may help maintain drug availability at the application site for a prolonged period.

Kinetic study:

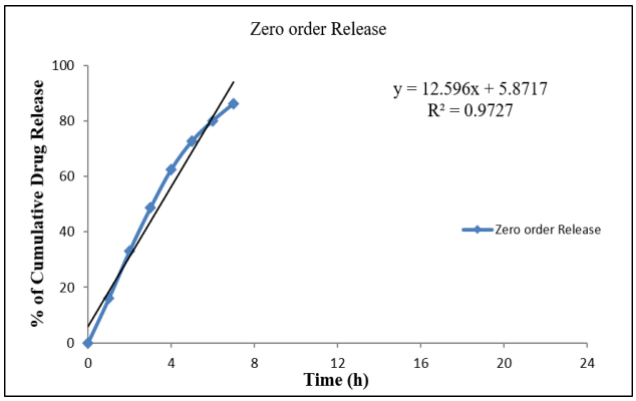


Figure 9: Zero order

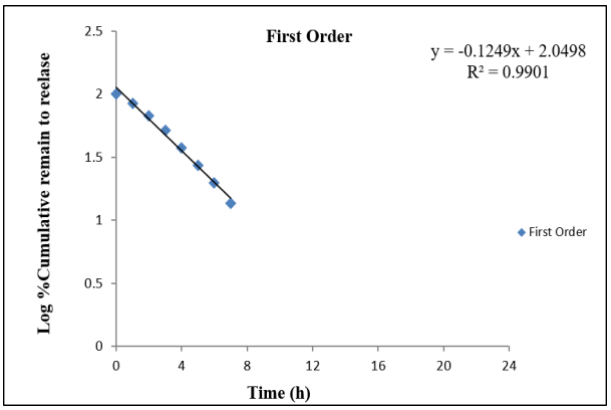


Figure 10: First order

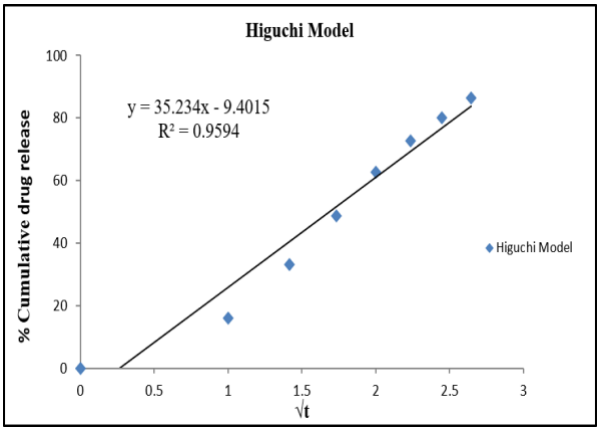


Figure 11: Higuchi model

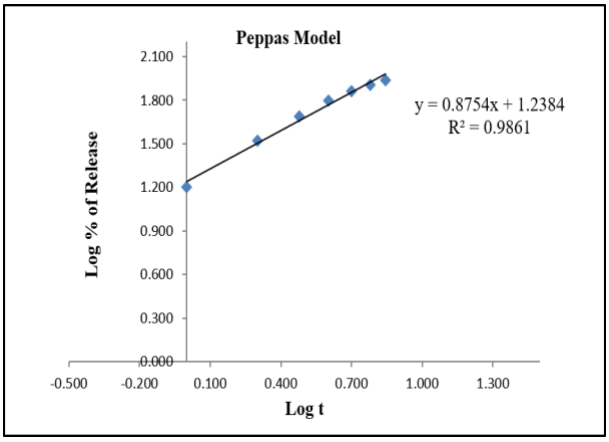


Figure 12: Peppa's model

The release data of the optimized formulation were fitted to different mathematical models, including zero-order, first-order, Higuchi and Korsmeyer–Peppas models. The regression coefficients obtained were 0.9727 for zero-order, 0.9901 for first-order, 0.9594 for Higuchi and 0.9861 for Korsmeyer–Peppas model according to the kinetics table.

Among the models studied, the first-order model showed the highest R^2 value of 0.9901, indicating the best correlation with the experimental release data. Therefore, the release of clindamycin hydrochloride from F6 was predominantly consistent with first-order kinetics over the studied period. This suggests that the drug was released at a relatively uniform rate and was less dependent on the remaining drug concentration during the period studied.

The relatively lower R^2 values for the zero-order and Higuchi models indicate that these models provided a comparatively poorer fit to the obtained release data. Thus, the kinetic analysis supports the controlled-release characteristics of the optimized formulation.

Antimicrobial activity:

F6 produced a distinct zone of inhibition against the test organism, clearly larger than anything seen around the untreated control well — evidence that the drug's antibacterial action survived formulation into the gel.

Test

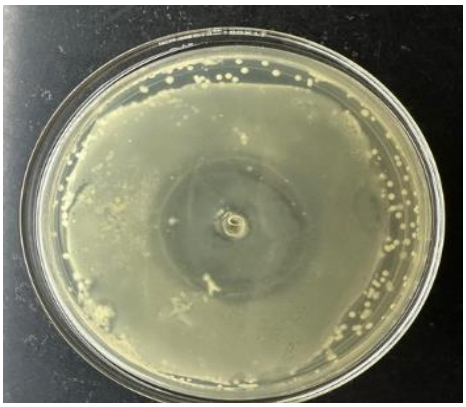


Figure 13: Antimicrobial activity by zone inhibition method

Skin irritation study:

The skin-irritation study was performed to evaluate the topical tolerability of the optimized F6 formulation. The hair was removed before the study, and the experiment was conducted after an appropriate period to minimize any irritation caused by the hair-removal procedure. The animals were observed at different time intervals, including before application, 1 hour, 24 hours, 48 hours, 7 days and 14 days. The irritation scores for both the control and F6-treated groups were recorded as 0 at all observation intervals. No visible signs of erythema or edema were observed during the study period. These findings indicate that the optimized clindamycin hydrochloride proniosomal gel was non-irritant under the conditions of the study and exhibited good topical tolerability.

Stability studies:

Over four weeks, F6's entrapment efficiency slipped only slightly under both storage conditions, with the refrigerated samples losing marginally less than the accelerated set .

Table 6: Stability of formulation F6 over 4 weeks (entrapment efficiency, %)

Storage condition	Initial EE (%)	EE after 4 weeks (%)
8 ± 2 °C (refrigerated)	86.2	85.36
30 ± 2 °C / 65 ± 5% RH (accelerated)	89.26	88.23

CONCLUSION

In the present research work an attempt was made to formulate and evaluate Clindamycin hydrochloride loaded proniosomal gel. From the reproducible results of executed experiments. It can be concluded that;

- Preformulation studies of Clindamycin hydrochloride comply with the reported literature

limits.

- The UV spectrum of Clindamycin hydrochloride showed a peak at 210nm. Standard calibration curve was constructed in the concentration range of 2-10µg/ml using phosphate buffer with pH 7.4 as a medium.
- FTIR studies revealed no chemical interaction between the drug and the excipient and indicate stability of drug in proniosomal formulation.
- Clindamycin hydrochloride proniosomes gel was prepared using co-acervation phase separation method.
- The Seven formulations produced by co-acervation phase separation. From this F6 selected as the optimized formulation.
- The particle size for the optimized formulation was found to be 249.69nm.
- Zeta potential for the optimized formulation was found -35.7 mV which indicates the stability of proniosomes.
- The entrapment efficiency for the optimized formulation was 86.7%.
- From the diffusion study it was found that formulation F6 shows the highest drug permeation was found to be 86.34%.
- F6 followed first order release profile, kinetic profile.
- Formulations stored at refrigeration condition (8±2 °C) showed a higher drug entrapment when compared to the formulations stored at accelerated condition (30 ± 20 ºC at (65±5% RH) after a period of 4weeks.
- Hence the formulated proniosomes were found to be stable and showed prolonged release of Clindamycin hydrochloride.

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