

DEVELOPMENT AND EVALUATION OF ETHOSOMAL DELIVERY SYSTEM OF CLOSTEBOL ACETATE AND NEOMYCIN SULPHATE FOR DERMATOLOGICAL APPLICATIONS

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Abstract

The present study was aimed at the development and evaluation of an ethosomal delivery system containing Clostebol acetate and Neomycin sulphate for enhanced dermatological applications. Ethosomes were prepared using varying concentrations of phospholipid and ethanol by the ethanol injection method and subsequently incorporated into Carbopol-based gel formulations. The prepared ethosomes were evaluated for vesicle size, entrapment efficiency, zeta potential, and stability, while the ethosomal gels were assessed for physicochemical characteristics, drug content, and in vitro drug release behavior. Among the prepared formulations, F5 exhibited the highest entrapment efficiency of 84.80% for Clostebol acetate and 80.50% for Neomycin sulphate, with a vesicle size of 215.80 nm and a zeta potential of -39.85 mV, indicating good stability. The optimized ethosomal suspension was incorporated into gel formulation EF2, which showed excellent homogeneity, suitable spreadability, good extrudability, skin-compatible pH (6.72), and high drug content for both drugs. In vitro drug release studies demonstrated sustained release, with cumulative drug release of 90.20% for Clostebol acetate and 94.75% for Neomycin sulphate over 10 hours. Drug release kinetics revealed that the release followed the Korsmeyer–Peppas model, indicating a diffusion-controlled mechanism. Stability studies confirmed that the formulation remained stable under refrigerated conditions. The findings suggest that the developed ethosomal gel is a promising topical delivery system capable of enhancing skin penetration, improving drug retention, and providing sustained therapeutic action for dermatological applications.

Introduction

Skin infections and wounds remain significant healthcare concerns worldwide, often resulting in delayed healing, secondary infections, and reduced quality of life. Effective topical therapy requires adequate drug penetration into the deeper layers of the skin while maintaining sufficient local drug concentration at the site of action [1].

However, conventional topical formulations such as creams, ointments, and gels frequently exhibit limited skin permeation due to the barrier properties of the stratum corneum, thereby reducing therapeutic efficacy [2].

Clostebol acetate is a synthetic anabolic steroid widely used in dermatological

preparations to promote tissue repair, stimulate epithelial regeneration, and accelerate wound healing. It enhances protein synthesis and cellular proliferation, thereby facilitating the restoration of damaged skin tissues [3]. Neomycin sulphate is a broad-spectrum aminoglycoside antibiotic effective against a variety of Gram-positive and Gram-negative bacteria commonly associated with skin infections. The combination of clostebol acetate and neomycin sulphate has been extensively utilized for the treatment of infected wounds, burns, ulcers, and post-surgical skin lesions due to its dual action of tissue regeneration and antimicrobial protection [4].

Despite their therapeutic benefits, the clinical effectiveness of conventional formulations containing clostebol acetate and neomycin sulphate may be limited by inadequate skin penetration and poor retention within deeper skin layers [5]. Therefore, the development of advanced drug delivery systems capable of enhancing dermal and transdermal transport has gained considerable attention. Ethosomes are novel phospholipid-based vesicular carriers containing high concentrations of ethanol. The presence of ethanol imparts flexibility to the vesicular membrane and disrupts the

lipid organization of the stratum corneum, thereby enhancing skin permeability [6].

Ethosomal systems have demonstrated superior skin penetration, improved drug entrapment efficiency, enhanced stability, and prolonged drug release compared with conventional liposomes and topical formulations. These characteristics make ethosomes particularly suitable for delivering both hydrophilic and lipophilic drugs into deeper skin tissues [7]. The incorporation of clostebol acetate and neomycin sulphate into an ethosomal delivery system may improve drug permeation, enhance local bioavailability, prolong drug retention at the target site, and ultimately improve therapeutic outcomes in dermatological applications. Furthermore, ethosomal formulations may reduce dosing frequency and minimize systemic exposure, thereby improving patient compliance and safety.

Therefore, the present study was undertaken to develop and evaluate an ethosomal delivery system of clostebol acetate and neomycin sulphate for dermatological applications. The formulated ethosomes were characterized for their physicochemical properties, including vesicle size, zeta potential, entrapment efficiency, and in vitro drug release behavior, with the objective of

establishing an effective and stable topical delivery system for enhanced skin therapy.

Material and Methods

Material

Clostebol acetate and Neomycin sulphate were used as the model drugs for the preparation of the ethosomal delivery system. Soya phospholipid was employed as the vesicle-forming lipid, while ethanol served as the penetration enhancer and essential component of the ethosomal vesicles. Polyethylene glycol (PEG) was incorporated as a stabilizing agent during formulation. Carbopol 934 was used as the gelling agent for the preparation of ethosomal gel formulations. Propylene glycol was utilized as a humectant and permeation enhancer, whereas triethanolamine was added for pH adjustment and gel neutralization. Methyl paraben was used as a preservative, and distilled water served as the aqueous phase. All chemicals and reagents used in the study were of analytical grade and were used without further purification.

Methods

Preparation of Ethosomes of Clostebol acetate and Neomycin sulphate

Six different ethosomal formulations (F1–F6) were prepared using the cold method by varying the concentrations of soya

phosphatidylcholine (0.5–1.5% w/v) and ethanol (10–20% w/v), while keeping the drug concentration constant. For each formulation, accurately weighed quantities of Clostebol acetate (1g) and Neomycin sulphate (1g) were used. Initially, the required amount of soya phosphatidylcholine (as per formulation, i.e., 0.5%, 1.0%, or 1.5% w/v) was dissolved in the specified concentration of ethanol (10% or 20% w/v) in a closed vessel.

The mixture was heated gently to $30 \pm 1^\circ\text{C}$ using a water bath to ensure complete dissolution of the lipid. Separately, the drug solution was prepared by dissolving Clostebol acetate and Neomycin sulphate in an appropriate quantity of distilled water containing PEG (20% w/v). This aqueous phase was also maintained at $30 \pm 1^\circ\text{C}$ to match the temperature of the ethanolic lipid phase. The aqueous drug solution was then added slowly in a dropwise manner to the ethanolic phospholipid solution under continuous stirring using a magnetic stirrer at 1000 rpm. The addition was carried out carefully to promote uniform vesicle formation and prevent aggregation.

After complete addition, the mixture was continuously stirred for an additional 5 minutes to ensure proper mixing and formation of ethosomal vesicles. The

resulting dispersion was then allowed to cool at room temperature ($25 \pm 1^\circ\text{C}$) for about 45 minutes, leading to the formation of stable ethosomal vesicular systems [8]. The same procedure was followed for all formulations (F1–F6), with variation only in the concentration of phospholipid and

ethanol, as specified in the composition table. These variations were designed to study their effect on vesicle size, entrapment efficiency, and drug release behavior. The composition of the different ethosomal dispersions is summarized in Table 1.

Table 1: Different Composition of ethosomes formulation

F. Code	Drug (g)		Phospholipid (% w/v)	Ethanol (% w/v)	PEG (%w/v)	Water (%w/v)
	Clostebol acetate	Neomycin sulphate				
F1	1	1	0.5	10	20	100
F2	1	1	0.5	20	20	100
F3	1	1	1.0	10	20	100
F4	1	1	1.0	20	20	100
F5	1	1	1.5	10	20	100
F6	1	1	1.5	20	20	100

Evaluation of Clostebol acetate and Neomycin sulphate loaded Ethosomes

Microscopic observation of prepared ethosomes

An optical microscope (Cippon, Japan) with a camera attachment (Minolta) was used to observe the shape of the prepared ethosomes formulation [9].

Vesicle size and zeta potential

Vesicle size and zeta potential of the Ethosomes were measured by photon correlation spectroscopy using a horiba

scientific, nanoparticle analyzer instrument the results shown in table.

Entrapment efficiency

Entrapment efficiency was determined by measuring the concentration of untrapped free drug in aqueous medium [10]. About 1 ml of the drug loaded ethosomes dispersion was placed in the eppendorf tubes and centrifuged at 10,000 rpm for 30 min. The ethosomes along with encapsulated drug were separated at the bottom of the tubes. Plain ethosomes without drug was used as blank sample and centrifuged in the same

manner. In order to measure the free drug concentration, the UV absorbance of the supernatant was determined using simultaneous equation method.

Formulation of ethosomal loaded gel

Carbopol (0.5%, 1%, and 2%) was dispersed in distilled water and allowed to hydrate for 1–2 hours. Propylene glycol (10%) and methyl paraben (0.1%) were added with continuous stirring. The pH was adjusted

using triethanolamine (q.s.) to form a clear gel base.

The optimized drug-loaded ethosomal suspension (equivalent to 0.5% of both drugs) was then incorporated into the gel by slow mechanical stirring at 25 rpm for 10 minutes. Finally, the volume was adjusted with distilled water to obtain uniform ethosomal gel formulations (EF1–EF3) [11].

Table 2: Composition of different gel base

S. No.	Formulation	Carbopol (%)	Propylene Glycol (%)	Triethanolamine (%)	Methyl Paraben (%)	Distilled Water (%)
1	EF1	0.5	10	q.s	0.1	q.s to 100
2	EF2	1.0	10	q.s	0.1	q.s to 100
3	EF3	2.0	10	q.s	0.1	q.s to 100

Evaluation of gel

Physical characteristic

The Physical Characteristic was checked for gel formulations (homogeneity and texture) and observations were shown in Table.

Determination of pH

The pH of the gel was determined by digital pH meter. One gram of gel was dissolved in 25 ml of distilled water and the electrode was then dipped in to gel formulation for 30 min until constant reading obtained [12]. And constant reading was noted. The measurements of pH of each formulation were replicated two times.

Washability

Formulations were applied on the skin and then ease and extent of washing with water were checked manually and observations were shown in Table.

Extrudability study

Gel formulations were filled into collapsible metal tubes or aluminium collapsible tubes [13]. The tubes were pressed to extrude the material and the extrudability of the formulation was checked in table.

Assay

Weight equivalent to 10 mg of ethosomal gel dissolved in 5 ml methanol in 10 ml volumetric flask, sonicate it for 10 min and volume make up to 10 ml and dilute suitably to 10µg/ml and take the absorbance and calculate using simultaneous equation method [14].

Spreadability

Two glass slides of standard dimensions (6×2) were selected. The gel formulation whose spreadability had to be determined was placed over one of the slides. The second slide was placed over the slide in such a way that the formulation was sandwiched between them across a length of 6 cms along the slide. 100 grams of weight

was placed up on the upper slide so that the gel formulation between the two slides was traced uniformly to form a thin layer.

The weight was removed and the excess of the gel formulation adhering to the slides was scrapped off. The lower slide was fixed on the board of the apparatus and one end of the upper slide was tied to a string to which 20 gram load could be applied 50 with the help of a simple pulley. The time taken for the upper slide to travel the distance of 6 cms and separate away from lower slide under the direction of the weight was noted. The experiment was repeated and the average of 6 such determinations was calculated for each gel formulation.

$$\text{Spreadability} = \frac{m.l}{t}$$

Where, S=Spreadability (gcm/sec)

m = weight tied to the upper slide (20 grams)

l= length of glass slide (6cms).

t = time taken is seconds.

Viscosity

The measurement of viscosity of the prepared gel was done using Brookfield digital viscometer. The viscosity was measured using spindle no. 6 at 10 rpm and 25°C. The sufficient quantity of gel was filled in appropriate wide mouth container. The gel was filled in the wide mouth

container in such way that it should sufficiently allow to dip the spindle of the Viscometer. Samples of the gels were allowed to settle over 30 min at the constant temperature (25±1°C) before the measurements.

***In-vitro* drug release studies using the semipermeable membrane preparation of semi permeable membrane for the diffusion studies:**

A semipermeable membrane measuring approximately 25 cm × 2 cm was carefully washed under running water and then soaked in distilled water for 24 hours prior to use. This step was performed to remove any glycerin present on the membrane. The prepared membrane was subsequently mounted on the diffusion cell for further studies [15-16].

The prepared ethosomal delivery system was evaluated for *in vitro* drug release using a modified Franz diffusion cell. The dissolution study was carried out in 24 mL of dissolution medium, maintained at 37±0.2°C and stirred at 50 rpm. Samples were withdrawn at predetermined time intervals and replaced with an equal volume of fresh dissolution medium to maintain sink conditions. The withdrawn samples were diluted to a final volume of 10 mL using PBS (pH 7.4) and assayed spectrophotometrically. The percentage drug release was calculated using simultaneous equation method.

Stability Study

The stability study of the optimized formulation was carried out in accordance

with ICH guidelines to evaluate its physicochemical stability over time under different storage conditions. The prepared ethosomal formulation was filled in suitable, airtight glass containers and stored at two different conditions: refrigerated condition (4 ± 2°C) and room temperature (25–28 ± 2°C). The samples were stored for a period of 3 months, and analysis was performed at predetermined time intervals of 1 month, 2 months, and 3 months.

Results and Discussion

The present study was aimed at developing and evaluating an ethosomal delivery system containing Clostebol acetate and Neomycin sulphate for enhanced dermatological applications. Ethosomes were prepared using varying concentrations of phospholipid and ethanol to optimize vesicular characteristics, drug entrapment, and drug release behavior. The compositions of the prepared ethosomal formulations (F1–F6) and gel formulations (EF1–EF3) are presented in Table 1 and Table 2, respectively.

The prepared ethosomes exhibited vesicle sizes ranging from 165.30 to 215.80 nm with satisfactory drug entrapment efficiencies for both Clostebol acetate and Neomycin sulphate (Table 3). The results demonstrated that increasing phospholipid

concentration enhanced entrapment efficiency due to the availability of a larger lipid matrix for drug encapsulation. Formulation F5, containing 1.5% phospholipid and 10% ethanol, exhibited the highest entrapment efficiencies of $84.80 \pm 0.96\%$ for Clostebol acetate and $80.50 \pm 0.74\%$ for Neomycin sulphate, along with a vesicle size of 215.80 nm. These findings indicate efficient encapsulation of both drugs within the ethosomal vesicles. Similar observations have been reported in previous studies where increased phospholipid concentration improved drug loading capacity and vesicle stability.

Based on vesicle size and entrapment efficiency, formulation F5 was selected as the optimized formulation. Further characterization revealed a zeta potential value of -39.85 mV (Table 4), indicating good colloidal stability due to sufficient electrostatic repulsion between vesicles. A zeta potential value greater than ± 30 mV is generally considered indicative of a stable dispersion system.

The optimized ethosomal suspension was incorporated into Carbopol gel bases prepared with different polymer concentrations (Table 2). Evaluation of the gel formulations demonstrated satisfactory physical characteristics. All formulations

exhibited excellent homogeneity and smooth texture without any signs of phase separation (Table 5). Spreadability values ranged from 11.10 to 16.20 g·cm/sec, indicating easy application on the skin surface. As the Carbopol concentration increased, viscosity increased and spreadability decreased, which is a commonly observed phenomenon in gel formulations. Formulation EF2 showed an optimum balance between spreadability and extrudability, making it suitable for topical administration.

The pH values of all gel formulations ranged between 6.35 and 6.90, which are compatible with the physiological pH of the skin and are unlikely to cause irritation (Table 6). Viscosity values increased with increasing polymer concentration, confirming the thickening effect of Carbopol. Drug content analysis demonstrated uniform distribution of both drugs throughout the gel matrix. EF2 showed the highest drug content, with $98.50 \pm 0.25\%$ of Clostebol acetate and $99.12 \pm 0.74\%$ of Neomycin sulphate (Table 6), indicating excellent formulation uniformity.

The in vitro release study of the optimized ethosomal gel EF2 demonstrated sustained drug release behavior over a period of 10

hours (Table 7). Clostebol acetate exhibited cumulative drug release of 90.20%, while Neomycin sulphate showed 94.75% release at the end of the study period. The release profiles suggest that the ethosomal gel system was capable of providing prolonged drug release, which may enhance therapeutic efficacy and reduce the frequency of application. The cumulative release profiles are illustrated in Figure 1.

Drug release kinetic analysis was performed to determine the mechanism of drug release from the optimized formulation (Table 8 and Figure 2). Regression analysis revealed that the release data best fitted the Korsmeyer–Peppas model, with correlation coefficients (R^2) of 0.9970 for Clostebol acetate and 0.9996 for Neomycin sulphate (Table 9). These values were higher than those obtained for zero-order and first-order kinetics, indicating that drug release followed a diffusion-controlled mechanism.

The Peppas model suggests that both drugs were released through a combination of diffusion and matrix relaxation processes from the gel network.

Stability studies conducted over a period of three months demonstrated that the optimized ethosomal formulation remained relatively stable under refrigerated conditions ($4\pm 2^\circ\text{C}$) compared with room temperature storage ($25\text{--}28\pm 2^\circ\text{C}$) (Table 10). A gradual increase in particle size was observed during storage, which was more pronounced at room temperature. Physical appearance remained normal under refrigerated conditions, whereas slight turbidity and aggregation were observed at room temperature after prolonged storage. These findings indicate that refrigeration is the preferred storage condition for maintaining the physicochemical stability of the ethosomal formulation.



F1



F2



F3



F4

F5

F6

Figure 1: Microscopic observation of prepared ethosomes formulation**Table 3: Result for Vesicle size and Entrapment efficiency of Clostebol acetate and Neomycin sulphate loaded Ethosomes**

Formulation Code	Vesicle Size (nm)	% Entrapment Efficiency (Clostebol acetate)	% Entrapment Efficiency (Neomycin sulphate)
F1	182.45	72.30±0.45	68.30±0.36
F2	165.30	70.00±0.63	65.90±0.45
F3	198.75	78.40±0.74	74.40±0.33
F4	176.40	75.10±0.58	70.80±0.63
F5	215.80	84.80±0.96	80.50±0.74
F6	192.65	81.60±0.45	77.40±0.74

Table 4: Vesicle size and entrapment efficiency of optimized ethosomes formulation

Formulation Code	Vesicle size (nm)	Entrapment Efficiency		Zeta potential
		Clostebol acetate	Neomycin sulphate	
F5	215.80	84.80	80.50	-39.85

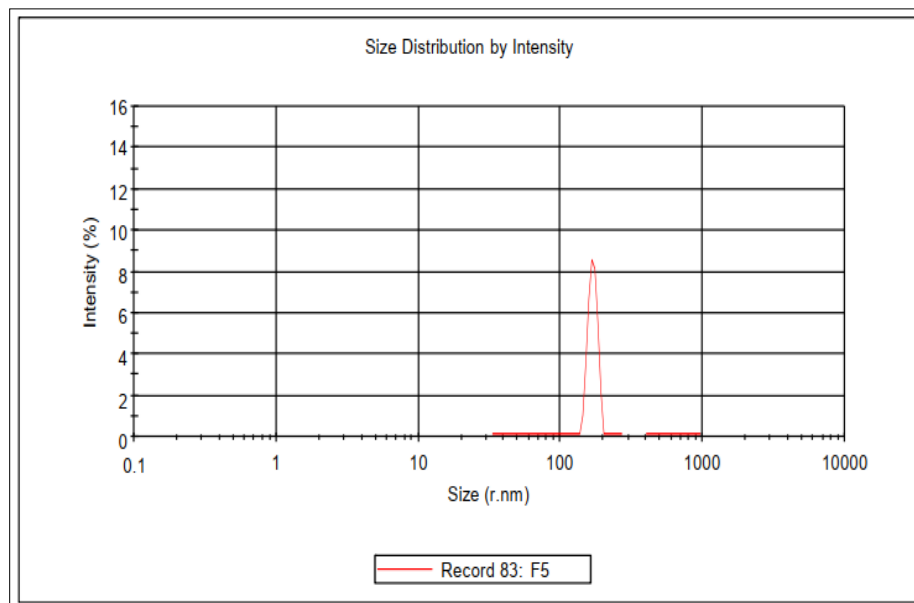


Figure 2: Graph of Vesicle size of optimized formulation F5

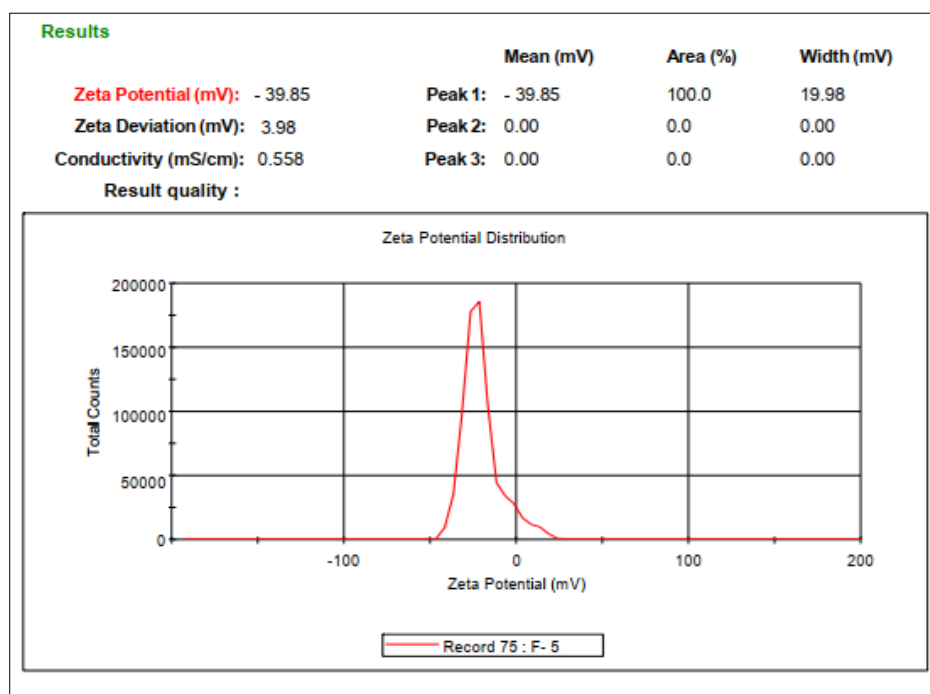


Figure 3: Graph of Zeta Potential of optimized formulation F5

Table 5: Results of Homogeneity, Extrudability, Spreadability of gel formulation

Code	Homogeneity and Texture	Spreadability (gm·cm/sec.)	Extrudability	Washability
EF1	+++	16.20	++	Good
EF2	+++	13.40	+++	Good
EF3	+++	11.10	++++	Good

Table 6: Results of pH, Viscosity and % Drug content

Code	pH	Viscosity (cps)	% Drug Content	
			Clostebol acetate	Neomycin sulphate
EF1	6.35±0.04	3250±18	94.20±0.30	96.65±0.65
EF2	6.72±0.05	3485±20	98.50±0.25	99.12±0.74
EF3	6.90±0.06	3720±22	96.10±0.28	95.65±0.96

Table 7: Results of cumulative % drug release of Clostebol acetate and Neomycin sulphate from optimized ethosomes gel formulation EF2

S. No.	Time (hrs)	Cumulative % drug release	
		Clostebol acetate	Neomycin sulphate
1	0.5	8.25	12.40
2	1	15.60	21.85
3	2	28.75	36.20
4	4	45.30	55.80
5	6	62.40	71.25
6	8	78.65	85.40
7	10	90.20	94.75

Table 8: *In vitro* drug release data for Clostebol acetate of optimized gel formulation EF2

S. No.	Time (H)	Square Root of Time	Log Time	Cumulative* Percentage Drug Release ± SD	Log Cumulative Percentage Drug Release	Cumulative Percent Drug Remaining	Log cumulative Percent Drug Remaining
1	0.5	0.707	-0.301	8.25	0.916	91.75	1.963
2	1	1.000	0	15.60	1.193	84.40	1.926
3	2	1.414	0.301	28.75	1.459	71.25	1.853
4	4	2.000	0.602	45.30	1.656	54.70	1.738
5	6	2.449	0.778	62.40	1.795	37.60	1.575
6	8	2.828	0.903	78.65	1.896	21.35	1.329

7	10	3.162	1	90.20	1.955	9.80	0.991
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Table 9: Results of regression analysis data of ethosomal formulation EF2

Formulation	Zero order	First order	Pappas plot
Clostebol acetate	0.9893	0.9613	0.9970
Neomycin sulphate	0.9717	0.9597	0.9996

Table 10: Results of stability study of optimized formulation of ethosomes

Characteristic	1 Month		2 Month		3 Month	
Temperature	4±2°C	25–28±2°C	4±2°C	25–28±2°C	4±2°C	25–28±2°C
Average Particle Size (nm)	118.25±1.20	132.45±2.15	135.60±2.85	178.40±3.20	152.80±3.65	215.75±4.50
Physical Appearance	Normal	Slightly turbid	Normal	Turbid	Normal	Slight aggregation

Conclusion

The present study successfully developed and evaluated an ethosomal delivery system of Clostebol acetate and Neomycin sulphate for dermatological applications. The prepared ethosomal formulations exhibited desirable physicochemical characteristics, including nanosized vesicles, high drug entrapment efficiency, and good stability. Among the formulations, F5 was identified as the optimized ethosomal formulation, showing maximum entrapment efficiency and a favorable zeta potential value. The optimized ethosomes were successfully incorporated into a Carbopol gel base, and the resulting formulation (EF2) demonstrated excellent homogeneity,

suitable pH, satisfactory viscosity, good spreadability, and uniform drug content. In vitro drug release studies revealed sustained and controlled release of both Clostebol acetate and Neomycin sulphate over a prolonged period. Release kinetic analysis indicated that drug release followed the Korsmeyer–Peppas model, suggesting a diffusion-controlled mechanism. Stability studies further confirmed that the formulation remained stable under refrigerated storage conditions. The developed ethosomal gel represents a promising topical drug delivery system that may enhance skin penetration, improve local drug availability, provide prolonged therapeutic action, and improve the

management of skin infections and wound-healing conditions. Further in vivo and clinical investigations are warranted to establish its therapeutic efficacy and safety in dermatological applications.

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