



**FORMULATION AND EVALUATION OF LIPID BASED VESICULAR SYSTEM  
ETHOSOMES FOR TREATMENT OF SKIN INFECTION**

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**ABSTRACT**

This study investigates the formulation and evaluation of lipid-based ethosomes loaded with Linezolid for the treatment of skin infections. Ethosomes were prepared and characterized for vesicle size, entrapment efficiency, and stability. Among the formulations tested, F5 emerged as the optimized formulation with a vesicle size of 110.25 nm and an entrapment efficiency of 85.45%. The selected ethosome formulation (EF2) was incorporated into a gel base, evaluated for physical characteristics including pH, viscosity, and drug content, as well as practical properties such as spreadability and extrudability. EF2 exhibited favorable attributes, including a pH of 6.74, viscosity of 3315 cps, and 98.12% drug content, alongside good spreadability and extrudability. In vitro drug release studies demonstrated sustained release kinetics over 10 hours, fitting well with zero-order and Pappas plot models. These findings highlight EF2 as a promising candidate for topical delivery of Linezolid, with potential implications for enhanced treatment outcomes in dermatological conditions.

**Keywords:** Ethosomes, Linezolid, lipid-based vesicular systems, topical drug delivery, skin infections.

**INTRODUCTION**

Skin infections represent a significant healthcare challenge globally, exacerbated by the emergence of antibiotic-resistant pathogens and limitations of conventional therapies. Conventional treatments such as topical creams and oral antibiotics often face barriers in achieving effective drug delivery to the infection site within the skin layers, leading to suboptimal therapeutic outcomes and potential systemic side effects. In recent years, lipid-based vesicular systems have garnered considerable attention as promising alternatives for enhancing the topical delivery of antimicrobial agents. Among these, ethosomes stand out for their ability to encapsulate both hydrophilic and lipophilic drugs within phospholipid bilayers, thereby facilitating deeper penetration into the skin

and enhancing drug efficacy. Ethosomes are composed of phospholipids, ethanol, and water, forming soft vesicles that can deform and penetrate through the lipid layers of the skin more efficiently than traditional liposomes (Touitou *et al.*, 2000; Elsayed *et al.*, 2006).

This property makes ethosomes particularly suitable for delivering drugs to target sites of infection, potentially reducing the frequency of application and improving patient compliance. Research has demonstrated the efficacy of ethosomes loaded with antimicrobial agents against various pathogens, including drug-resistant strains (Fang *et al.*, 2018; Chen *et al.*, 2021). However, despite these advancements, challenges remain in optimizing ethosomal

formulations for stability, scalability, and regulatory approval.

This study aims to address these challenges by systematically formulating ethosomes and evaluating their physicochemical properties, drug release profiles, and therapeutic efficacy for the targeted treatment of skin infections.

## **MATERIALS AND METHODS**

### **Materials**

Linezolid, provided as a gift sample by Bioplus Life Science, Bangalore, served as the antimicrobial agent. Solvents included methanol, ethanol, and chloroform sourced from Qualigens Fine Chemicals, Mumbai, for various formulation and extraction purposes. Phospholipid, obtained from Loba Chemie Pvt Ltd Mumbai, was used as a key component in the formulation of ethosomes. Carbapol, methyl paraben, and propyl paraben, also sourced from Loba Chemie Pvt Ltd Mumbai, were employed as viscosity enhancers and preservatives in the formulation. Additionally, propylene glycol from Qualigens Fine Chemicals, Mumbai, was used as a solubilizing agent. These materials were selected to formulate and evaluate lipid-based vesicular systems, specifically ethosomes, for the targeted delivery of Linezolid to treat skin infections effectively.

### **Methods**

#### **Preparation of Ethosomes of Linezolid**

Soya PC (0.5 to 1.5% w/v) was dissolved in ethanol (10-20% v/v) and heated up to  $30 \pm 1^\circ\text{C}$  in a water bath in a closed vessel. Distilled water or drug solution in distilled water (0.1% w/v solution), which is previously heated up to  $30 \pm 1^\circ\text{C}$ , was added slowly in a fine stream to the above ethanolic lipid solution with continuous mixing using a

magnetic stirrer at 900 rpm. Mixing was continued for another 5 minutes and finally, the vesicular dispersions resulted was left to cool at room temperature ( $25 \pm 1^\circ\text{C}$ ) for 45 minutes (Touitou *et al.*, 2000). Different ethosomal dispersions and their composition are shown in table 1.

#### **Evaluation of Linezolid 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 (Elsayed *et al.*, 2006).

#### **Vesicle size and zeta potential**

Vesicle size and zeta potential of the Ethosomes were measured by photon correlation spectroscopy (Paolino *et al.*, 2005) using a horiba scientific, nanoparticle analyzer instrument.

#### **Entrapment efficiency**

Entrapment efficiency was determined by measuring the concentration of untrapped free drug in aqueous medium. 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 Linezolid 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 at 248nm (Dayan and Touitou, 2000).

#### **Formulation of ethosomal gel**

The incorporation of the drug loaded ethosomes (equivalent to 0.1%) into gels was achieved by slow mechanical mixing at 25 rpm (REMI type BS stirrer) for 10 minutes.

The optimized formulation was incorporated into three different carbapol gel concentration 0.5, 1 and 2% w/w (Sujitha *et al.*, 2014).

### Evaluation of ethosomal gel

#### Physical characteristic

The **physical** characteristic was checked for gel formulations (homogeneity and texture).

#### 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. And constant reading was noted (Ketul *et al.*, 2012). 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.

#### Extrudability study

The gel formulations were filled into collapsible metal tubes or aluminium collapsible tubes (Jain *et al.*, 2004). The tubes were pressed to extrude the material and the extrudability of the formulation was checked.

#### 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 10 $\mu$ g/ml and take the absorbance at 248 nm and calculate using calibration curve of linearity (Vivek and Dharendra, 2010).

#### Spreadability

Two glass slides of standard dimensions (6 $\times$ 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 (Jain *et al.*, 2015). The experiment was repeated and the average of 6 such determinations was calculated for each gel formulation.

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

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<sup>0</sup>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 ( $25\pm 1^{\circ}\text{C}$ ) before the measurements.

### **In-vitro drug release studies using the semipermeable membrane**

#### **Preparation of semipermeable membrane for the diffusion studies:**

The semipermeable membrane approximately 25 cm x 2cm was taken and washed in the running water. It was then soaked in distilled water for 24 hours, before used for diffusion studies to remove glycerin present on it and was mounted on the diffusion cell for further studies. The prepared Ethosomes delivery system was evaluated for *in vitro* drug release. The drug release studies were carried out using modified franz diffusion cell. The dissolution study was carried out in 24 ml dissolution medium which was stirred at 50 rpm maintained at  $37\pm 0.2^{\circ}\text{C}$ . Samples were withdrawn at different time interval and compensated with same amount of fresh dissolution medium. Volume of sample withdrawn was made up to 10ml by PBS (pH 7.4). The samples withdrawn were assayed spectrophotometrically at 248nm for Linezolid and using UV visible spectrophotometer. The release of Linezolid was calculated with the help of Standard curve of Linezolid (Vivek *et al.*, 2010).

### **RESULTS AND DISCUSSION**

The formulation and evaluation of lipid-based vesicular systems, specifically ethosomes loaded with Linezolid for the treatment of skin infections, are detailed through several key tables. These tables provide critical insights into the physical characteristics, drug encapsulation efficiency, formulation optimization, and performance of the ethosomal gel.

Firstly, from Table 3, formulations such as F5 stand out with a vesicle size of 110.25 nm and an impressive entrapment efficiency of 85.45%. This combination indicates optimal vesicle characteristics for skin penetration and efficient drug delivery, essential for therapeutic efficacy against skin infections.

Table 4 confirms that formulation F5 was optimized, maintaining its vesicle size and high entrapment efficiency, further validated as the chosen formulation. The negative zeta potential (-38.5 mV) suggests good stability and potential for controlled release upon application.

Moving to the gel formulation (Table 5), EF2 demonstrates excellent attributes such as high homogeneity (+++), good spreadability (12.32 gm.cm/sec.), and easy extrudability (+++), which are crucial for patient compliance and effective topical application. Combined with a skin-friendly pH of 6.74 and a high drug content of 98.12%, EF2 promises effective drug delivery and skin tolerance.

The drug release profile of EF2 shows a sustained release pattern over 10 hours, with cumulative drug release steadily increasing from 20.32% to 93.32% at 10 hours. This sustained release is supported by regression analysis, which indicates a good fit to zero-order and Pappas plot models, ensuring predictable and controlled drug release kinetics.

The study underscores the potential of ethosomal formulations, particularly EF2, in delivering Linezolid for the treatment of skin infections. Its optimized characteristics in terms of vesicle size, entrapment efficiency, gel formulation attributes, and sustained drug release profile position EF2 as a promising candidate for further preclinical and clinical

investigations. Future studies should focus on assessing in vivo efficacy, skin penetration capabilities, and long-term stability to fully

ascertain its clinical applicability and therapeutic benefits.

**Table 1: Different Composition of ethosomes formulation**

F. Code	Drug (mg)	Phospholipid (% w/v)	Ethanol (% w/v)	PEG (%w/v)	Water (%w/v)
F1	200	0.5	10	20	100
F2	200	0.5	20	20	100
F3	200	1.0	10	20	100
F4	200	1.0	20	20	100
F5	200	1.5	10	20	100
F6	200	1.5	20	20	100

**Table 2: Composition of different gel base**

S. No.	Formulation	Carbapol (%)
1.	EF1	0.5
2.	EF2	1
3.	EF3	2

**Table 3: Result for Vesicle size and Entrapment efficiency of Linezolid loaded Ethosomes**

Formulation Code	Vesicle size (nm)	% Entrapment Efficiency
F1	165.58±0.25	69.98±0.22
F2	192.21±0.32	72.25±0.15
F3	169.98±0.15	67.78±0.36
F4	162.35±0.44	73.32±0.10
F5	110.25±0.36	85.45±0.33
F6	130.34±0.25	71.15±0.22

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

Formulation Code	Vesicle size (nm)	Entrapment Efficiency	Zeta potential
F5	110.25±0.32	85.45±0.33	-38.5

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

Code	Homogeneity and Texture	Spreadability (gm.cm/sec.)	Extrudability	Washability
EF1	+++	15.45	+++	Good
EF2	+++	12.32	+++	Good
EF3	+++	10.55	+++	Good

+++ Good

++ Average

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

Code	pH	Viscosity (cps)	% Drug content
EF1	6.32±0.05	3458±15	94.65±0.22
EF2	6.74±0.06	3315±23	98.12±0.32
EF3	6.96±0.08	3210±18	95.65±0.15

**Table 7: Results of cumulative % drug release of Linezolid from optimized ethosomes gel formulation EF2**

S. No.	Time (hrs)	% Cumulative drug release ethosomal gel
1	0.5	20.32±0.35
2	1	35.45±0.25
3	2	45.85±0.15
4	4	56.69±0.33
5	6	68.78±0.41
6	8	76.65±0.36
8	10	93.32±0.45

**Table 8: Results of regression analysis data of ethosomal formulation**

Formulation	Zero order	First order	Pappas plot
EF2	R <sup>2</sup> = 0.9580	R <sup>2</sup> = 0.8979	R <sup>2</sup> = 0.9753

## CONCLUSION

The study underscores the feasibility and effectiveness of ethosomes as carriers for Linezolid delivery, highlighting their capability to enhance drug stability, skin penetration, and sustained release characteristics. Future research should focus on further optimizing formulation parameters, conducting in vivo efficacy studies, and assessing long-term stability to validate the clinical applicability and therapeutic benefits of these lipid-based vesicular systems in treating skin infections.

## DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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