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Original Research Article

FORMULATION AND CHARACTERIZATION OF ORNIDAZOLE-LOADED ETHOSOMAL GEL

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ABSTRACT

Ornidazole-loaded ethosomal gel formulations were developed and optimized for enhanced topical delivery and sustained drug release. Ethosomes were prepared using varying concentrations of soya phosphatidylcholine and ethanol and evaluated for vesicle size, entrapment efficiency, and zeta potential. Among the six formulations (F1–F6), F5 showed the smallest vesicle size (118.74 ± 0.25 nm), highest entrapment efficiency (84.95 ± 0.28%), and a zeta potential of –39.85 mV, indicating excellent stability. The optimized ethosomal formulation (F5) was incorporated into a Carbopol gel (EF1–EF3) and assessed for physicochemical properties, including pH, viscosity, homogeneity, spreadability, extrudability, and drug content. EF2 exhibited the most favorable characteristics with pH 6.74 ± 0.06, viscosity 3315 ± 23 cps, and drug content 98.12 ± 0.32%. *In vitro* drug release studies showed a controlled and sustained release of Ornidazole, reaching 96.65 ± 0.63% at 10 hours, following a non-Fickian diffusion mechanism. Stability studies revealed that the formulation remained physically and chemically stable under refrigerated conditions for three months. The optimized ethosomal gel (EF2) demonstrated enhanced drug entrapment, sustained release, and stability, making it a promising candidate for topical delivery of Ornidazole.

Keywords: Ornidazole, Ethosomes, Topical drug delivery, Vesicle size, Entrapment efficiency, Sustained release, Zeta potential, Carbopol gel, Stability study, Non-Fickian diffusion.

INTRODUCTION

Ornidazole is a nitroimidazole derivative widely used for the treatment of anaerobic bacterial and protozoal infections. Despite its therapeutic effectiveness, conventional topical and transdermal delivery of Ornidazole is limited by poor skin permeation and low bioavailability at the site of infection. These limitations often necessitate frequent dosing and may result in suboptimal patient compliance. To overcome such barriers, advanced vesicular drug delivery systems have been investigated to improve drug penetration through the stratum corneum, enhance local drug concentration, and sustain release at the target site (Raju *et al.*, 2019).

Ethosomes are novel soft vesicular carriers composed of phospholipids, ethanol in relatively high concentrations, and water. Unlike traditional liposomes, the high ethanol content in ethosomal systems imparts greater fluidity and deformability to the vesicles, enabling them to penetrate deeper layers of

the skin by interacting with the lipid matrix of the stratum corneum (Garg *et al.,* 2017).

This enhanced penetrative capability has made ethosomes a promising platform for transdermal and dermal drug delivery, showing improved permeation compared to conventional carriers in a variety of drugs including anti-infectives, anti-inflammatories, and antifungals (Touitou *et al.,* 2000).

Incorporation of ethosomal suspensions into a gel base further enhances their applicability as topical formulations. Ethosomal gels combine the advantages of nanocarrier systems with the ease of administration and sustained contact time offered by polymeric gels such as Carbopol or hydroxypropyl methylcellulose. These gels exhibit desirable characteristics like controlled release, improved spreadability, enhanced retention at the application site, and potentially greater therapeutic efficacy over traditional formulations.

Therefore, the development of an Ornidazole-loaded ethosomal gel aims to harness the deformable nature of ethosomes and the advantageous properties of gel bases to improve transdermal delivery, maximize local drug availability, and reduce dosing frequency. Successful formulation and characterization of such a system may offer an effective strategy for the management of bacterial infections with improved patient compliance and therapeutic outcomes.

MATERIALS AND METHODS

Materials

Ornidazole was used as the model drug for the development of the ethosomal gel formulation. Phospholipid (soya phosphatidylcholine) was employed as the vesicle-forming agent, while ethanol served as a penetration enhancer and imparted flexibility to the ethosomal vesicles. Propylene glycol was used as a co-solvent and permeation enhancer. Carbopol 934 was selected as the gelling agent for incorporation of the optimized ethosomal suspension into a topical gel base. Triethanolamine was used to neutralize the gel and adjust the pH. Methanol and distilled water were used as solvents during formulation and analytical studies. All chemicals and reagents used were of analytical grade and used without further purification.

Methods

Preparation of Ethosomes of Ornidazole

Six different ethosomal formulations (F1–F6) were prepared with varying concentrations of soya phosphatidylcholine (Soya PC, 0.5–1.5% w/v) and ethanol (10–20% w/v). For each formulation, Soya PC was dissolved in the specified concentration of ethanol in a closed vessel and heated to 30 ± 1°C using a water bath (Das *et al.,* 2018). In parallel, distilled water or drug solution (Ornidazole, 0.5% w/v) preheated to 30 ± 1°C was slowly added dropwise to the ethanolic lipid solution while continuously stirring at 900 rpm using a magnetic stirrer. Mixing was maintained for an additional 5 minutes to ensure uniform dispersion. The resulting vesicular dispersions were then allowed to cool at room temperature (25 ± 1°C) for 45 minutes (Touitou *et al.,* 2000). The composition of the different ethosomal dispersions is summarized in Table 1.

Evaluation of Ornidazole loaded Ethosomes

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.

Entrapment efficiency

Entrapment efficiency was determined by measuring the concentration of unentrapped free drug in aqueous medium (Li *et al.,* 2012). 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 Ornidazole 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 270nm.

Formulation of ethosomal loaded gel

The incorporation of the drug loaded ethosomes (equivalent to 0.5%) into gels was achieved by slow mechanical mixing at 25 rpm (REMI type BS stirrer) for 10 minutes (Zhang *et al.,* 2018). The optimized formulation was incorporated into three different Carbopol gel concentration 0.5, 1 and 2% w/w (Table 2).

Evaluation of gel

Physical characteristic

The Physical Characteristic was checked for gel formulations (homogeneity and texture) and observations were shown in Table 5.

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 (Zhai *et al.,* 2015). 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.

Extrudability study

The gel formulations were filled into collapsible metal tubes or aluminium collapsible tubes (Mbah *et al.,* 2019). 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 10µg/ml and take the absorbance at 270 nm and calculate using calibration curve of linearity (Ascenso *et al.,* 2015).

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 25ºC. 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±/1ºC) 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 (Behl *et al.*, 1980; Ghafourian *et al.*, 2004).

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 at 270 nm for Ornidazole using a UV-visible spectrophotometer. The percentage drug release was calculated using the standard calibration curve of Ornidazole.

RESULTS AND DISCUSSION

The results clearly demonstrate that formulation F5 and its corresponding gel EF2 exhibited optimal vesicle characteristics, high drug entrapment, good physicochemical stability, and controlled drug release behavior, confirming the suitability of ethosomal gel as an effective topical delivery system for Ornidazole.

The vesicle size and entrapment efficiency data (Table 3) revealed a significant influence of formulation composition on the physicochemical characteristics of Ornidazole-loaded ethosomes. Vesicle sizes ranged from 118.74 ± 0.25 nm to 189.65 ± 0.30 nm, indicating successful formation of nanosized vesicles across all batches. Among the formulations, F5 exhibited the smallest vesicle size (118.74 ± 0.25 nm) along with the highest entrapment efficiency (84.95 ± 0.28%). This can be attributed to the optimized ratio of phospholipid and ethanol,

where ethanol increases membrane fluidity and deformability, enabling better drug accommodation within the vesicular bilayer. Formulations with comparatively larger vesicle sizes (F1–F3) showed lower entrapment efficiency, possibly due to suboptimal lipid concentration and reduced vesicle stability.

Based on these findings, formulation F5 was selected as the optimized ethosomal system. Further characterization (Table 4) demonstrated a high negative zeta potential (−39.85 mV), indicating strong electrostatic repulsion between vesicles and thus good physical stability with minimal aggregation. The narrow vesicle size distribution and sufficient surface charge confirm the robustness and colloidal stability of the optimized ethosomal formulation. The graphical representation of vesicle size (Figure 1) and zeta potential (Figure 2) further supports the uniformity and stability of formulation F5.

The optimized ethosomal formulation was incorporated into a gel base to enhance topical applicability. Evaluation of gel formulations EF1–EF3 (Table 4) showed excellent homogeneity and texture (+++), indicating uniform dispersion of ethosomes within the polymeric matrix. Spreadability values ranged from 10.55 to 15.45 g·cm/sec, suggesting good ease of application. EF2 exhibited balanced spreadability and extrudability (+++), ensuring ease of removal from the container and uniform application on the skin. All formulations demonstrated good washability, an essential parameter for patient acceptability and convenience.

Physicochemical evaluation of ethosomal gels (Table 5-6) showed pH values between 6.32 and 6.96, which are compatible with skin pH and unlikely to cause irritation upon topical application. Viscosity values indicated the formation of a stable gel with appropriate consistency, where EF2 exhibited moderate viscosity (3315 ± 23 cps), favoring both retention at the site of application and ease of spread. Drug content analysis revealed high and uniform drug distribution, with EF2 showing the highest drug content (98.12 ± 0.32%), indicating minimal drug loss during formulation and excellent content uniformity.

In-vitro drug release studies of the optimized ethosomal gel EF2 (Table 7) demonstrated a sustained and controlled release pattern. An initial release of 22.36 ± 0.52% within 0.5 h may be attributed to the release of surface-associated drug, followed by a gradual increase in drug release up to 96.65 ± 0.63% at 10 h. This sustained release behavior is advantageous for topical therapy as it can maintain therapeutic drug levels for prolonged periods, reduce dosing frequency, and enhance patient compliance. The presence of ethosomes within the gel matrix acts as a dual-controlled system, where the vesicular carrier and gel network together modulate drug diffusion.

Drug release kinetic analysis (Table 8) further elucidated the release mechanism of Ornidazole from the ethosomal gel. Regression analysis (Table 9) showed that the optimized formulation EF2 best fitted the Korsmeyer–Peppas (Pappas) model (R^2 = 0.982), followed by zero-order kinetics (R^2 = 0.9564). This indicates a non-Fickian or anomalous transport mechanism, suggesting that drug release was governed by a combination of diffusion through the ethosomal bilayer and relaxation of the gel

matrix. Such a release pattern is highly desirable for topical antimicrobial therapy, ensuring prolonged drug availability at the infection site.

The study confirms that Ornidazole-loaded ethosomal gel, particularly formulation EF2, possesses favorable vesicle characteristics, excellent stability, acceptable gel properties, and controlled drug release behavior. These findings highlight the potential of ethosomal gel systems as an effective and patient-friendly approach for topical delivery of Ornidazole, offering improved therapeutic efficacy and enhanced skin permeation compared to conventional formulations.

Table 1: Different Composition of ethosomes formulation

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

Table 2: Composition of different gel base

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

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

Formulation Code	Vesicle Size (nm)	% Entrapment Efficiency
F1	168.42 ± 0.28	70.25 ± 0.24
F2	189.65 ± 0.30	74.12 ± 0.18
F3	160.87 ± 0.20	68.45 ± 0.30
F4	155.36 ± 0.35	76.58 ± 0.15
F5	118.74 ± 0.25	84.95 ± 0.28
F6	128.22 ± 0.22	78.35 ± 0.20

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

Formulation Code	Vesicle size (nm)	Entrapment Efficiency	Zeta potential
F5	118.74 ± 0.25	84.95 ± 0.28	-39.85

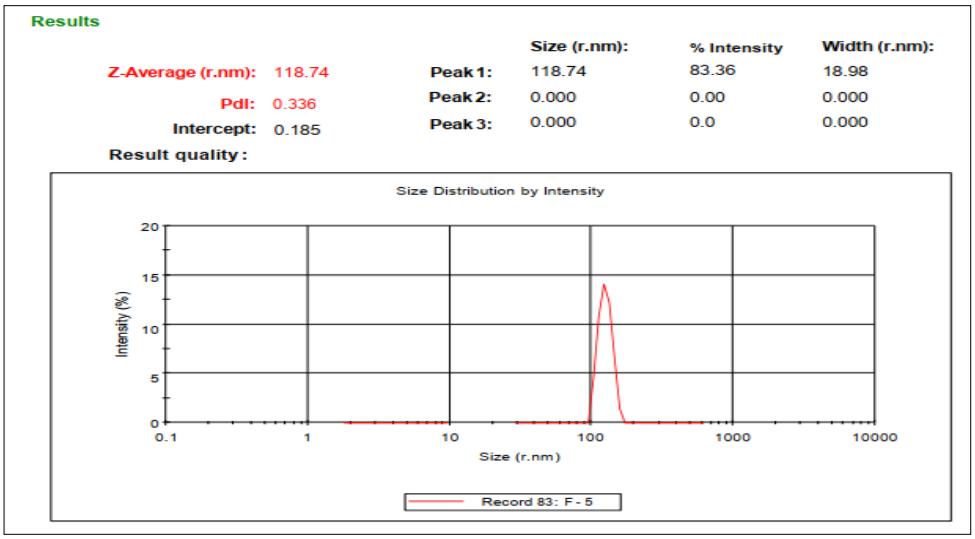

Figure 1: Graph of Vesicle size of optimized formulation F5

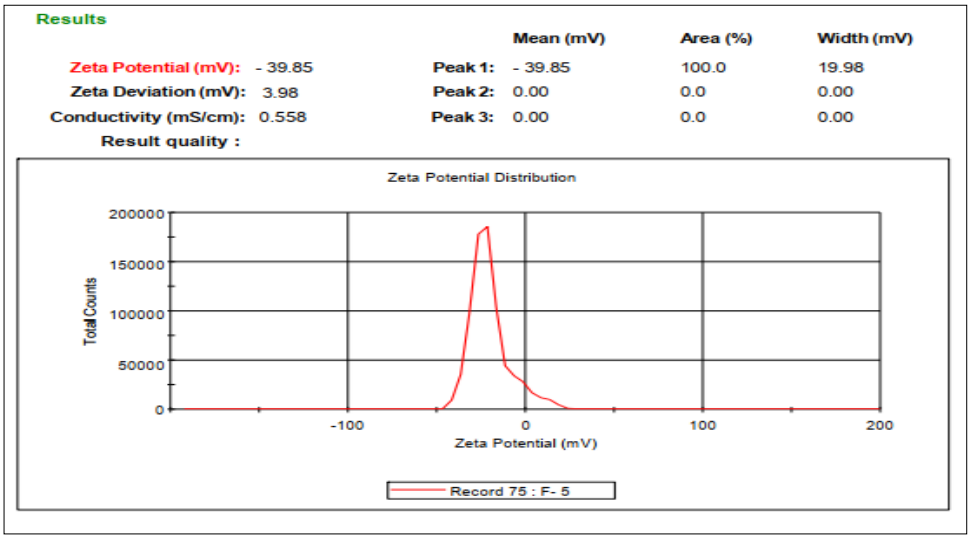

Figure 2: Graph of Zeta Potential of optimized formulation F5

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

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

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 Ornidazole from optimized ethosomes gel formulation EF2

S. No.	Time (hrs)	% Cumulative drug release ethosomal gel
1	0.5	22.36±0.52
2	1	35.65±0.36
3	2	48.85±0.45
4	4	59.98±0.23
5	6	69.98±0.85
6	8	78.85±0.74
8	10	96.65±0.63

Table 8: *In vitro* drug release data for 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	0.5	-0.301	22.36	1.349	77.64	1.890
2	1	1	0.000	35.65	1.552	64.35	1.809
3	2	2	0.301	48.85	1.689	51.15	1.709
4	4	4	0.602	59.98	1.778	40.02	1.602
5	6	6	0.778	69.98	1.845	30.02	1.477
6	8	8	0.903	78.85	1.897	21.15	1.325
7	10	10	1.000	96.65	1.985	3.35	0.525

Table 9: Results of regression analysis data of ethosomal formulation

Formulation	Zero order	First order	Pappas plot
EF2	0.9564	0.8361	0.982

CONCLUSION

The study successfully formulated an Ornidazole-loaded ethosomal gel with desirable vesicle size, high entrapment efficiency, good stability, and suitable gel characteristics. The optimized formulation (EF2) exhibited skin-compatible pH, uniform drug content, and sustained drug release following non-Fickian diffusion. Overall, the ethosomal gel proved to be a promising and effective topical delivery system for Ornidazole with potential for improved therapeutic efficacy and patient compliance.

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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