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Original Article

Fabrication and Characterisation of Azithromycin-Loaded Ethosomes as an Advanced Vesicular Carriers for Acne Therapy

Abishek Kumar Anant Patil[1], Prashant Shivanand Halagali[1], Rahul Ramesh Koli[1], Nisha Shirkoli[1,*], Veerkumar Prakash Japti[1], Kishori Prasanna Sutar[2]

[1]Department of Pharmaceutical Quality Assurance, KLE College of Pharmacy, KLE Academy of Higher Education and Research, Nehru Nagar, Belagavi, Karnataka, INDIA.
[2]Department of Pharmaceutics, KLE College of Pharmacy, KLE Academy of Higher Education and Research, Nehru Nagar, Belagavi, Karnataka, INDIA.

ABSTRACT

Aim: This study is aimed to design and evaluate azithromycin-loaded ethosomes as an innovative approach to address the limitations of conventional azithromycin topical dosage forms in the treatment of acne. **Materials and Methods:** Azithromycin-loaded ethosomes were prepared using the ethanol injection method. Their physicochemical properties were evaluated using techniques like FTIR, DSC, particle size analysis and drug content determination. The formulation was optimized using a central composite design to achieve optimal drug entrapment and release. **Results:** The optimized formulation, Azithromycin loaded ethosomes 04 (AZT-ETH 04), demonstrated excellent performance, with an entrapment efficiency of 95.56±2.21%, drug content of 81.18±2.00%, a polydispersity index of 0.31±0.01, a zeta potential of -26.21 mV and a particle size of 174.73±2.16 nm. Aloe vera juice was employed as the base for an ethosomal gel incorporating Azithromycin loaded ethosomes 04 (AZT-ETH 04), formulated using Carbopol 934 (1.8%) as the gelling agent. The ethosomal gel exhibited desirable properties, including a pH of 6.53±0.15, viscosity of 756±3.27 cPs, spreadability of 3±0.19 g cm/s and an impressive drug release of 89.18±1.01% over 9 hr. **Conclusion:** The optimized azithromycin-loaded ethosomes exhibited superior physicochemical properties, including high drug entrapment and small particle size. The ethosomal gel, formulated with aloe vera juice and Carbopol 934, demonstrated desirable properties like optimal pH, viscosity and spreadability. It also exhibited a sustained drug release profile and potent antibacterial activity, making it a promising topical delivery system for acne treatment.

Keywords: Azithromycin, Central-Composite Design, Design of experiment, Ethosomes, *P. acne*.

Correspondence:
Ms. Nisha Shirkoli
Department of Pharmaceutical Quality Assurance, KLE College of Pharmacy, Belagavi, KLE Academy of Higher Education and Research, Nehru Nagar, Belagavi-590010, Karnataka, INDIA.
Email: nishashirkoli@klepharm.edu

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INTRODUCTION

The skin, the body's largest and most accessible organ, plays a significant role in systemic drug administration.[1] It serves as a habitat for microorganisms and is susceptible to contamination by various microbial species.[2] Acne, a prevalent chronic inflammatory skin condition, is caused by bacterial colonization on the skin's epithelium. This colonization is facilitated by the bacteria's ability to adhere to the skin, thrive in its dry and acidic environment and rapidly reattach.[3] Acne is strongly associated with excessive sebum production and predominantly affects adolescents, with an incidence of up to 80%, though it can occur at any age. While not life-threatening, acne often results in significant emotional distress.[4,5]

Topical treatments are commonly preferred for acne management due to their targeted delivery, but the limited penetration of drugs through the stratum corneum (skin barrier) reduces their effectiveness.[6,7] To address this challenge, nanocarrier-based drug delivery systems are gaining attention.[8] Among various nanocarriers, lipid-based vesicular systems such as liposomes, niosomes, ethosomes, transferosomes and cubosomes are particularly notable for their ability to enhance subcutaneous penetration and drug permeation.[9] Ethosomes, in particular, stand out due to their unique properties such as high deformability, flexibility and superior skin delivery capabilities.[10] These characteristics enable ethosomes to facilitate the localized delivery of drugs to the skin while also addressing the growing concern of drug resistance by allowing antibiotics to penetrate bacterial cell membranes or interiors.[11]

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Pallavi Wadaskar's liposome formulation exhibited low drug encapsulation efficiency, limiting its potential for effective acne treatment.[12] Tamilvanan Shunmugaperumal's microemulsion, while promising, presented formulation complexities and stability concerns.[13] Ethosome-based drug delivery systems represent a novel and highly innovative approach in dermatological therapies, particularly in acne management. This platform offers not only improved drug solubility and sustained release but also enhanced therapeutic efficacy. Among the first-line antibiotics for acne treatment, macrolides like Azithromycin (AZT) are notable, especially for individuals who cannot tolerate tetracyclines, such as pregnant women and children under eight years of age.[14] Azithromycin, a broad-spectrum macrolide antibiotic, is effective in treating acne through various routes of administration, including oral, intravenous and topical. However, as of now, no studies have reported the development of azithromycin-loaded ethosomes for the treatment of acne, making this a novel and groundbreaking approach.

The encapsulation of azithromycin in ethosomes enhances its solubility, ensures sustained drug release and boosts antibacterial effectiveness, positioning it as a cutting-edge strategy for combating acne. This innovative method not only addresses the limitations of traditional topical treatments but also highlights the potential of ethosomal systems to revolutionize acne therapy by improving patient outcomes and reducing the risks associated with drug resistance.[15]

MATERIALS AND METHODS

Materials

Materials used in the study were Azithromycin as a gift sample from Empree Medicaments Pvt. Ltd., Belagavi. Soya lecithin and Carbopol934 from HiMedia Lab Pvt. Ltd., Analytical grade ethanol was employed. Triethanolamine from SDFCL Pvt. Ltd., and other materials were procured from K.L.E College of Pharmacy, Belagavi.

Determination of absorption maxima (Λ_{Max}) of the drug

The maximum peak of the spectrum was reported as the lambda max of the drug. The drug's stock sample was produced with an ethanol concentration of 100 µg/mL. The solution was scanned from 200-400 nm in the SHIMADZU UV spectrophotometer. Max of the drug was used to determine the spectrums highest point.[16]

Fourier-Transform Infrared (FTIR) Spectroscopy

The acquired drug samples Fourier Transform Infrared (FT-IR) spectrum was compared to a typical FT-IR spectrum of pure drug as a reference. IR spectroscopy was also conducted to investigate the compatibility of the pure drug Azithromycin and physical mixtures of polymers such as Azithromycin, Soya lecithin and the blend of azithromycin and soya lecithin.[17]

Differential Scanning Calorimetry (DSC)

DSC analysis evaluated the thermal behavior of azithromycin and its mixture with excipients. Around 5 mg samples were heated in aluminum pans (10°C/min, N2 purge: 100 mL/min) from 40°C to 300°C. Thermograms were compared to assess potential physicochemical interactions.

Experimental design by Quality by Design approach

The experiments were designed using Design-Expert software (version 13.0, Stat-Ease Inc., Minneapolis, MN, USA).[18,19] A total of nine formulations were generated based on a Central Composite Design (CCD) with factors at 3 levels (3^2) to optimize the ethosomal formulations. The independent variables included the concentration of soy lecithin (X1) and ethanol (X2), while the response variables were vesicle size (Y1) and entrapment efficiency (Y2). The experimental design incorporated three levels of soy lecithin (200, 300 and 400 mg) and ethanol (5, 7 and 9 mL) to evaluate their effects systematically. The statistical details are summarized in Table 1.

Development of Ethosomes via the Ethanol Injection Method

The ethanol injection method was employed to prepare the ethosomal formulation loaded with azithromycin. The required amounts of soy lecithin and azithromycin were accurately weighed and dissolved in ethanol in a sealed beaker, using a magnetic stirrer set at 700 rpm at room temperature. Phosphate buffer solution (pH 6.8) was then added dropwise into the ethanolic solution at a rate of 200 µL/min while maintaining continuous stirring at 500 rpm. The mixture was stirred for an additional 10 min to ensure thorough integration of the two solutions. The resulting milky ethosomal suspension was left to swell overnight. To reduce the vesicle size, the ethosomal suspension was subjected to probe sonication for 15 min. In total, nine formulations were prepared using different ratios of soy lecithin and ethanol, as outlined in Table 2.[20]

Estimation of Vesicle Size

The vesicle size of the ethosomal preparation was analyzed using a Malvern Zetasizer. For this analysis, the sample was diluted with Milli-Q water to ensure accurate determination of vesicle size and Polydispersity Index (PDI). Specifically, 0.1 mL of the supernatant from the prepared formulation was carefully withdrawn and diluted with 1 mL of Milli-Q water. The diluted sample was then subjected to Dynamic Light Scattering (DLS) measurement to evaluate the vesicle size and uniformity of the dispersion.[21]

Determination of Percentage Entrapment Efficiency (% EE)

The ethosomal suspension was centrifuged at 4°C for 3 hr to separate the free drug from the ethosomal vesicles. The concentration of azithromycin in the supernatant was determined using UV spectrophotometry. The percentage of Entrapment Efficiency (% EE) was calculated using the following equation:

$$\% EE = \frac{\text{Total Drug} - \text{Unentrapped Drug}}{Total\ drug} \times 100 \quad \text{..... Eq. 1}$$

Preparation of Gel from Aloe Vera Juice

Aloe vera mucilage (acidic) was washed with water and phosphate buffer (pH 6.8) to remove any impurities. To form a gel, Carbopol 934 (1.8%) was dispersed in the mucilage using triethanolamine. Triethanolamine serves to adjust pH, thereby stabilizing aloe vera bioactives and the structures of ethosomal gels. It enhances emulsification, ensures skin compatibility and prevents degradation. Precise control is essential to maintain stability and efficacy in formulations. The perfume was then incorporated into the gel and the final formulation was stored in an airtight container to preserve its stability.

Development of Ethosomal Gel

The azithromycin-entrapped ethosomal gel was prepared by soaking the gelling agent (Carbopol 1.8%) in a measured quantity of aloe vera juice for 24 hr. The optimized ethosomal formulation was then dissolved in the aloe vera juice and allowed to swell for an additional 24 hr. The mixture was stirred using a magnetic stirrer at 50 rpm. Methylparaben was added dropwise as a preservative and triethanolamine was used which adjusts pH, ensuring aloe vera bioactivity, ethosomal stability and skin compatibility. Controlled addition prevents over-alkalization, maintaining formulation effectiveness and preserving active ingredient integrity. The final gel was stored in an airtight container for further examination (Table 3).

Evaluation of pH

Ethosomal gel pH was measured using a pre-calibrated pH meter (phosphate buffer, pH 6.8). 1 g of gel was dispersed in 10 mL Milli-Q water.

Evaluation of Viscosity

The Brookfield viscometer was used to measure the viscosity of the prepared azithromycin ethosomal gel at room temperature and rpm of 10, 15 and 20, respectively. Gel viscosity was measured and recorded.[22]

Evaluation of Spreadability

The spreadability of Azithromycin ethosomal gel was measured using the parallel plate method. 0.5 g of gel (1 cm diameter) was placed between two glass plates with a 500 mg weight for 5 min. Increased diameter indicates good spreadability.[23] Spreadability is calculated as mentioned in equation 2:

$$S = W \times L/T \text{..... Eq. 2}$$

Where, S=Spreadability, W=Weight on upper slide, L=Length of the slide, T=Upper and lower slide separation time.

Measurement of drug content of Azithromycin ethosomal gel formulation

To determine the drug content of formulated ethosomal gel UV spectrophotometric analysis is carried out at a wavelength of 205 nm. 1 g of Formulated gel was dissolved in the ethanol. The mixture was sonicated for 15-30 min. From this 1 mL was pipetted out in a 10 mL volumetric flask and volume was made up of ethanol, analyzed at 205 nm wavelength.

Estimation of Antimicrobial (ATM) Activity

Minimum Inhibitory Concentration (MIC) (Resazurin-based turbidometric method)

The resazurin-based Turbidometric (TB) assay was adopted to demonstrate the inhibition effects of Azithromycin Ethosomes. A pure culture of a specified microorganism grown overnight then

Table 1: Coded Values of 3^2 Central-Composite Design for Formulation of Ethosomes.

Independent variables	Levels		
	Low (-1)	Medium (0)	High (+1)
X1=Soya lecithin	200	300	400
X2=Ethanol	5	7	9
	Constraints	Importance	
X1=Soya lecithin	In range	+++	
X2=Ethanol	In range	+++	
Dependent variables			
Y1=vesicle size	Minimum	+++	
Y2=Entrapment Efficiency	Maximum	+++	

Table 2: Central Composite Design employed experimental runs.

Formulation code	Amount of soya lecithin (g)	Amount of Ethanol (mL)	Vesicle Size (nm)	Entrapment efficiency (%)
AZT- ETH 1	400	5	245.24	92.96
AZT- ETH 2	200	9	188.50	94.83
AZT- ETH 3	400	9	198.18	94.73
AZT- ETH 4	300	5	174.73	95.56
AZT- ETH 5	400	7	253.10	94.74
AZT- ETH 6	300	7	199.92	96.22
AZT- ETH 7	200	5	203.58	95.66
AZT- ETH 8	300	9	185.56	93.76
AZT- ETH 9	200	7	199.63	95.46

diluted in growth-supporting broth (typically Mueller Hinton Broth) to a concentration between $1x10^5$ and $1x10^6$ cfu/mL. A stock dilution of the antimicrobial test substance is made at approximately 100 times the expected MIC (if known). Further 1:1 dilution is made in test tubes or 96 well microtiter plates. All dilutions of the test product(s) are inoculated with equal volumes of the specified microorganism. A positive and negative control tube or well is included for every test microorganism to demonstrate adequate microbial growth over the course of the incubation period and media sterility, respectively. An aliquot of the positive control is plated and used to establish a baseline concentration of the microorganism used. The tubes or microtiter plates are then incubated at the appropriate temperature and duration. After an overnight incubation at 37°C, 5 µL resazurin (6.75 mg mL^{-1}) was added to all wells and incubated at 37°C for another 4 hr. Changes of colour was observed and recorded. The lowest concentration prior to colour change was considered as the Minimum Inhibitory Concentration (MIC).

Minimum Bactericidal Concentration (MBC) (Agar Diffusion Method)

MBC is the minimal antibacterial density necessary to kill bacteria, that is, bactericidal as opposed to merely bacteriostatic densities**.** MIC was further spotted on Brain heart infusion agar plates. After incubation period of 24 hr at 37°C the colony forming units were counted. the MBC is interpreted as the concentration of the antimicrobial agent contained in the series that inhibits visible growth.[24]

In vitro Diffusion studies

The study compared the release of Azithromycin from in house formulated Azithromycin Loaded Ethosomal Gel and Marketed Gel. The release was measured using a Franz cell diffusion apparatus with a cellophane membrane and phosphate buffer at 37°C. The amount of released Azithromycin was quantified spectrophotometrically at 205 nm. This study aimed to determine if the Ethosomal Gel could improve the controlled release of Azithromycin.[25]

Table 3: Formulation Table of Azithromycin loaded entrapped ethosomal gel.

Ingredients	Quantity
Aloe Vera Juice	10 mL
Ethosomal Suspension	10 mL
Carbopol 934	1.8%
Triethanolamine	0.005%
Methyl Paraben	0.01%
Perfume	Q. S

Ex vivo Diffusion Studies

A Franz diffusion cell with pre-soaked sheep skin was used to study the release of Azithromycin Loaded Ethosomal gel through skin. The experiment was conducted at 37°C with continuous stirring in phosphate buffer (pH 6.8). The amount of released Azithromycin was measured using UV spectrophotometry. This study aimed to evaluate the potential of the Ethosomal gel for controlled skin delivery of Azithromycin.[26]

Scanning Electron Microscopy

SEM creates images of the sample under study using an electron beam. Typically, vesicles were first frozen at -20°C, then freeze dried. To avoid charge accumulation, the specimen sample needs to be grounded electrically and electrically conductive at least at the surface.

Therefore, a freeze-dried sample is coated with a conducting material, such as gold/palladium alloy, platinum, etc., using a low vacuum sputter coating technique or vacuum evaporation. The sample is placed on an SEM stub equipped with conducting pads under the scanning electron microscope. With SEM, an electron beam is used to image the specimen; however, rather than passing

through the specimen, the beam is "scanned" across it to produce an incredibly deep-field image of the sample's surface.[27,28]

RESULTS

Determination of Absorption Maxima (Λ Max) of The Drug

Absorption maxima of azithromycin were determined using a wavelength sweep from 400 to 200 nm. At 205 nm to 208 nm, the highest absorption was recorded. This maximum was employed in the subsequent study.

Compatibility Studies By FT-IR Spectroscopy

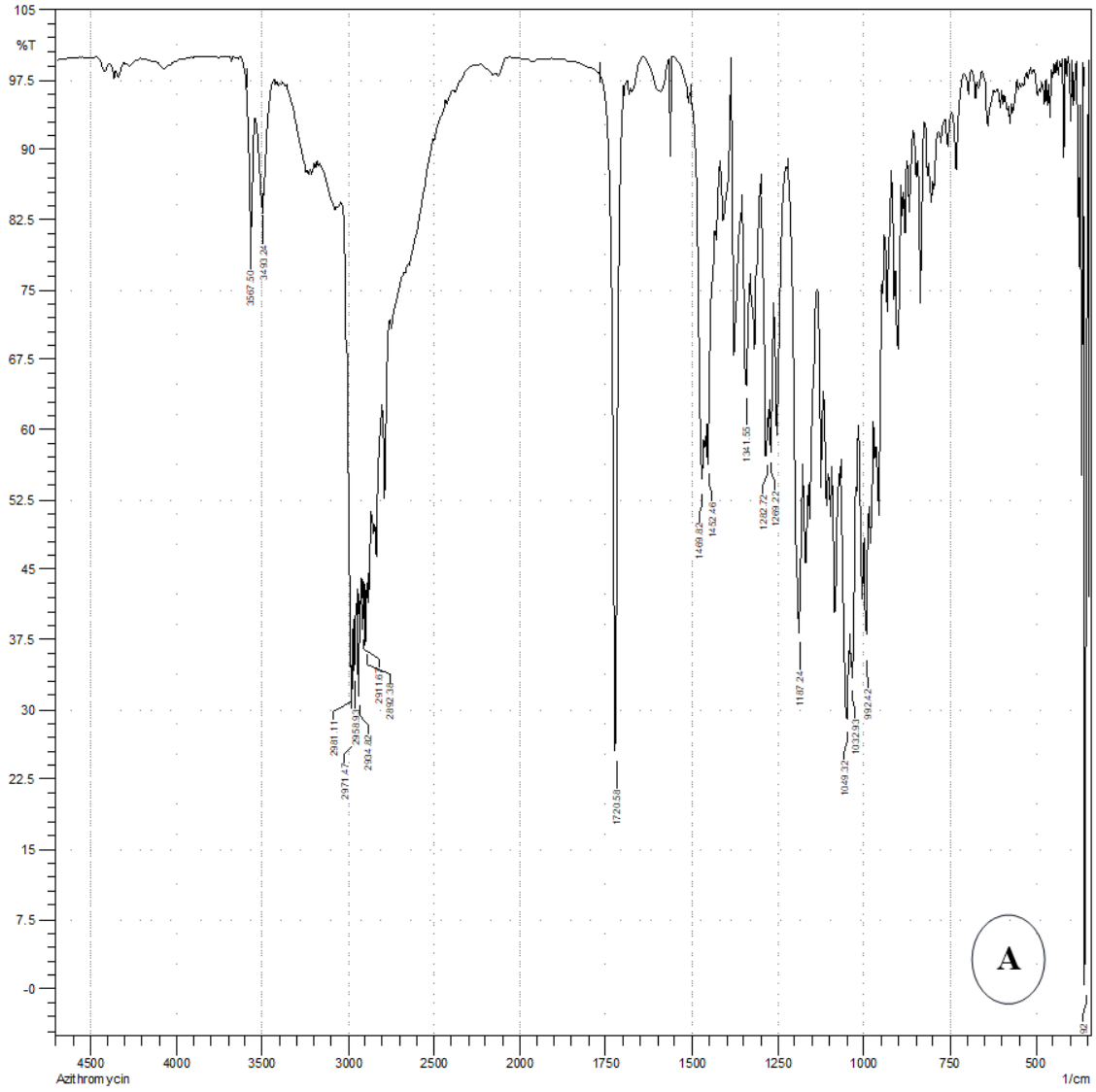
The functional group peaks of the azithromycin ethosomal formulation did not interfere with FTIR analysis indicating they were chemically compatible. The stretch of the OH bond was found to be 3415.12 cm^{-1} (Figure 1A and 1B respectively).

The pure drug azithromycin's melting point was determined by DSC analysis to be 123.93°C, while the blend's melting point was determined to be 124.67°C, as indicated by the sharp endotherm in each thermogram (Figure 2A and Figure 2B respectively).

Optimizing Process Performance Utilizing ANOVA

Based on the regression coefficients obtained from the ANOVA study and the analysis of Equations 3 and 4, it was observed that the independent variables X1 (Soy lecithin) and X2 (Ethanol) exhibit antagonistic effects on Response Y1 (vesicle size). In contrast, both X1 (Soy lecithin) and X2 (Ethanol) negatively affect Response Y2 (% EE).

The response surface plots in Figures 3 and 4 illustrates these effects. Specifically, as the concentrations of soy lecithin and ethanol increase, the particle size also increases, while the % EE decreases. The overlay plot shown in Figure 5 highlights the region of successful operating ranges, which is indicated by the yellow-colored shaded zone.



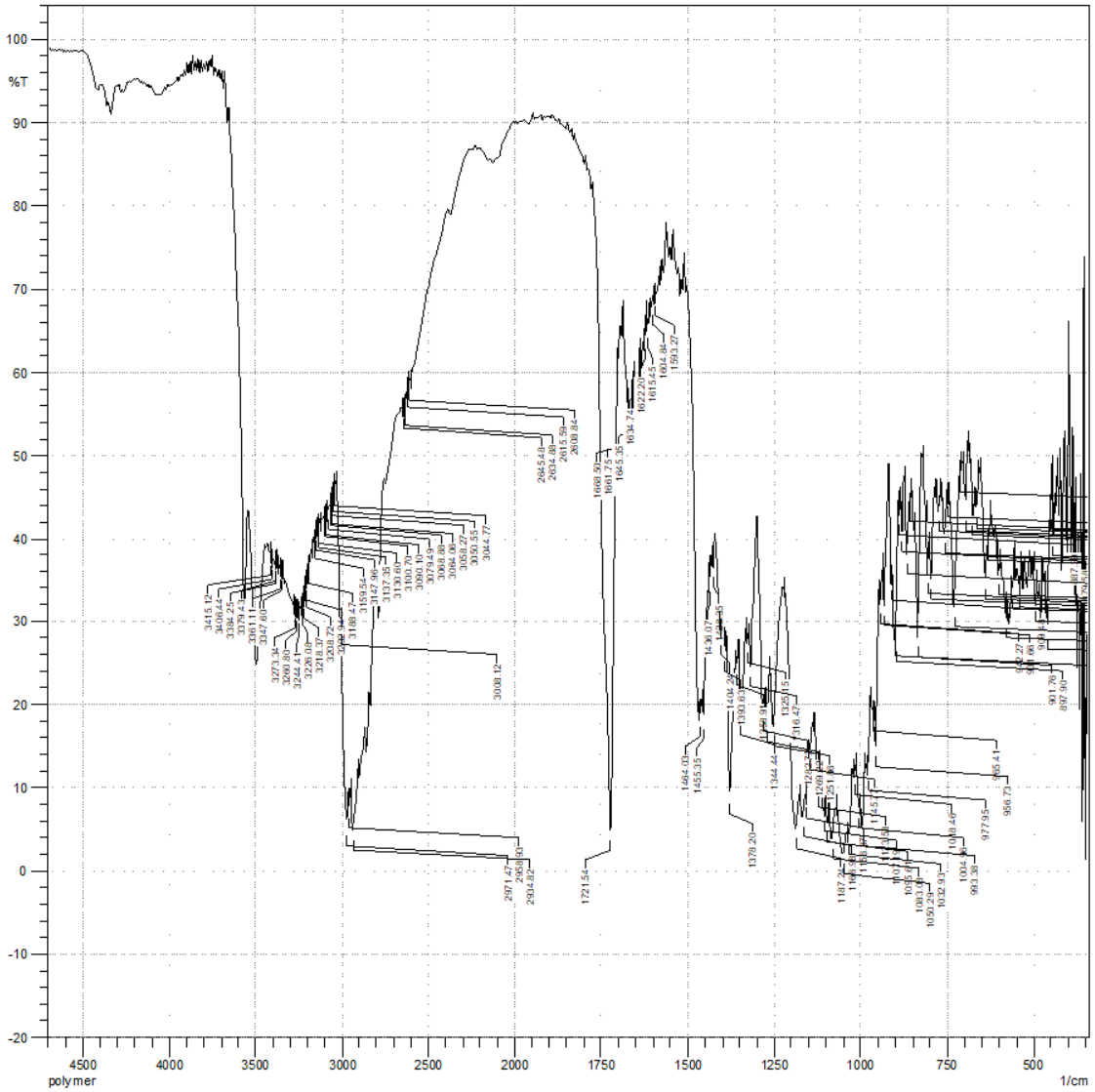


Figure 1: (A) FTIR spectrum of pure drug Azithromycin, (B) FTIR spectrum of physical mixture of Azithromycin and Soya Lecithin.

Polynomial Equation

$$\text{Visicle size} = +167.43 + 43.13*A - 7.72*B + 3.37*AB \ldots$$

…… Equation. 3

$$\text{Entrapment efficiency} = +86.88 + 5.52*A - 3.45*B - 0.5250*AB - 8.19*A^2 + 0.5300*B^2$$

…….. Equation. 4

Estimation of Vesicle Size, Polydispersity Index (PDI) And Entrapment Efficiency, Zeta Potential

Ethosomes were prepared with an average size of 174.73±2.16 to 253.10±3.17 nm and entrapment efficiency of 92.96±1.99 to 96.22±2.21. The average PDI for the ethosomes ranged from 0.23±0.01 to 0.31±0.01. The optimized formulation exhibited a desirable profile with an average vesicle size of 174.73 nm (±2.16), a high entrapment efficiency of 95.56% (±2.21) and a narrow size distribution (PDI) of 0.31 (±0.01) and Zeta potential was found in the range of -22 to -37 (-mV).

Estimation of Drug Content of Ethosomal Dispersion

The drug content of all the formulations was recorded in the range of 70.69±2.03 to 81.18±2.00. The maximum drug content was observed in the formulation of AZT- ETH 04 i.e. 81.18±2.00 whereas the lowest drug content was found in formulation AZT-ETH 03 69.37±1.08 as indicated in Table 4.

pH Measurement

The pH of azithromycin-loaded ethosomal formulations was found to be 6.35±0.15.

Viscosity Measurement

The viscosity of formulation was carried out by using a Brookfield viscometer. The viscosity of Azithromycin loaded ethosomal gel was found to be 756±3.27 cPs.

Spreadability Measurement

The therapeutic effect of the azithromycin ethosomal gel depends on the spreading value. The spreadability of azithromycin-loaded ethosomal gel was found to be 3±0.19 g.cm/sec. The results are shown to be in Table 5.

In vitro Diffusion Study

The *in vitro* skin penetration of an azithromycin-loaded ethosomal gel formulation over a dialysis membrane was investigated using a Franz diffusion cell. The total cumulative amount of drug released in 9 hr was calculated. The results revealed that the cumulative amount of drug release in the marketed cream was recorded in the range of 9.17±1.34% to 61.03±7.59% whereas the Azithromycin loaded ethosomes was recorded in the range of 9.89±1.19% to 89.18±1.03% which clearly depicts enhanced skin permeation of optimized batch which is significantly higher. Figure 6 shows the comparative release of azithromycin-marketed gel with an optimized batch of azithromycin-loaded ethosomal gel.

Ex vivo Skin Permeation Studies

An *ex vivo* permeation study using a Franz diffusion cell was conducted to evaluate the transdermal delivery potential of the optimized Azithromycin loaded Ethosomal Gel (AZT- ETH 04) across sheep abdominal skin, a model closely resembling human skin. Over a 9 hr period, the Ethosomal Gel formulation significantly outperformed the free-drug Ethosomal gel, achieving a 72.34% penetration rate compared to 52.8% (Figure 7). These results highlight the potential of the nanosponge hydrogel as a promising transdermal drug delivery system.

Scanning Electron Microscopy

The morphology of the ethosomal formulation (AZT ETH 4) was analyzed using a scanning electron microscope, as shown in Figure 8. The SEM image reveals spherical to slightly irregular-shaped particles with a relatively smooth surface. The particle sizes were measured and annotated in the image, ranging between 42.49 nm and 97.54 nm, indicating nanoscale dimensions. The compact and smooth structure of the particles

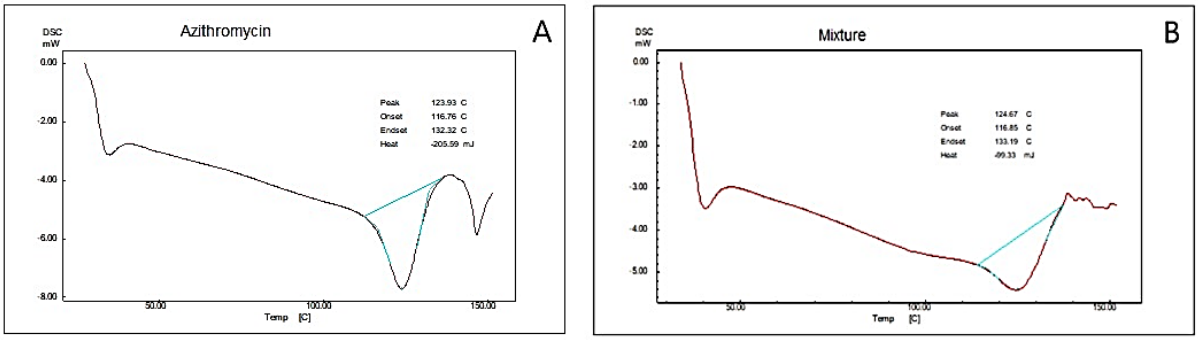


Figure 2: (A) DSC Thermogram of Azithromycin, (B) DSC Thermogram physical mixture of Azithromycin and Soya Lecithin.

Table 4: Characterization of the ethosomes.

Formulation	Vesicle size (nm)	Entrapment efficiency (%)	Polydispersity index	Drug content (%)	Zeta Potential (mV)
AZT- ETH 1	245.24±2.88	92.96±1.97	0.25±0.01	71.37±1.51	-22.37
AZT- ETH 2	188.50±3.93	94.83±2.25	0.26±0.02	70.79±0.49	-23.91
AZT- ETH 3	198.18±2.87	94.73±2.10	0.23±0.01	69.37±1.08	-25.97
AZT- ETH 4	174.73±2.16	95.56±2.21	0.31± 0.01	81.18± 2.00	-26.21
AZT- ETH 5	253.10±3.17	94.74±2.01	0.26±0.01	70.69±2.03	-28.56
AZT ETH 6	199.92±1.53	96.22±1.55	0.26±0.01	72.99±1.10	-30.98
AZT ETH 7	203.58±2.81	95.66±1.32	0.29±0.02	75.84±1.08	-32.77
AZT ETH 8	185.56±3.33	93.76±1.99	0.27±0.02	70.99±1.63	-34.96
AZT ETH 9	199.63±2.44	95.46±0.84	0.26± 0.02	73.98±1.72	-37.03

n=3 (mean± SD).

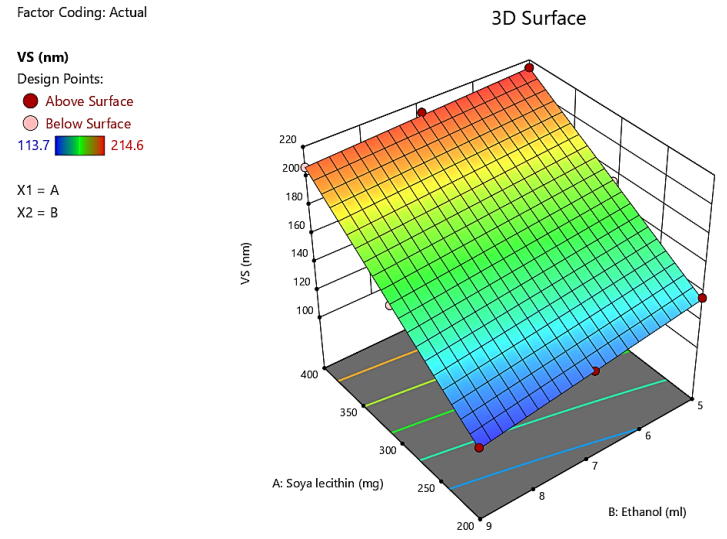


Figure 3: Response surface plot of vesicle size.

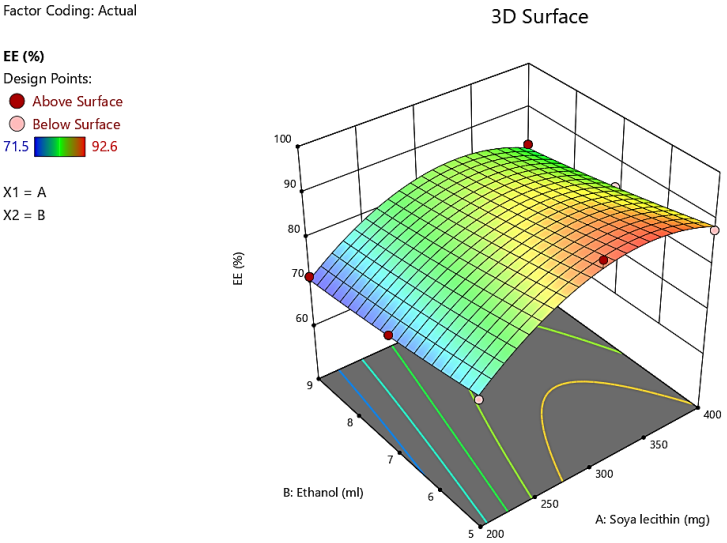


Figure 4: Response surface plot of entrapment efficiency.

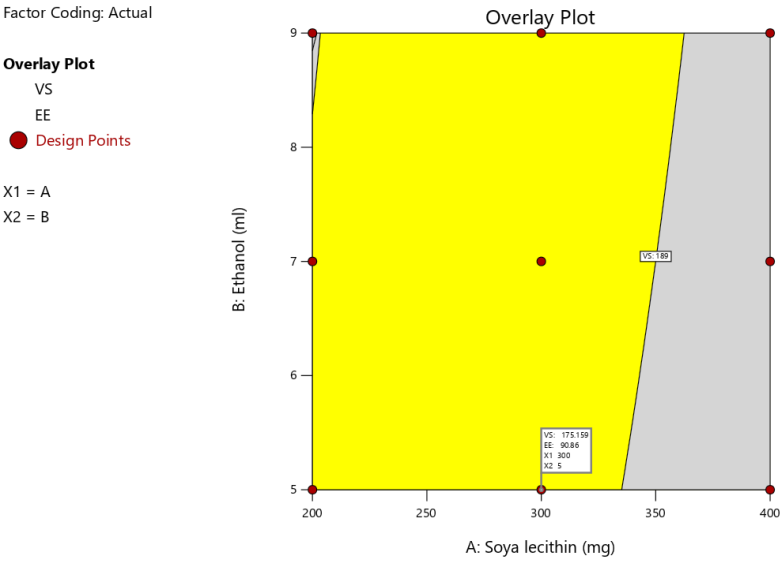


Figure 5: Design space overlay plot.

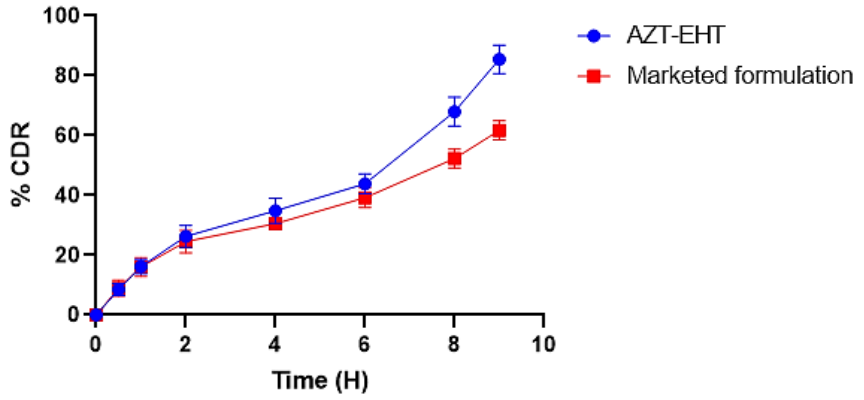


Figure 6: Comparative *In vitro* drug release.

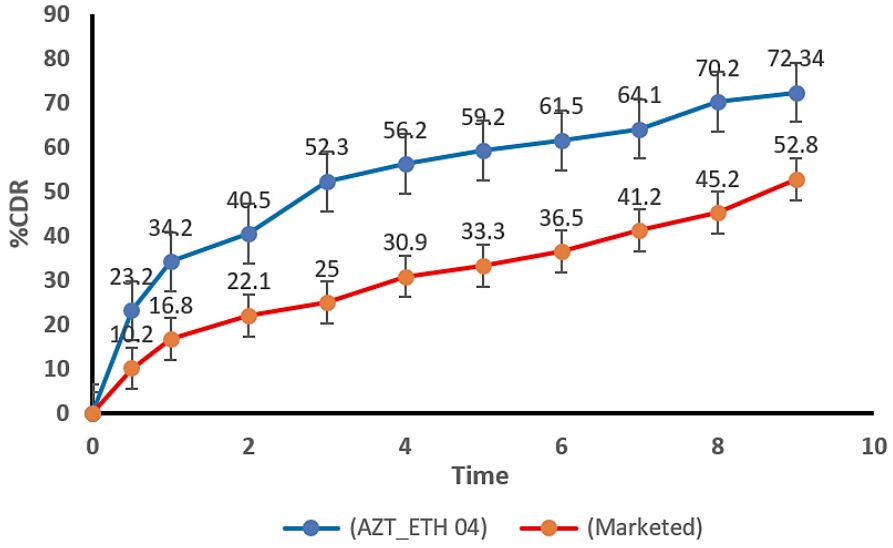


Figure 7: Comparative *ex vivo* Skin Permeation studies.

suggests uniform dispersion, which is desirable for ethosomal formulations to enhance stability and delivery efficiency.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The value of MIC of ethosomal suspension was found to be 25 µg/mL and standard it was 42 µg/mL as depicted in Figure 9A. Minimum Bacterial concentration of Azithromycin loaded ethosomal gel against Propionibacterium acnes was found to be 10th dilution, up to 3rd dilution there was total inhibition of growth whereas from 4th dilution it is noticed that some inhibition found up to 10th dilution. The value of MBC of ethosomal suspension was found to be 50 µg/mL, as this concentration had no visibility of bacterial growth (Figure 9B and Figure 10).

DISCUSSION

The FTIR spectrum and DSC thermogram of the physical mixture did not exhibit significant changes in the peak configurations, confirming the compatibility between the drug and the polymer. Ethanol is known to soften the vesicle membrane, facilitating the interpenetration of ethanol hydrocarbon chains into the lipid bilayer of vesicles. This interaction results in a marked reduction in vesicle membrane thickness, as previously reported.[29]

The response surface plot provided insights into the relationships between the independent variables and the responses. A positive correlation was observed between X1 (soy lecithin concentration) and Y1 (drug entrapment efficiency), whereas a negative correlation was noted between X2 (ethanol concentration) and Y1. Similarly, X1 positively influenced Y2 (vesicle size), while X2 exerted a negative influence on this response. The optimization analysis conducted using Design-Expert software yielded acceptable results across all measured responses. The identified design space indicated optimal levels for soy lecithin (300 mg)

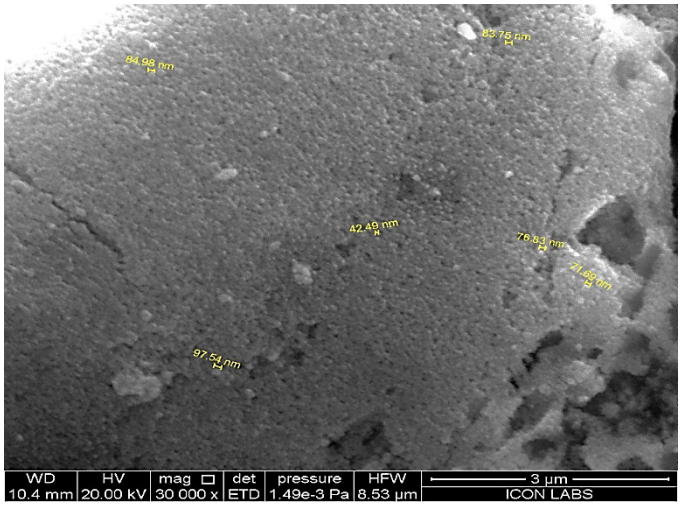


Figure 8: Scanning Electron Microscopy. (Scale bar=100 nm. Original magnification Â 15000).

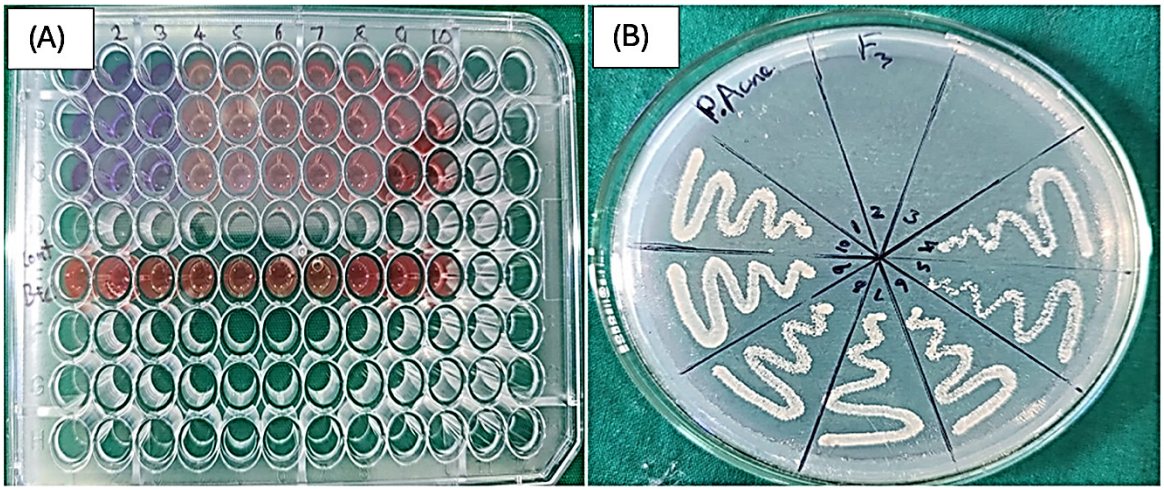


Figure 9: (A): Estimation of Minimum Inhibitory Concentration, (B): Estimation of Minimum Bactericidal Concentration.

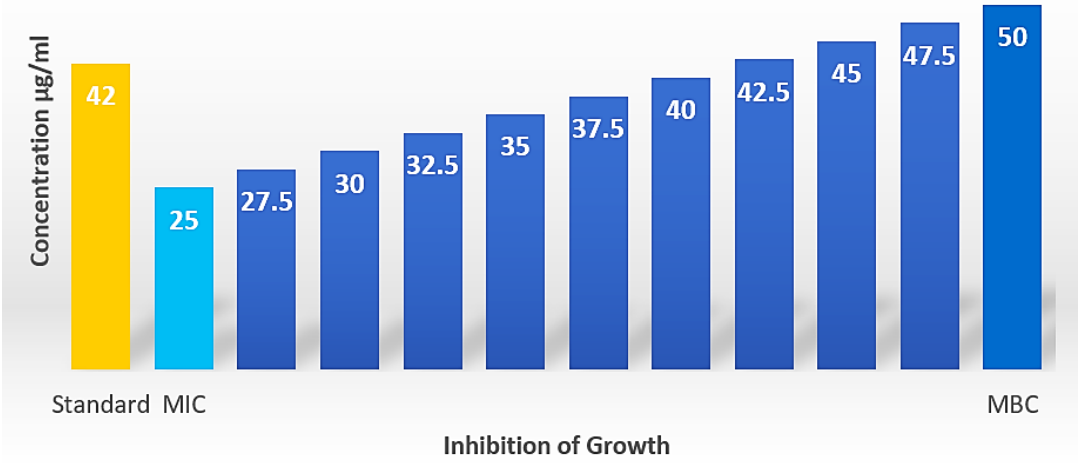


Figure 10: Graph showing Comparison between concentration (µg/mL) of Standard, MIC and MBC to inhibit the growth of bacteria *P. Acne*.

Table 5: Gel appearance, Spreadability, pH, Viscosity.

Parameters	Azithromycin-loaded ethosomal gel
Appearance	Creamish white
Spreadability (gm.cm/sec)	3±0.19
Ph	6.35±0.15
Viscosity (cPs)	756± 3.27

n=3 (Mean±SD).

and ethanol (5 mL), which balanced the desired outcomes. The predictive capacity of the model was robust, demonstrated by a low prediction error of 5%.

Ethosomes prepared with high ethanol concentrations initially induced a shrinkage of the lipid bilayer structure, as previously documented.[30] Ethanol was also found to interact with the lipids of the stratum corneum, disrupting their ordered arrangement and significantly enhancing skin permeability. Permeability increased by up to 35% at moderate ethanol concentrations but declined at higher concentrations, exceeding 50% ethanol.[31]

The ethosomal formulations exhibited a narrow vesicle size distribution and moderate stability under experimental conditions. While increasing ethanol concentration reduced drug entrapment efficiency, it enhanced the solubilization and entrapment of hydrophilic drugs such as azithromycin. Among the formulations, the ethosomal gel AZT-ETH 04 demonstrated superior drug release profiles compared to the marketed formulation. Additionally, the ethosomal gel maintained a physiological pH suitable for skin application and exhibited excellent spreadability. Cellophane membrane diffusion studies are used to mimic skin permeation. By studying how substances move through a simple membrane, researchers can understand factors affecting skin penetration, like molecular size and lipid solubility. This knowledge helps in designing better drug delivery systems. The enhanced permeation in *ex vivo* studies is attributed to the presence of ethanol, a known enhancer of stratum corneum penetration. The antimicrobial efficacy of the gel was determined by evaluating its Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). MIC was defined as the lowest concentration of the antimicrobial agent required to inhibit visible microbial growth (25 µg/mL), while MBC was the lowest concentration capable of completely eradicating the microbial population (50 µg/mL).

CONCLUSION

The current study successfully developed azithromycin-loaded ethosomes aimed at enhancing transdermal drug delivery by improving drug permeability. Efforts were made to optimize the formulation and thoroughly evaluate its physicochemical and functional properties. The FTIR spectral analysis confirmed the absence of any significant interaction between azithromycin and the phospholipid (soya lecithin), demonstrating their compatibility based on experimental data. Particle size analysis revealed that the ethosomal formulations exhibited vesicle sizes ranging from 185.56±3.33 nm to 253.10±3.17 nm. Among the tested formulations, the optimized formulation, AZT-ETH 04, demonstrated superior performance with a vesicle size of 174.73±2.16 nm, the highest drug content (81.18±2.00%) and the highest entrapment efficiency (95.56±2.21%). The zeta potential of -26.21 mV indicated good stability of the ethosomal suspension. *In vitro* drug release and *ex vivo* skin permeation studies showed that the ethosomal gel significantly enhanced azithromycin release compared to the marketed formulation. The optimized azithromycin-loaded ethosomal gel demonstrated potent antimicrobial activity with MIC and MBC values of 25 µg/mL and 50 µg/mL, respectively, confirming its efficacy. This result suggests that ethosomes effectively facilitate improved drug delivery, underscoring their potential as a promising carrier system for transdermal applications.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

AZT: Azithromycin; **UV:** Ultra-violet; **FTIR:** Fourier-Transform Infrared; **DSC:** Differential Scanning Calorimetry; **USA:** United States of America; **PBS:** Phosphate-Buffered Saline; **%EE:** Percentage Entrapment Efficiency; **ATM:** Antimicrobial; **MIC:** Minimum Inhibitory Concentration; **TB:** Turbidometric; **MBC:** Minimum Bactericidal Concentration; **SEM:** Scanning Electron Microscopy; **PDI:** Polydispersity Index; **pH:** Potential of Hydrogen; **CCD:** Central Composite Design; **IR:** Infrared.

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