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Research Article

Formulation And Characterization of Ethosomal Drug Delivery System for Co-Delivery of Amoxicillin and Clavulanic Acid to Treat Bacterial Skin Infection

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ABSTRACT

Bacterial skin infections are among the most common microbial infections affecting the human population and are often associated with pain, inflammation, and tissue damage. Conventional oral antibiotic therapy may lead to systemic side effects, poor site-specific delivery, and reduced patient compliance. The present study aimed to formulate and characterize an ethosomal drug delivery system for the co-delivery of amoxicillin and clavulanic acid for topical treatment of bacterial skin infections. Ethosomes were prepared by the hot method using soya lecithin, ethanol, and propylene glycol. Preformulation studies including solubility, melting point, partition coefficient, and FTIR analysis were performed for both drugs. Optimization studies were carried out for aqueous-organic phase ratio, temperature, drug loading, and sonication time. The optimized formulation showed particle size of 188 nm with PDI of 0.102, indicating narrow particle size distribution. Entrapment efficiency of amoxicillin and clavulanic acid was found satisfactory. In-vitro drug release study demonstrated sustained drug release up to 12 h as compared to plain drug dispersion. Stability studies confirmed that the formulation remained stable with minimal changes in particle size and PDI. The developed ethosomal formulation exhibited potential for site-specific topical delivery and enhanced therapeutic effectiveness in bacterial skin infections.

INTRODUCTION

Bacterial skin infections are common infectious disorders caused by pathogenic microorganisms invading the skin tissues. These infections may result in pain, swelling, inflammation, discomfort, and changes in skin appearance. Among different bacterial skin infections, impetigo is one of the most frequently occurring contagious infections. Conventional antimicrobial therapy mainly

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involves oral or topical administration of antibiotics. However, several limitations such as systemic side effects, poor site-specific action, rapid metabolism, low stability, and antimicrobial resistance reduce the effectiveness of conventional therapies. Beta-lactam antibiotics are widely used for the treatment of gram-positive and gram-negative bacterial infections. Amoxicillin, a penicillin-class antibiotic, is extensively used either alone or in combination with clavulanic acid for the treatment of various infections including skin infections. Clavulanic acid acts as a beta-lactamase inhibitor and protects amoxicillin from degradation by beta-lactamase-producing bacteria. Topical drug delivery systems provide advantages such as localized drug action, reduced systemic exposure, improved patient compliance, and enhanced drug accumulation at the infected site. Ethosomes are soft vesicular carriers composed mainly of phospholipids, ethanol, and water. Due to high ethanol content, ethosomes possess enhanced deformability and improved skin penetration capability. Therefore, the present study was aimed at the formulation and characterization of ethosomal drug delivery system containing amoxicillin and clavulanic acid for improved topical delivery against bacterial skin infections.

2. Materials and Methods

2.1 Materials

Amoxicillin, clavulanic acid, isopropyl alcohol (IPA), ethanol, and soya lecithin were procured from Sigma Aldrich.

2.2 Preformulation Studies

2.2.1 Organoleptic Properties

Colour, odour, and texture of amoxicillin and clavulanic acid were evaluated visually.

2.2.2 Melting Point Determination

The melting point of both drugs was determined using capillary method with melting point apparatus.

2.2.3 Solubility Study

Solubility of both drugs was determined in different solvents including water, ethanol, methanol, and acetone.

2.2.4 Partition Coefficient

Partition coefficient study was performed using equal volumes of aqueous and organic phases followed by UV spectrophotometric analysis.

2.2.5 FTIR Spectroscopy

FTIR spectra of pure drugs were recorded in the range of 4000–400 cm^{-1} using potassium bromide disc method.

2.2.6 Analytical Method Development

Simultaneous UV spectrophotometric method was developed for estimation of amoxicillin and clavulanic acid at 231 nm and 210 nm respectively. Calibration curves were prepared in concentration range of 10–50 µg/ml.

3. Preparation of Ethosomes

Ethosomes were prepared using hot method. Soya lecithin and propylene glycol were dissolved in ethanol and maintained at 30°C. Water was added dropwise with continuous stirring at 700 rpm. The formulation was further sonicated for 10 min at controlled temperature.

4. Optimization of Ethosomal Formulation

4.1 Optimization of Aqueous and Organic Phase Ratio

Different aqueous to organic phase ratios (1:2, 1:3, and 1:4) were evaluated. The formulation with 1:4 ratio showed minimum particle size and was selected.

4.2 Optimization of Temperature

The effect of temperature (20°C, 30°C, and 40°C) on particle size was evaluated. The formulation prepared at 40°C showed optimized particle size.

4.3 Optimization of Drug Loading

Drug loading studies indicated that increase in drug concentration resulted in increase in particle size and reduction in entrapment efficiency. Optimized formulation was obtained with 6% drug loading.

4.4 Optimization of Sonication Time

Two sonication times (10 min and 15 min) were evaluated. Sonication for 10 min resulted in lower particle size and PDI.

5. Characterization of Ethosomes

5.1 Particle Size and Polydispersity Index

Particle size and PDI were measured using dynamic light scattering method with Zetasizer.

5.2 Entrapment Efficiency

Entrapment efficiency was determined by centrifugation method followed by UV spectrophotometric analysis.

5.3 Stability Study

Stability study was carried out by monitoring particle size and PDI during storage.

5.4 In-vitro Drug Release Study

In-vitro drug release study was performed using dialysis bag diffusion method in phosphate buffer pH 6.8.

6. Results and Discussion

The developed ethosomal formulation was systematically evaluated through preformulation studies, analytical method development, optimization studies, characterization parameters, stability studies, and in-vitro drug release studies. The obtained results demonstrated successful preparation of nanosized ethosomal vesicles suitable for topical delivery of amoxicillin and clavulanic acid.

6.1 Preformulation Studies

Preformulation studies were carried out to determine the physicochemical properties of amoxicillin and clavulanic acid. Parameters such as colour, odour, melting point, molecular weight, pKa, and solubility were evaluated to ensure suitability of the drugs for ethosomal formulation development.

Table 1: Preformulation Parameters of Amoxicillin(Table arranged according to IJPS journal format with centered headings, scientific units, and uniform presentation.)

Parameter	Observation
Colour	White
Odour	Odourless
Melting Point	197–204°C
Molecular Weight	315
pH/pKa	Approx. 4 / 0.8
Solubility	Highly soluble in water; soluble in ethanol and methanol; insoluble in acetone

Table 2: Preformulation Parameters of Clavulanic Acid(Table arranged according to journal style with proper scientific formatting and aligned observations.)

Parameter	Observation
Colour	Brown

Odour	Odourless
Melting Point	228–235°C
Molecular Weight	199.5
pH/pKa	Approx. 6 / 1
Solubility	Highly soluble in water; soluble in ethanol and methanol; insoluble in acetone

FTIR Interpretation

FTIR spectroscopy was performed to confirm the identity and compatibility of the drugs with formulation excipients. The characteristic peaks observed in the spectra confirmed the presence of functional groups corresponding to amoxicillin and clavulanic acid. No significant shifting or disappearance of peaks was observed, indicating absence of drug–excipient interaction and good compatibility within the formulation system. FTIR spectra of both drugs showed characteristic peaks corresponding to functional groups without any major shift, indicating compatibility of drugs with formulation excipients.

Figure 1: FTIR Spectrum of Amoxicillin Reference Standard
Figure 2: FTIR Spectrum of Amoxicillin Sample
Figure 3: FTIR Spectrum of Clavulanic Acid Reference Standard
Figure 4: FTIR Spectrum of Clavulanic Acid Sample

Analytical Method Development

A simultaneous UV spectrophotometric method was developed and validated for quantitative estimation of amoxicillin and clavulanic acid. Calibration curves were prepared using standard concentrations ranging from 10–50 µg/ml. The absorbance values showed linear correlation with concentration, confirming the suitability of the developed analytical method.

Calibration curves were prepared for amoxicillin and clavulanic acid in the concentration range of 10–50 µg/ml using UV spectrophotometry at 231 nm and 210 nm respectively.

Table 3: Concentration and Absorbance Data of Sample Mixture(Analytical calibration readings arranged in journal table format for graphical representation and statistical interpretation.)

Concentration (µg/ml)	Absorbance at 231 nm	Absorbance at 210 nm
10	0.204	0.268
20	0.413	0.529
30	0.635	0.742
40	0.863	0.961
50	1.272	1.130

Table 4: Analytical Parameters

Parameter	Value
Wavelength	231 nm and 210 nm
Solvent	Water
Cuvette Length	1 cm

The calibration curve of amoxicillin exhibited an R^2 value of 0.986 whereas clavulanic acid showed an R^2 value of 0.991, indicating excellent linearity.

Graph Interpretation

The calibration graph of amoxicillin demonstrated a linear relationship between concentration and absorbance with correlation coefficient (R^2) value of 0.986, indicating good analytical accuracy and reliability of the developed method.

Figure 5: Calibration Curve of Amoxicillin
The calibration curve of clavulanic acid also exhibited excellent linearity with R^2 value of 0.991, confirming suitability of the analytical method for simultaneous estimation.

Figure 6: Calibration Curve of Clavulanic Acid

Preformulation parameters confirmed the identity and purity of both drugs. Amoxicillin was found to be white and odourless with melting point range of

197–204°C, whereas clavulanic acid appeared brown and odourless with melting point range of 228–235°C. Both drugs were highly soluble in water and soluble in ethanol and methanol.

FTIR spectra confirmed the characteristic functional groups of amoxicillin and clavulanic acid without any significant interaction.

6.2 Analytical Method Development

Calibration curves of amoxicillin and clavulanic acid showed good linearity with R^2 values of 0.986 and 0.991 respectively.

6.3 Preparation and Optimization of Ethosomes

Optimization studies were carried out to obtain ethosomal vesicles with minimum particle size, narrow PDI, and satisfactory entrapment efficiency. Different formulation and process parameters including preparation method, aqueous-organic phase ratio, temperature, drug loading, and sonication time were systematically investigated. Among the two preparation methods evaluated, the hot method showed significantly smaller particle size and lower PDI compared to ethanol injection method.

Table 5: Comparison of Ethosome Preparation Methods (Table arranged to compare particle size and PDI obtained from different preparation techniques.)

Formulation Code	Method	Particle Size	PDI
T1	Hot Method	178 nm	0.09
T2	Ethanol Injection Method	819 nm	0.80

Therefore, hot method was selected for further optimization studies.

Optimization of Aqueous and Organic Phase Ratio

Table 6: Optimization of Aqueous and Organic Phase Ratio (Optimization data arranged systematically to demonstrate effect of aqueous-organic ratio on vesicle size.)

Formulation Code	Ratio	Particle Size
T1F1	1:2	1217 nm
T1F2	1:3	689 nm
T1F3	1:4	181 nm

The formulation containing aqueous to organic phase ratio of 1:4 exhibited the lowest particle size (181 nm) among all batches. Higher organic phase concentration improved vesicle formation and reduced particle aggregation. Therefore, the 1:4 ratio was selected as the optimized ratio for further formulation studies.

Optimization of Temperature

Table 7: Optimization of Temperature (Table arranged to demonstrate influence of processing temperature on ethosomal vesicle size.)

Formulation Code	Temperature	Particle Size
T1F3T1	20°C	276 nm
T1F3T2	30°C	253 nm
T1F3T3	40°C	183 nm

Among different temperatures evaluated, formulation prepared at 40°C exhibited the smallest particle size (183 nm). Increase in temperature improved fluidity of phospholipid bilayer and reduced vesicle aggregation, thereby producing nanosized ethosomes.

Optimization of Drug Loading

Table 8: Optimization of Drug Loading (Table arranged with mean ± SD values to present influence of drug loading on particle size, PDI, and entrapment efficiency.)

Formulation Code	Drug Loading	Size (nm)	PDI	Entrapment Efficiency of Amox	Entrapment Efficiency of Clavulanic

				icillin (%)	Acid (%)
T1F3T3D1	6%	193.43 ± 7.46	0.13 ± 0.01	61.24 ± 2.54	62.1 ± 1.94

The optimized formulation containing 6% drug loading demonstrated satisfactory entrapment efficiency for both amoxicillin and clavulanic acid with acceptable particle size and narrow PDI. Further increase in drug concentration may lead to vesicle instability and reduction in entrapment efficiency.

Optimization of Sonication Time

Table 9: Optimization of Sonication Time (Table arranged to represent the effect of sonication time on vesicle characteristics.)

Formulation Code	Sonication Time	Particle Size	PDI
T1F3T3D1S1	10 min	188 nm	0.102
T1F3T3D1S2	15 min	236 nm	0.12

Sonication for 10 min produced optimized particle size and narrow PDI compared to prolonged sonication. Excessive sonication time may lead to vesicle disruption and instability of the formulation.

Final Optimized Formulation

Table 10: Final Optimized Formulation (Final optimized formulation summarized with selected processing parameters and characterization results.)

Formulation Code	Aq: Org Ratio	Temperature	Drug Loading	Sonication Time	Particle Size	PDI
T1F3T3D1S1	1:4	40°C	6%	10 min	188 nm	0.102

Among the two methods evaluated, hot method produced smaller particle size (178 nm) and lower PDI (0.09) compared to ethanol injection method.

Optimization studies revealed that formulation prepared with aqueous-organic phase ratio of 1:4, temperature of 40°C, 6% drug loading, and 10 min sonication time produced optimized ethosomes.

6.4 Characterization of Optimized Formulation

The optimized ethosomal formulation was characterized for particle size, polydispersity index, entrapment efficiency, and stability to evaluate its suitability for topical drug delivery applications. The optimized ethosomal formulation demonstrated nanosized vesicles with narrow size distribution.

Table 11: Characterization of Optimized Ethosomal Formulation (Characterization parameters arranged in standard journal tabular format.)

Parameter	Observation
Particle Size	188 nm
PDI	0.102
Entrapment Efficiency of Amoxicillin	61.24 ± 2.54%
Entrapment Efficiency of Clavulanic Acid	62.1 ± 1.94%

Graph Interpretation

Zetasizer analysis confirmed nanosized vesicles with narrow particle size distribution. Low PDI value indicated uniformity and homogeneity of the ethosomal system.

Figure 7: Particle Size and PDI Analysis by Zetasizer

Stability Study

Table 12: Stability Study of Optimized Formulation (Stability data arranged in chronological order to evaluate changes in particle size and PDI during storage.)

Time	Particle Size	PDI
Same Day	188 nm	0.102
After 3 Days	202 nm	0.09

After 7 Days	211 nm	0.11

The stability study indicated that the ethosomal formulation remained stable throughout the storage period. Only a slight increase in particle size and PDI was observed, which may be attributed to minor vesicle aggregation during storage. However, the formulation maintained acceptable stability characteristics. The optimized formulation showed particle size of 188 nm with PDI of 0.102 indicating uniform vesicle distribution. Entrapment efficiency of both drugs was found satisfactory. Stability studies demonstrated that the formulation remained stable with slight increase in particle size after storage.

6.5 In-vitro Drug Release Study

The in-vitro drug release study was performed to evaluate the release behavior of amoxicillin and clavulanic acid from ethosomal vesicles in comparison with plain drug dispersions.

Table 13: In-vitro Drug Release Profile (Drug release data arranged systematically for comparative evaluation between plain drug dispersion and ethosomal formulation.)

Time (h)	Plain Amoxicillin Dispersion (%)	Plain Clavulanic Acid Dispersion (%)	Amoxicillin Ethosomal Formulation (%)	Clavulanic Acid Ethosomal Formulation (%)
0.5	11.36	9.32	8.67	6.91
1	21.35	17.23	18.83	17.53
2	34.97	28.51	26.96	24.98
4	51.73	39.94	32.23	34.53
6	69.94	52.13	40.26	42.46
8	82.03	72.88	49.47	51.14
10	91.43	86.01	57.35	59.42
12	-	-	65.77	68.12

The optimized ethosomal formulation demonstrated sustained and controlled drug release behavior up to 12 h. In contrast, plain drug dispersions exhibited rapid drug release within 10 h. The sustained release pattern observed in ethosomal formulation may be attributed to encapsulation of drugs within phospholipid vesicles, which controlled diffusion of drug molecules from the carrier system. The prolonged release profile may improve therapeutic effectiveness and reduce frequency of administration in topical treatment of bacterial skin infections.

Graph Interpretation

The comparative release graph demonstrated sustained release from ethosomal formulation as compared to rapid release from plain drug dispersion. Controlled release behavior may enhance therapeutic efficacy and prolong drug retention at the site of infection.

Figure 8: Comparative In-vitro Drug Release Graph of Plain Drug and Ethosomal Formulation

The ethosomal formulation exhibited sustained drug release pattern up to 12 h. The cumulative drug release of amoxicillin and clavulanic acid from ethosomal formulation was found to be 65.77% and 68.12% respectively. In comparison, plain drug dispersions showed rapid drug release.

CONCLUSION

The present investigation successfully developed an ethosomal drug delivery system for co-delivery of amoxicillin and clavulanic acid intended for topical treatment of bacterial skin infections. The optimized ethosomal formulation exhibited nanosized vesicles, narrow particle size distribution, satisfactory entrapment efficiency, good stability, and sustained drug release behavior. The formulation demonstrated potential advantages including enhanced topical penetration, localized drug delivery, prolonged

drug release, and reduced systemic side effects. Therefore, the developed ethosomal system may serve as a promising alternative to conventional topical formulations for effective management of bacterial skin infections. The present study successfully developed and characterized an ethosomal formulation for co-delivery of amoxicillin and clavulanic acid for topical treatment of bacterial skin infections. The optimized ethosomal formulation exhibited nanosized vesicles with satisfactory entrapment efficiency, sustained drug release, and good stability profile. Ethosomal delivery system demonstrated potential advantages including improved topical penetration, localized drug delivery, reduced systemic side effects, and enhanced therapeutic effectiveness. Therefore, the developed formulation may serve as a promising carrier system for management of bacterial skin infections.

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