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Rational Design of Ciprofloxacin-Loaded Niosomes via Ether Injection Method: Enhanced Drug Release, Oral Bioavailability, and Antimicrobial Efficacy

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ABSTRACT

Ciprofloxacin is a widely prescribed fluoroquinolone antibiotic; however, its therapeutic efficacy following oral administration may be limited by variable bioavailability and gastrointestinal adverse effects. The present study aimed to develop ciprofloxacin-loaded niosomes using the modified ether injection method and evaluate their drug content, entrapment efficiency, in vitro drug release, and oral bioavailability in comparison with marketed ciprofloxacin tablets. Nine niosomal formulations (F1–F9) were prepared using Tween 20, Span 20, or their combinations with different surfactant-to-cholesterol ratios. Drug content and entrapment efficiency were determined by UV-visible spectrophotometry, while in vitro drug release was evaluated in phosphate buffer (pH 6.8) for 60 minutes using a USP type II dissolution apparatus. Optimized formulations (F5, F8, and F9) were further assessed for relative oral bioavailability in Swiss albino mice. The prepared niosomes exhibited drug content ranging from 65.2 ± 0.12% to 95.2 ± 0.22% and entrapment efficiency from 57.6 ± 0.17% to 91.4 ± 0.21%, with formulation F8 demonstrating the highest entrapment efficiency (91.4 ± 0.21%). In vitro cumulative drug release after 60 minutes ranged from 77.2% to 98.3% among the niosomal formulations, while marketed products showed 95.1–99.4% release. Among the developed formulations, F8 achieved the highest cumulative drug release (98.3%) and the greatest relative bioavailability (50.59%), compared with 45.10% for F5, 39.47% for F9, and 40.00% for the standard ciprofloxacin formulation. The combination of Tween 20 and Span 20 with an optimized cholesterol ratio significantly improved drug entrapment and oral bioavailability. These findings demonstrate that ciprofloxacin-loaded niosomes prepared by the modified ether injection method, particularly formulation F8, represent a promising vesicular drug delivery system capable of enhancing ciprofloxacin delivery and improving oral bioavailability, with potential application for more effective antimicrobial therapy.

Keywords: Bioavailability; Ciprofloxacin; Drug Entrapment Efficiency; Drug Release; Ether Injection Method; Niosomes; Non-Ionic Surfactants; Vesicular Drug Delivery.

1. Introduction

Ciprofloxacin is a second-generation fluoroquinolone antibiotic widely used for the treatment of various bacterial infections owing to its broad-spectrum activity, favorable tissue penetration, and bactericidal mode of action [1]. Although ciprofloxacin proves to be highly useful, there are some limitations pertaining to the oral route of administration of this medication due to its poor and inconsistent absorption rate, gastrointestinal discomfort, and variability of the drug level in the blood plasma. This may reduce the efficacy of the drug and lower the compliance rate among patients. Hence, the development of an alternative drug delivery system for the said drug becomes imperative [2-3].

Different vesicular drug delivery systems have gained much attention as possible candidates for delivering pharmaceuticals and improving their pharmacokinetics and pharmacodynamics. Notable among them is the niosome, a vesicle made from a non-ionic surfactant. This vesicle is known for its strengths, which include chemical stability, biocompatibility, ease of preparation, and the ability to entrap both hydrophilic and lipophilic substances [4]. Applications of niosomes have been explored in-depth based on their ability to provide sustained drug release, protect the drug from degradation, and improve the bioavailability of the drug [5].

The ether injection method is another accepted method in niosome formulation to ensure a homogeneous distribution of vesicles with high entrapment efficiency. The variables involved in niosome formulation, which include surfactant, surfactant to cholesterol ratio, and solvent, can be varied to adjust the release properties of niosomes. The inclusion of cholesterol in this formulation is essential as it demonstrates differences in release properties of the drug due to its effect on cell membrane hardness, permeability, and cell membrane integrity [6].

Previous studies have already proved the effectiveness of niosomal dosage forms in enhancing the release properties and bioavailability of poorly soluble and variably absorbed drugs [7]. With regard to ciprofloxacin, the incorporation of niosomes may lead to the reduction of GI irritation, increased absorption, and sustained blood concentration levels, which would eventually result in improved antimicrobial action and decreased dosage frequency. In addition, it is vital to compare its effectiveness with other marketed formulations [1,8].

In this context, the objective of the current study is the preparation of ciprofloxacin niosomes by ether injection technique using different non-ionic surfactants (Tween 20, Span 20, and their combination) and cholesterol in different proportions. This experiment seeks to investigate the in vitro release behavior of the drug, ascertain the effectiveness of the newly synthesized formulations, and assess their efficacy compared to the existing ciprofloxacin tablets.

Moreover, some of the optimized formulations have been tested in-vitro to examine their relative bioavailability in comparison to a standard drug. This study aims to explore the development of ciprofloxacin-loaded niosomes as a promising and efficient drug delivery system for improving antimicrobial therapy and facilitating pharmaceutical development in the future.

1.1. Study Objectives

In this study, the main goal was to prepare ciprofloxacin-loaded niosomes by utilizing the Modified Ether Injection technique and to analyze their physicochemical characteristics. This study aimed at accomplishing the following objectives:

1) Formulation of niosomes incorporating ciprofloxacin via the ether injection technique with different non-ionic surfactants (Tween 20, Span 20, and their combinations) in different surfactant to cholesterol ratios.

2) Assessment of the impact of surfactants' ratio and cholesterol amount on the physicochemical properties of prepared niosome systems.

3) Investigation of in vitro drug release profile of the developed niosome preparations and its comparison with the commercial ciprofloxacin tablets.

4) Determination of the potency of the developed niosomes with the use of dialysis separation technique and the subsequent comparison of obtained results with those of commercial formulations.

5) Identification of optimal niosomal formulation by drug release and potency data.

6) Evaluation of in vivo oral bioavailability of the selected optimized formulations by determination of area under the plasma concentration-time curve (AUC) in albino mice.

7) Comparative investigation of the efficacy of ciprofloxacin-loaded niosomes with standard oral administration of ciprofloxacin tablets.

8) Assessment of the capability of niosomal loading to enhance ciprofloxacin bioavailability, drug release sustaining action, and antimicrobial treatment efficiency.

9) Exploration of the possibility to use the ether injection technique as a simple, reproducible, and scalable procedure for the preparation of ciprofloxacin-loaded niosomal drug delivery systems.

10) Providing the experimental data for the application of niosomes as nanocarriers for ciprofloxacin to enhance its oral delivery efficiency.

2. Literature Review

Ciprofloxacin is a second-generation fluoroquinolone antibiotic that are commonly used for treatment of various infections caused by Gram-positive and Gram-negative bacteria because of their broad spectrum of antimicrobial activity and good penetration into different tissues [9]. Unfortunately, the effectiveness of the medication can be reduced by poor oral absorption, gastro-intestinal irritation, relatively short biological half-life, and the necessity of frequent doses [10-11]. Therefore, many scientists decided to conduct research on development of innovative systems for drug delivery that could increase effectiveness and pharmacokinetics of ciprofloxacin [1].

Among the novel techniques of drug delivery, nanotechnologies can help solve problems associated with traditional methods of medication. Thereby, niosomes became especially popular since they have such characteristics as biodegradability, biocompatibility, chemical stability, low cost and capability to encapsulate hydrophilic and lipophilic medications. Due to protection against degradation, increased permeation through membranes, increased circulation time, and controlled drug release, niosomes can improve therapeutic effectiveness and reduce systemic toxicity [12-13].

There are several ways of preparing niosomes which include thin-film hydration, reverse phase evaporation, bubble method, microfluidization, ethanol injection and ether injection. Among them, ether injection is one of the most reproducible methods that provide relatively homogeneous vesicles, high drug entrapment and controlled size of particles. In particular, during the process, gradual evaporation of ether promotes formation of bilayers and creation of vesicles suitable for sustained drug delivery [14].

However, physicochemical properties of niosomes depend on the nature of the non-ionic surfactant used, cholesterol concentration, ratio of the surfactant to cholesterol, and method of vesicle formation. Vesicles prepared using Tween-type surfactants are more flexible, thus exhibit higher permeability and release rates due to high hydrophilic-lipophilic balance (HLB), while Span-type surfactants prepare more rigid bilayers resulting in slower release of drugs. The role of cholesterol is that of membrane stabilizing agent that prevents bilayer permeability and drug leakage [12-13].

Recently it was established that encapsulation of ciprofloxacin in niosomes considerably increases its antibacterial properties. Niosomal encapsulation of ciprofloxacin leads to high efficiency of its encapsulation, improved release rate, increased antibacterial activity against multidrug-resistant bacteria, effective inhibition of bacterial biofilms

and prevention of the emergence of antibiotic resistance in comparison with the free form of ciprofloxacin. Thus, this nanovesicular system allows to enhance antimicrobial therapy, reduce the dose of drugs needed, as well as adverse effects [1, 15-16].

Finally, it should be noted that recently conducted review articles demonstrate that niosomes are among the most promising nanovesicular drug delivery systems owing to their scalability, easy preparation, increase of oral bioavailability and possibility of targeted drug delivery. Advancements in such areas as formulation optimization, surface modifications and multifunctional nanocarriers contribute to their usage in antimicrobial therapy, cancer therapy, eye drug delivery, pulmonary drug delivery, and oral controlled drug delivery [13].

Even though various research works have been carried out on ciprofloxacin loaded niosomes for topical and parenteral administration, very few references have been reported concerning the preparation of ciprofloxacin niosomes through ether injection technique utilizing varying concentrations of Tween 20, Span 20, and cholesterol. Moreover, the in vitro drug release, activity and in vivo bioavailability comparison of these niosomes in comparison with marketed ciprofloxacin tablets is less studied. The current research work has been planned in order to formulate and evaluate the ciprofloxacin loaded niosomes using ether injection technique.

3. Materials and Methods

3.1. Materials

Ciprofloxacin was gifted as a sample. The nonionic surfactants used in the formulation of niosomes were Tween 20 and Span 20, Cholesterol, and Chloroform. All the reagents used in the research work were of analytical reagent quality. Ciprofloxacin tablets (Ciprocin, Ciprox, and Flontin) were purchased from local medical stores. Albino mice were used in the in vivo work to check the bioavailability of the formulation. Distilled water was used in the experiment.

3.2. Experimental Animals

Eight-week-old Swiss albino mice (20-30g) purchased from Jahangirnagar University, Dhaka, Bangladesh and were housed in animals' cages under standard environmental conditions (22-25°C, humidity 60-70%, 12 hr light: 12 hr dark cycle) [17]. The mice were feed with standard pellet diet taken from, Jahangirnagar University Dhaka. The animals used in this study were cared in accordance with the guidelines on animal experimentation of our institute.

3.3. Preparation of Ciprofloxacin-Loaded Niosomes

Niosomes were prepared using the modified ether injection method. Ciprofloxacin (500 mg), non-ionic surfactant (Tween 20, Span 20, or their combination), and cholesterol were dissolved in chloroform in a composition similar to that of the preparations (F1 to F9). The organic fluid was gradually added to the heated aqueous solution, and niosomes were formed as a consequence of the evaporation of chloroform. Different proportions of non-ionic surfactant and cholesterol (2:1, 2.5:1, and 3:1) were used to study their effect on the release of the drug and bioavailability. The prepared niosomes were allowed to equilibrate and were stored in a refrigerator prior to evaluation [17]. Each formulation was coded as indication in Table 1.

Table 1. Composition of ciprofloxacin-loaded niosomal formulations prepared by the modified ether injection method using single and combination form of tween 20 and span 20 surfactants.

Formulation Code	Drug Ciprofloxacin (mg)	Surfactant (mg)		Cholesterol (mg)	Drug: Surfactant: Cholesterol	Chloroform (mL)
F1	500	Tween 20	1000	500	1:2:1	20
F2			1250		1:2.5:1	
F3			1500		1:3:1	
F4		Span 20	1000		1:2:1	
F5			1250		1:2.5:1	
F6			1500		1:3:1	
F7		Tween 20 + Span 20	500 + 500		1:2:1	
F8			625 + 625		1:2.5:1	
F9			750 + 750		1:3:1	

3.4. Standard Calibration Curve of Ciprofloxacin

A graph of calibration for the standard ciprofloxacin was obtained using distilled water. The stock solution was diluted to obtain a concentration ranging from 1 to 9 µg/mL [17]. Absorbance for all the solutions was determined using a UV-visible spectrophotometer with a wavelength of 272 nm. The results were plotted on a graph, shown in Figure 1, as absorbance versus concentration, which was later used to calculate drug concentrations.

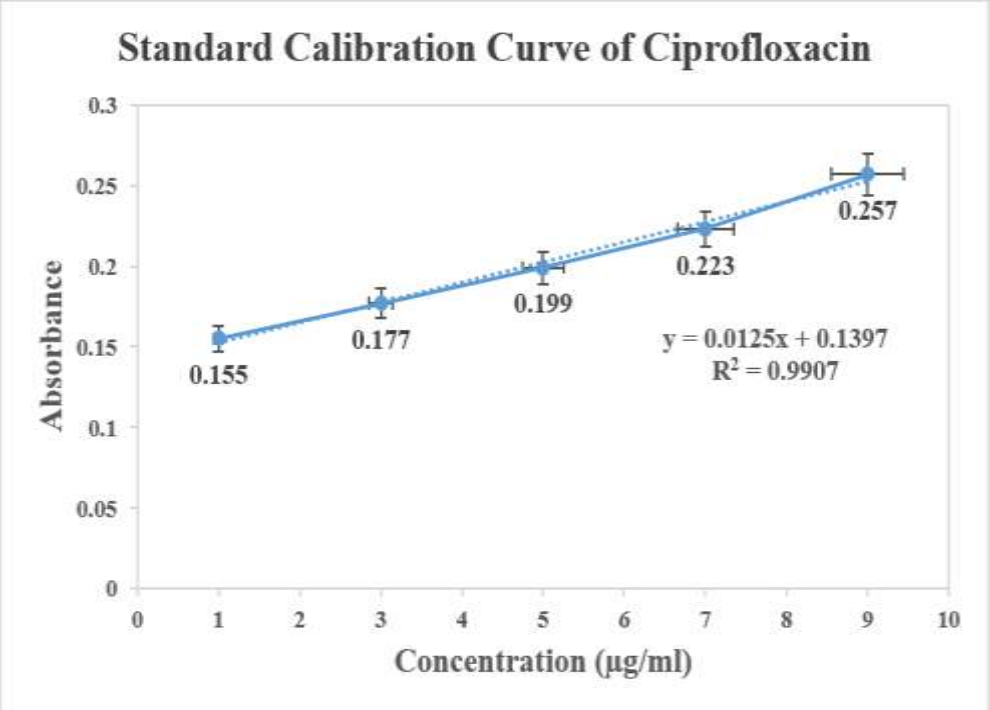

Figure 1. Standard calibration curve of ciprofloxacin.

3.5. Drug content

To determine the percentage of drug content, 10 mg equivalent of niosomal solutions were added to a 100 mL volumetric flask and diluted in phosphate-buffered solution (pH 7.4). Another 1 mL volume was taken from this mixture and diluted to make up a total volume of 10 mL using the phosphate-buffered solution [18]. Each of the five niosomes was studied to obtain the absorbance values at 272 nm using UV-visible spectrophotometry technique, and then the drug content percentage was determined.

3.6. Entrapment Efficiency

To calculate the entrapment efficiency, the unentrapped drug was isolated from the niosomal suspensions using the process of centrifugation. All formulations were subjected to centrifugation at 3000 rpm for 45 minutes to isolate the unentrapped drug. Centrifugation was carried out under the above-mentioned conditions because the sedimentation of the niosomal vesicles can be effectively achieved due to their relatively larger sizes compared to nanosized vesicles as previously reported [17]. Absorbance value of the supernatant was noted using UV–Visible spectrophotometer at 272 nm. Even though ultracentrifugation is preferred to separate nanosized vesicles, the used conditions appeared to be sufficient in this case.

3.7. In-Vitro Drug Release Study

The profile of in vitro drug release of a batch of nine niosomal formulations (F1 to F9) and commercial formulations (S1 to S3) were evaluated using membrane driven diffusion approach over a period of 1 hour. The membrane of each niosomal formulation was taken as donor and dissolved in it with an equivalent concentration of 20 mg of ciprofloxacin. The USP type II dissolution apparatus (paddle method) was employed; the membrane was placed in 900 mL of phosphate buffer solution maintained at 37 °C and adjusted to pH 6.8 using 1 M sodium hydroxide as the receptor medium. The stirring speed was maintained constant at 100 rpm for 60 minutes. In this process, 10 mL samples were withdrawn at specific time intervals (15, 30, 45, and 60 minutes) using micropipettes and replaced in the reaction mixture with the fresh medium. The filtrates were obtained from each of the sample solutions using Whatman filter paper and diluted [19-20]. Spectrophotometric analysis was performed at λmax of 272 nm in the presence of phosphate buffer (pH 6.8) as the blank solution. The cumulative percentage release of the drug from each formulation was calculated by using MS Excel.

3.8. In-Vivo Bioavailability Study

Based on potency, entrapment efficiency and in-vitro release studies, formulations F5, F8, and F9 were selected for in-vivo studies. The albino mice were orally given 10 mg/kg of the selected formulations, and the standard drug was given at 5 mg/kg. Blood samples were collected at fixed time intervals, and plasma drug levels were estimated using spectrophotometry after processing the samples. The area under the concentration-time curve (AUC) was calculated for each set of formulations, and the relative bioavailability (%) was calculated based on the comparison of AUC values of formulations with those of standard drug formulations.

In pharmacokinetics, AUC is usually calculated using the trapezoidal rule from plasma concentration–time data. For two consecutive time points:

$$AUC_{t1\text{-}t2}=\frac{(C1+C2)(t2\text{-}t1)}{2} \qquad (1)$$

Total AUC is the sum of all trapezoids:

$$AUC_{0\text{-}t}=\mathrm{AUC}_{0-0.5}+\mathrm{AUC}_{0.5-1}+\mathrm{AUC}_{1-2}+\mathrm{AUC}_{2-3} \qquad (2)$$

Relative bioavailability compares the bioavailability of a test formulation with a reference formulation.

$$\text{Relative Bioavailability (\%)} = \frac{\mathrm{AUCf}}{\mathrm{AUCs}} \times \frac{\mathrm{Ds}}{\mathrm{Df}} \times 100 \qquad (3)$$

Where,

C1 and C2 are the concentration at time t1 and t2 respectively.

AUCf and AUCs are the area under the concentration-time curve of standard and sample respectively.

Ds and Df are the drug dose of sample and standard respectively [7].

3.9. Statistical Analysis

The experiment has been conducted thrice, and the data has been provided in terms of mean and standard deviation values. Bioavailability of test as well as standard samples has been determined by calculating the ratio of AUC value. Test samples as well as samples in the market have been compared by using Descriptive Statistics analysis.

4. Results and Discussion

In this study, ciprofloxacin-loaded niosomal vesicles were fabricated by the modified ether injection technique, and the effects of surfactant composition and cholesterol proportion on drug content, entrapment efficiency, in vitro drug release, and oral bioavailability were investigated. As the results show, the properties of the prepared formulations were greatly affected by the variables of the formulation process.

4.1. Drug Content and Entrapment Efficiency

Table 2 and Figure 2 summarize the results of the drug content and entrapment efficiency for the nine niosomal formulations and three marketed drugs. Drug content values varied from 65.2 ± 0.12% to 95.2 ± 0.22% (Table 2). Formulations F5 (95.2 ± 0.22%), F8 (95.1 ± 0.22%), and F9 (92.7 ± 0.14%) had the highest drug content in comparison with other niosomal formulations. This means that high loading was obtained in these formulations. The commercial formulations (S1-S3) had the highest drug content values from 95.7 ± 0.27% to 99.2 ± 0.18%, which is explained by the good pharmaceutical characteristics of these formulations.

Entrapment efficiency greatly depended on the composition of niosomal formulations, varying from 57.6 ± 0.17% (F3) to 91.4 ± 0.21% (F8). The formulations with a combination of Tween 20 and Span 20 had higher drug encapsulation efficiency in comparison with the formulations with one surfactant. The highest entrapment efficiency was obtained for formulations F8 (91.4 ± 0.21%), F9 (88.4 ± 0.06%), and F5 (82.8 ± 0.13%). This can be explained by the optimal ratio of surfactant and cholesterol which increases bilayer stability and decreases drug loss.

Table 2. Drug content and entrapment efficiency of ciprofloxacin-loaded niosomal formulations (mean ± SD, n = 5).

Formulation	% Drug content (Mean ± SD)	% Drug entrapment efficiency (Mean ± SD)
F1	73.2 ± 0.11	68 ± 0.15
F2	70.9 ± 0.1	63.2 ± 0.12
F3	65.2 ± 0.12	57.6 ± 0.17
F4	79.8 ± 0.31	72.2 ± 0.12
F5	95.2 ± 0.22	82.8 ± 0.13
F6	75.1 ± 0.03	69.3 ± 0.11
F7	81.5 ± 0.23	76.2 ± 0.15
F8	95.1 ± 0.22	91.4 ± 0.21
F9	92.7 ± 0.14	88.4 ± 0.06
S1	96.3 ± 0.21	N/A
S2	99.2 ± 0.18	N/A
S3	95.7 ± 0.27	N/A

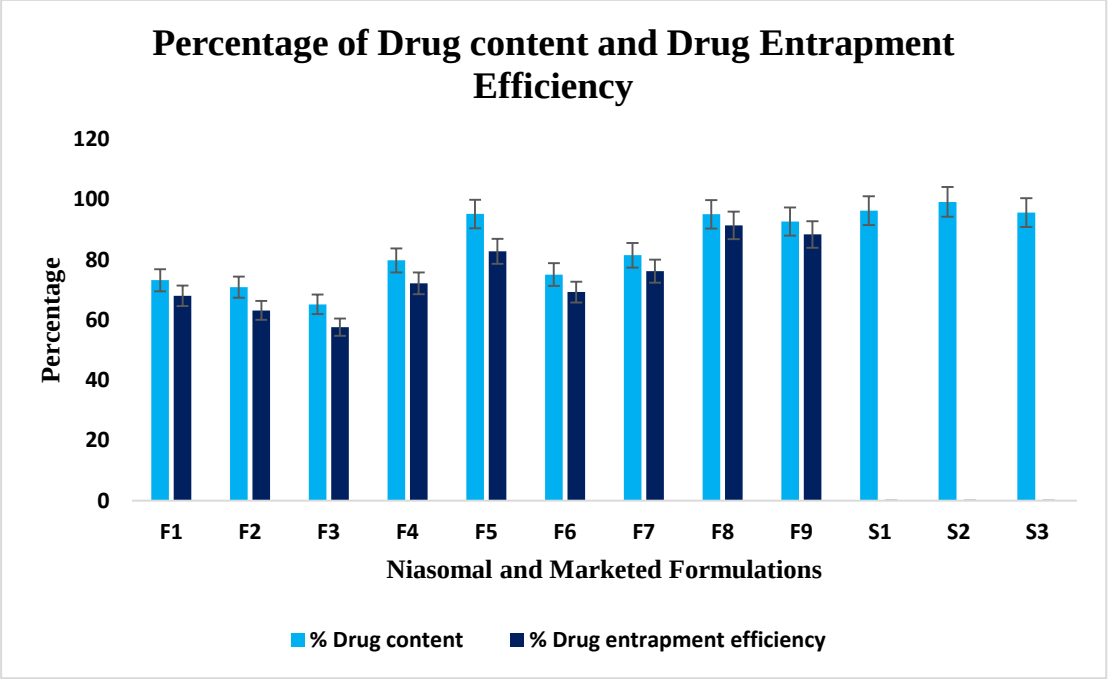

Figure 2. Comparison of drug content and entrapment efficiency of ciprofloxacin-loaded niosomal formulations (F1-F9) and marketed drug (S1-S3).

4.2. In Vitro Drug Release

Release profiles of drugs are depicted in Table 3 and represented in Figure 3. Release of drugs from all formulations was observed to increase gradually within 60 minutes. Cumulative release of drugs from formulations during 60 minutes varied from 77.2% to 98.3% in case of niosomal formulations. Formulation F8 was characterized by the highest drug release of 98.3%, whereas formulations F5 (97.4%) and F9 (96.7%) also demonstrated quite high

release rates. In their turn, formulations F1–F3 demonstrated comparatively low rates of release; this can be explained by the influence of surfactant composition on diffusion of the drug.

In marketed products cumulative drug release after 60 minutes reached 98.9% (S1), 99.4% (S2), and 95.1% (S3). Despite a slight superiority in terms of rate of drug release, marketed formulations provided similar values as optimized niosomal formulations (especially F8); however, vesicular encapsulation of the drug in niosomes may result in better absorption and performance of the drug. Enhanced release of formulation F8 can be related to combined application of Tween 20 and Span 20 at the optimal ratio between surfactant and cholesterol, providing favorable membrane characteristics and improved drug diffusion.

Table 3. In vitro cumulative drug release profiles of ciprofloxacin-loaded niosomal formulations (F1-F9) and marketed drug (S1-S3).

Sample Code	Percentage of Drug Release			
	15 min	30 min	45 min	60 min
F1	42.7	54.6	66.6	77.2
F2	43.5	46.5	68.7	79.5
F3	39.5	44.5	56.0	79.4
F4	38.8	59.5	80.7	91.7
F5	65.3	77.5	90.4	97.4
F6	49.1	60.2	71.7	83.7
F7	52.3	63.7	74.6	77.6
F8	76.9	89.0	94.2	98.3
F9	75.3	88.9	91.1	96.7
S1	70.4	88.2	95.7	98.9
S2	87.2	92.8	97.5	99.4
S3	71.6	84.6	93.8	95.1

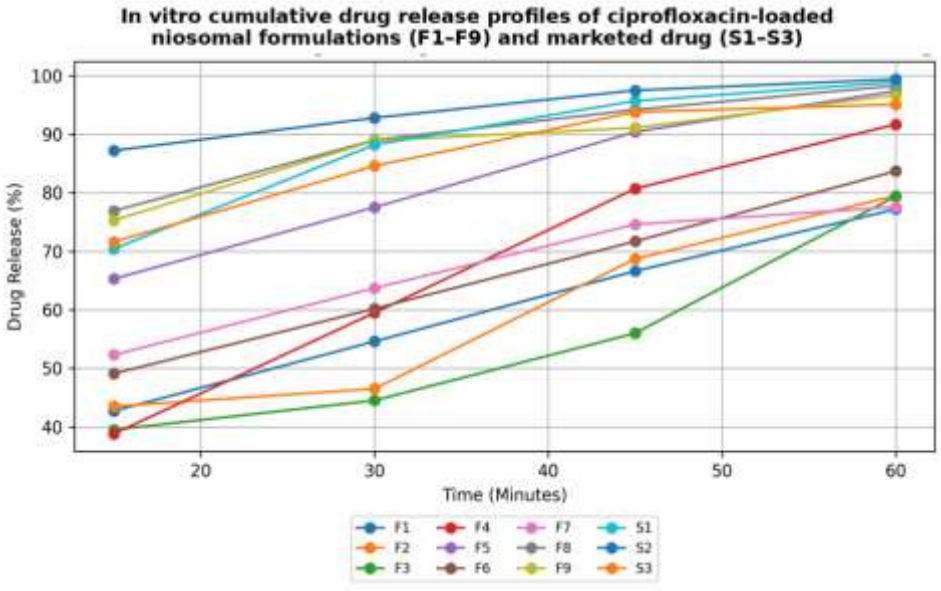

Figure 3. Comparative data for dissolution studies of ciprofloxacin-loaded niosomal formulations (F1-F9) and marketed drug (S1-S3).

4.3. Relative Oral Bioavailability

Taking into account drug content, entrapment efficiency, and in vitro dissolution results, three formulations (F5, F8, and F9) were chosen for further in vivo studies. It is evident from Table 4 and Figure 4 that formulation F8 had the highest area under the plasma concentration-time curve (AUC = 99.98) and the highest relative bioavailability (50.59%). Relative bioavailability, which is calculated using Equation 1, Equation 2 and Equation 3, of formulation F5 was equal to 45.10%, whereas formulation F9 had the value of 39.47%. Standard ciprofloxacin preparation had the relative bioavailability of 40%.

Higher bioavailability of F8 can be explained by higher entrapment efficiency and optimized vesicular composition, ensuring the highest effectiveness of drug protection and absorption. In spite of quite good in vitro drug release, F9 demonstrated inferior bioavailability as compared to F8.

Table 4. Relative bioavailability of the standard ciprofloxacine and the optimized formulations (F5, F8, F9).

Formulation	Drug dose (mg)	AUC	Relative Bioavailability (%)
Ciprofloxacin	0.125	98.81	40
F5	0.25	89.13	45.1
F8	0.25	99.98	50.59
F9	0.25	78.01	39.47

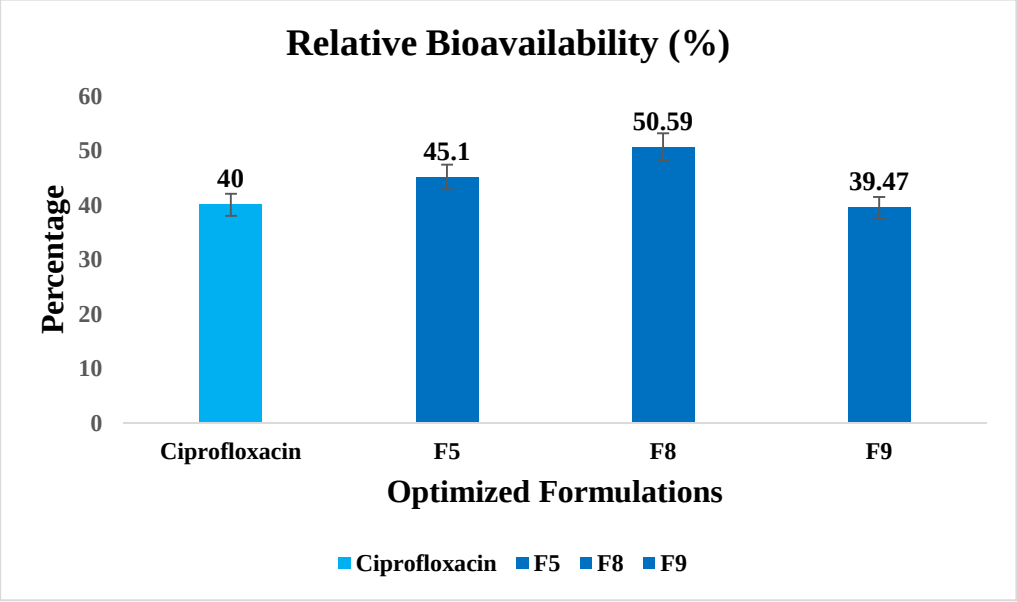

Figure 4. Comparative study among the relative bioavailability of the standard ciprofloxacine and the optimized formulations (F5, F8, F9).

4.4. Overall Performance of the Optimized Formulation

Out of the nine formulated niosomes, the F8 formulation showed greater performance with high drug loading (95.1%) along with high entrapment efficiency (91.4%), good in vitro release profile (98.3%), and highest relative oral bioavailability (50.59%). The results suggest that optimized surfactants like Tween 20, Span 20, and

cholesterol were able to form stable niosomes. This experiment shows that the ether injection method is a successful method for the preparation of ciprofloxacin loaded niosomes. There were significant changes in formulations due to optimization of surfactants and cholesterol concentration and the formulation F8 became the most promising one for future studies.

5. Conclusion and Future Perspectives

5.1. Conclusion

In the current research, successful production of ciprofloxacin-loaded niosomes via the modified ether injection method was accomplished and the effect of surfactants composition and surfactant to cholesterol ratio on the characteristics of the produced niosomes was revealed. The obtained niosomes possessed acceptable values of drug content, entrapment efficiency, in vitro drug release, and oral bioavailability. The drug content was found in the range of 65.2 ± 0.12% to 95.2 ± 0.22% and entrapment efficiency was found in the range of 57.6 ± 0.17% to 91.4 ± 0.21%; the formulation F8 had the highest entrapment efficiency value.

The results of in vitro dissolution experiment have shown the following cumulative release of drug –77.2% to 98.3% during 60 minutes. The highest drug release (98.3%) was observed for formulation F8, followed by F5 (97.4%) and F9 (96.7%). In the in vivo experiment, the highest relative oral bioavailability has been determined for formulation F8 as well (50.59% compared with 45.10% for F5, 39.47% for F9 and 40% for ciprofloxacin standard formulation).

Overall, it can be stated that the use of surfactants combination (Tween 20, Span 20 and cholesterol in optimal ratio) led to better entrapment and oral administration of ciprofloxacin. Among the produced niosomal formulations, F8 has shown the greater characteristics and can be considered as a prospective pharmaceutical preparation.

5.2. Future Perspectives

The results of the present study serve as an initial step for subsequent research on the topic of ciprofloxacin-loaded niosomes. In the future, the following issues should be addressed:

a) Comprehensive physicochemical characterization of the formulations including determination of their particle size, polydispersity index (PDI), zeta potential, and shape by means of scanning or transmission electron microscopy.

b) Assessment of the stability of the optimized formulations both accelerated and long-term storage under ICH guidelines.

c) Optimization of the formulation variables using Quality by Design (QbD) or Design of Experiments (DoE) methodology.

d) In-depth in vitro antimicrobial testing against clinically isolated Gram-positive and Gram-negative bacteria and multidrug-resistant strains.

e) Study of the antibiofilm activity and bacteria uptake by the optimized formulations.

f) Pharmacokinetics study aimed at determination of Cmax, Tmax, half-life, clearance, and mean residence time.

g) Toxicity, histopathology, and safety studies using repeated doses in proper animal models.

h) Biodistribution studies for elucidation of the tissue-targeting ability and drug disposition after oral administration.

i) Comparative study of the ether injection method and other methods of niosome preparation to determine the optimal method of preparation.

j) Regulatory assessment and clinical investigations to evaluate the therapeutic and commercial potential of the optimized formulation of ciprofloxacin-loaded niosomes.

Declarations

Source of Funding

This study received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of Interest

The authors declare that they have no competing interests related to this work.

Consent for Publication

The authors declare that they consented to the publication of this study.

Authors' Contribution

Md. Mahbubol Alam, Md. Shahinul Islam, and Suraiya Binta Kader took part in literature review, conceptualization, methodology, investigation, analysis, and manuscript writing, editing and reviewed the manuscript. Fahamida Binta Anower, Maliha Rahman, Md. Fardin Islam, and Afrin Farzana analyzed data. Fahamida Binta Anower involved in the design of the manuscript. All authors have read and approved the final manuscript.

Availability of Data and Materials

Supplementary information is available from the authors upon reasonable request.

Ethical Approval

All animal experiments were conducted in accordance with the institutional guidelines for the care and use of laboratory animals. Every effort was made to ensure the humane handling of animals and to minimize pain, discomfort, and stress throughout the experimental procedures.

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