

Development and In Vitro Evaluation of Diclofenac Sodium-Loaded Niosomes Prepared by the Ether Injection Method: Influence of Surfactant Composition on Drug Content, Entrapment Efficiency, Release Behavior, Kinetics, and Stability

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ABSTRACT

The primary focus of the present study is the preparation and evaluation of niosomes encapsulating diclofenac using the ether injection method, which is achieved through different surfactants and different ratios of surfactant to drug. There have been several works carried out on the preparation and evaluation of diclofenac-loaded niosomes; however, the present work has differentiated itself from others in terms of evaluating different niosome formulations prepared under various conditions. In total, twenty different formulations were studied for their drug loading, entrapment efficiency, in vitro drug release, and drug release kinetics. Formulation with 1:2:1 ratio (drug:surfactant:cholesterol) showed better results in terms of drug release of 98.06 percent within 2 hours, drug loading, and entrapment efficiency of 98 and 95 percent, respectively. The optimized release rate is near zero-order ($R^2=0.9987$), which shows that the drug released was controlled. The results were statistically analyzed by one-way and two-way ANOVA using Microsoft Excel software. One-way ANOVA analysis was conducted to test the differences in percentage drug content in 20 formulations (F1-F20) with replicates in two. In addition, two-way ANOVA (with replicates) was conducted to analyze the influence of formulation (F1-F20) and time (30-180 minutes) on percentage drug release with two replicates for each cell. Results obtained from the two-way ANOVA test showed that there were significant effects of both the formulation and time on the drug release rate ($p < 0.0001$). Specifically, there were highly significant effects of both formulation and time ($F(19,120) = 512.34$ and $F(5,120) = 1439.76$ respectively), while the interaction between the formulation and time factors were highly significant ($F(95,120) = 32.04$; $p < 0.0001$), showing that the drug release rates varied for each formulation over time. Statistical significance was considered to be when $p < 0.05$.

Keywords: Diclofenac; Dissolution; Drug Content; Entrapment Efficiency; Kinetics; Niosomes; Stability.

1. Introduction

Diclofenac is an oral non-steroidal anti-inflammatory drug (NSAID) that is effective for the total relief of pain, lowering inflammation, and curing joints, muscle and bone conditions [1]. However, the compound shows poor dissolution and absorption characteristics and falls into BCS class II drugs due to low aqueous solubility and high permeability [2].

With regard to this, many attempts have been made in designing new systems for administering drugs in the past few decades. A method adopted in administering drugs is by utilizing niosomes, which act as vehicles that facilitate the administration of drugs in a sustained manner so as to enhance the effectiveness of the drug [3]. Niosomes are surfactant vesicles consisting of non-ionic surfactants and cholesterol, in which either water-soluble or lipid-soluble drugs can be encapsulated in the aqueous compartment or the bilayer, making them efficient drug delivery systems [4-6]. Despite its many advantages, formulation optimization is essential because surfactant types, cholesterol levels, and preparation methods significantly influence the stability, loading capacity, and drug release kinetics of vesicles.

In this context, a particular research gap has emerged in terms of examining the impact of different combinations of surfactants on the physicochemical characteristics and drug release from diclofenac-containing niosomes prepared

by ether injection method. Hence, this investigation aimed to evaluate the best possible formulation of diclofenac encapsulated niosomes based on various parameters such as drug release, drug loading, entrapment efficiency, kinetics, and stability [7].

The uniqueness of this research can be highlighted as being the first one that compares twenty different niosome formulations made with varied surfactants and optimization based on multiple parameters, adding to the knowledge regarding interactions between surfactants and cholesterol and their influence on niosomal performance.

1.1. Objectives of the Study

The current research study was conducted to design and perform a complete evaluation of diclofenac sodium-loaded niosomes prepared through the ether injection technique. The study aimed at optimizing the formulation by investigating the effect of various non-ionic surfactants and surfactant-to-cholesterol ratios on the physicochemical characteristics, drug release profiles, and stability of the niosomal drug delivery system. The objectives of this current study can be stated as follows:

- 1) Preparing diclofenac sodium-loaded niosomes with the use of the ether injection technique using various non-ionic surfactants (Tween 20, Tween 80, Span 20, and Span 60) along with different surfactant-to-cholesterol ratios.
- 2) Examining the drug content and entrapment efficiency of the prepared niosomal formulations to estimate the drug loading and drug encapsulation efficiency of the formulations.
- 3) Studying the in vitro drug release profile of the formulations using the membrane diffusion method and comparison of the effect of various surfactant compositions on drug release from the niosomal formulations.
- 4) Studying the release kinetics of the optimized formulations using zero order, first order, second order, Higuchi, and Hixson–Crowell models to explain the drug release mechanism from the niosomal formulations.
- 5) Studying the short-term stability of the optimized niosomal formulations in terms of drug content and entrapment efficiency of the formulations stored at room temperature and refrigeration conditions.
- 6) Optimizing the diclofenac sodium-loaded niosomal formulation on the basis of drug content, entrapment efficiency, in vitro drug release, release kinetics, and stability for possible applications as a controlled drug delivery system.

2. Literature Review

Niosomes represent one of the most exciting vesicular drug delivery systems because of their ability to entrap both hydrophilic and lipophilic drugs along with increased effectiveness [8]. Niosomes are bilayer vesicles composed of non-ionic surfactants and cholesterol, which are able to enclose the drugs in the aqueous core or the lipid bilayer. Comparing with traditional dosage forms, niosomes possess a number of beneficial features, such as higher stability, prolonged circulation time, controlled drug release, higher bioavailability, lower toxicology and possibility of production on industrial scale. This explains their broad application in oral, topical, transdermal, ocular and targeted drug delivery [9].

The efficiency of niosomal formulations depends greatly on the composition, namely on the type of the surfactant, the ratio of surfactant to cholesterol, conditions of hydration and the preparation process itself. The non-ionic surfactants used in niosomal preparations belong to Tween and Span series and differ from each other by hydrophilic-lipophilic balance (HLB). The type of HLB affects greatly vesicles formation, drug loading, membrane permeability and release profile [10]. Cholesterol is crucial component of niosomes and its main function is to stabilize the bilayer membrane through decrease in its permeability and prevention of the leakage of the entrapped drug. Therefore, optimization of the above-mentioned parameters is necessary to get stable vesicles with desirable physicochemical characteristics [11].

There are several methods of niosome preparation, among which thin-film hydration, reverse-phase evaporation, sonication, microfluidization, bubble method, ether injection should be noted. The ether injection method is considered one of the most effective methods of obtaining relatively uniform vesicles with high encapsulation efficiency and reproducible particle size distribution. According to this technique, an organic solution with surfactant, cholesterol and drug is injected into the heated aqueous phase and after the evaporation of organic solvent, niosomal vesicles spontaneously form [12].

Prior researches have established that the optimization of surfactant mixture significantly impacts on the level of drug entrapment, efficiency of entrapment, stability of the vesicle system, and in vitro drug release from these systems. Surfactant mixtures comprising of Tween usually produce hydrated and more permeable bilayer systems because of their higher HLB value, while Span surfactant mixtures usually produce rigid vesicles, which help in slower and sustained release of drugs. In view of this, the comparison of various surfactant systems has been found to be very important to select the optimum formulation of the drug [13].

The advancements in pharmaceutical nanotechnology have led to the focus on formulation optimization rather than formulation development. The comparison of various formulations through the physicochemical characterization, drug release studies, kinetics, and stability tests provides a better insight into the performance of the formulations. This helps in choosing the optimum niosomal formulation with improved drug delivery attributes [14-15].

3. Materials and Methods

3.1. Reagents and Equipment

The following chemicals were used in the experiment: diclofenac sodium (99.7% pure), Tween 20, Span 20, Tween 80, Span 60 (all were analytical grade surfactants), cholesterol (97.5% pure), and chloroform (99.2% pure). These chemicals were purchased from the Pharmacy Department laboratory at Bangladesh University, Dhaka. The equipment used included a double beam ultraviolet-visible spectrophotometer (Shimadzu UV-1800, Japan), a dissolution device, and an electronic balance (AS 220.R2 PLUS).

3.2. Preparation of Diclofenac Niosomes (Ether Injection Method)

The required amount of cholesterol and surfactants (Tweens and Spans) was dissolved in a water bath at a temperature of 55-60 °C to form an organic phase. The aqueous phase was made up of distilled water (10 mL) and was kept at the same temperature (55-60 °C). Diclofenac-chloroform solution was separately made and slowly

introduced into the aqueous phase through a microsyringe at a flow rate of 1 mL/min while constantly stirring (600-800 rpm). The injection temperature was controlled by maintaining both phases in a thermostatically regulated water bath [2].

Then, the solution was continuously stirred using the magnetic stirrer. The temperature difference in the two layers gave rise to rapid evaporation of chloroform, thus giving rise to niosomes. Further stirring for another 30-45 min helped achieve complete evaporation of organic solvent. The total volume of the suspension was made up to 10 mL by adding distilled water [3].

Complete removal of the chloroform was accomplished through constant stirring and gentle heating, and its complete absence was assumed due to its high volatility and evaporation period; nonetheless, any quantitative estimation of its presence in the samples was not conducted using a specific method such as gas chromatography.

3.3. Standard Calibration Graph for Diclofenac Sodium

A measured amount of 10 mg of diclofenac sodium sample was accurately weighed using an electronic balance and placed into a volumetric flask of 100 mL. The final volume was filled till 100 mL with 0.1N HCl. 10 mL of the undiluted stock solution was poured into a volumetric flask of 100 mL and then diluted with distilled water to reach a level of 10 µg/mL. Aliquots containing 2, 4, 6, 8 and 10 mL were progressively moved into a volumetric flask of 10 mL, diluted to volume with water to achieve 2–10 µg/mL concentrations and their absorbance values were measured at 276 nm by employing UV–visible spectrophotometric analysis [2].

3.4. Drug Content

To estimate the percentage of drug content, a 10 mg equivalent of niosomal solution was poured in a volumetric flask measuring 100 mL and diluted with a phosphate-buffered solution (pH 7.4). Again, 1 mL volume of this mixture was taken and made up to the required 10 mL volume using a phosphate-buffered solution (pH 7.4). Then each of the twenty formulations was analyzed for absorbance at 276 nm through UV–visible spectrophotometric technique and the percentage of the drug content was determined [13].

3.5. Entrapment Efficiency

To determine entrapment efficiency, all twenty formulations underwent centrifugation at 3000 rpm for 45 minutes to separate the untrapped drug. The selected centrifugation conditions were sufficient to sediment the niosomal vesicles due to their relatively larger size compared to typical nanosized particles, as supported by previous reports. The supernatant absorbance was recorded using a UV–Vis spectrophotometer at 276 nm. Although ultracentrifugation is generally recommended for nanosized vesicles, the applied conditions were found adequate for phase separation in this study [2].

3.6. Drug Release Study (In-vitro)

The pattern of in vitro release of a batch of twenty diclofenac-based niosomal formulations was studied using the technique of membrane-driven diffusion for 3 hours. The release profiles of the formulations were assessed using egg membranes as a biological barrier. Egg membrane was selected as a low-cost, biocompatible, and

semi-permeable natural diffusion barrier that mimics biological membranes and is widely used for preliminary in vitro release studies [8]. The thickness of the membranes obtained was 50–100 μm , and it had micropores within the membrane which permitted selective passage of dissolved drug molecules through it without compromising the vesicle system, thus obtaining consistent diffusion-controlled release behavior, as reported in other researches [3].

In order to obtain the egg membranes, eggshells were immersed in an aqueous solution of 20% acetic acid for 24 hours. The egg membranes were then isolated from the eggshells and rinsed in distilled water. Membrane containing each niosome formulation was used as the donor phase and contained 20 mg equivalent concentration of diclofenac [2]. The USP type II dissolution setup (paddle method) was utilized; the membrane was put into 900 mL of phosphate buffer solution that was kept at 37 °C and adjusted to pH 6.8 using 1 M sodium hydroxide, serving as the receptor phase. The stirring rate was kept constant at 100 rpm for 180 minutes [16]. Throughout the experiment, 10 mL aliquots were transferred from the reaction mixture at 30-minute intervals for 180 minutes using micropipettes, replenished in the reaction mixture with fresh media [15]. The filtrates were obtained from each sampled solution by passing them through Whatman filter paper and diluted. Spectrophotometric measurements were carried out at λ_{max} of 276 nm using phosphate buffer (pH 6.8) as blank solution [2]. Percentage release of the drug from each formulation was analyzed using MS Excel, and results are presented in Table 3. The method exhibited reproducibility as the same membrane preparations were used and were of uniform thickness for all formulations.

3.7. Model Dependent Methods for Kinetics of Drug Release

The optimally designed formulations (F1, F3, F5 and F17) were further analyzed to comprehend their release kinetics. Comparison of dissolution profiles of optimized niosomal diclofenac sodium formulations was undertaken through five model-based techniques. The model-based techniques encompassed the first, second and zero-order model, Hixson-Crowell model and Higuchi dissolution model given in the Equation of 1, 2, 3, 4, and 5 respectively. The patterns of dissolution of the five model dependent methods can be represented by the following equations and results are shown in Table 4.

$$\text{Zero order kinetics: } D_t = D_o + kt \quad (1)$$

$$\text{First order kinetics: } \ln D_t = \ln D_o + kt \quad (2)$$

$$\text{Second order kinetics: } D_t^{-1} = D_o^{-1} + kt \quad (3)$$

$$\text{Hixson-Crowell model: } D_t^{1/3} + D_o^{1/3} = kt \quad (4)$$

$$\text{Higuchi model: } D = K \sqrt{(t-t_0)} \quad (5)$$

Where, D_o is primary quantity of drug, D_t is the drug quantity at t time and K is the constant of the kinetic drug release [15].

3.8. Stability Studies

The optimally designed formulations (F1, F3, F5, and F17) were stored in separate glass tubes, each sealed with aluminum foil, at ambient temperature and under refrigeration (4°C) for 30 days. After one month, all samples were

assessed and compared to the initial data regarding the percentage drug content and entrapment efficiency (Table 5) of diclofenac sodium [14].

4. Result and Discussion

4.1. Preparation of Diclofenac Sodium Niosomes

To establish a noble strategy in drug delivery system, twenty formulations (F1- F20) of diclofenac loaded niosomes, included in Table 1, were synthesized by ether injection method utilizing various proportions of surfactants (Tween 20, Tween 80, Span20, and Span80) with cholesterol and API.

Table 1. Composition of Diclofenac sodium-loaded niosomal formulations prepared by the Ether Injection method.

Formulation Code	Diclofenc Sodium (mg)	Surfactant	Cholesterol (mL)	Drug: Surfactant: Cholesterol	Chloroform (mL)	Total volume (mL)
F1	200	Tween 20 (mL)	200	1 : 1 : 1	20	600
F2	200		300	1 : 1.5 : 1	20	700
F3	200		400	1 : 2 : 1	20	800
F4	200		500	1 : 2.5 : 1	20	900
F5	200		600	1 : 3 : 1	20	1000
F6	200	Tween 80 (mL)	200	1 : 1 : 1	20	600
F7	200		300	1 : 1.5 : 1	20	700
F8	200		400	1 : 2 : 1	20	800
F9	200		500	1 : 2.5 : 1	20	900
F10	200		600	1 : 3 : 1	20	1000
F11	200	Span 20 (mg)	200	1 : 1 : 1	20	600
F12	200		300	1 : 1.5 : 1	20	700
F13	200		400	1 : 2 : 1	20	800
F14	200		500	1 : 2.5 : 1	20	900
F15	200		600	1 : 3 : 1	20	1000
F16	200	Span 60 (mg)	200	1 : 1 : 1	20	600
F17	200		300	1 : 1.5 : 1	20	700
F18	200		400	1 : 2 : 1	20	800
F19	200		500	1 : 2.5 : 1	20	900
F20	200		600	1 : 3 : 1	20	1000

4.2. Preparation of Standard Calibration Curve

After that, all of the formulations were evaluated by different parameters including drug content percentage and the efficiency of drug entrapment, dissolution profiles, kinetics of drug release and stability studies. To evaluate these parameters, a standard calibration curve of diclofenac sodium was prepared (Figure 1).

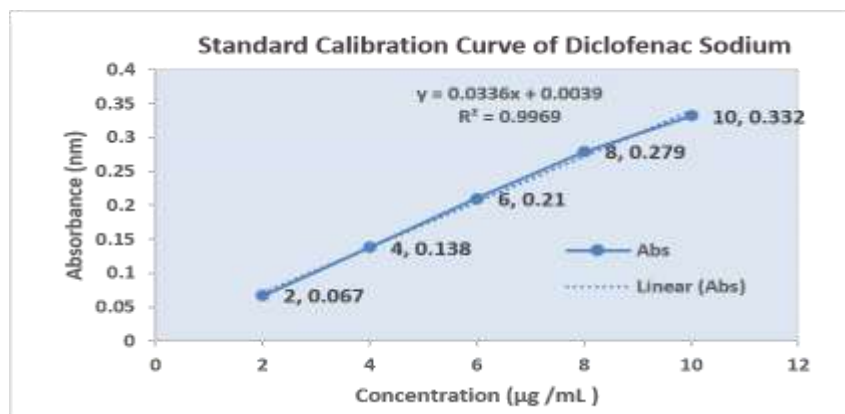


Figure 1. Standard Calibration Curve of Diclofenac Sodium.

4.3. Drug Content and Entrapment Efficiency

The content of drug and the efficiency of entrapment for each of twenty formulation was estimated in Table 2 and presented in Figure 2 and Figure 3 respectively. The study showed that the niosomes prepared by tween20 (F3) at 1:2:1 ratio of API, surfactant and cholesterol was superior with highest drug content (98.2%) and drug entrapment efficiency (95.6%). Furthermore, the F1, F5 and F17 formulations were revealed that the drug content and the efficiency of drug entrapment was determined to lie between approximately 85.2% to 88.2% and 82% to 87% respectively.

Table 2. Characterization of formulations (F1-F20) by drug content and entrapment efficiency in percentage.

Formulation	% Drug Content (Mean ± SD)	% Drug Entrapment Efficiency (Mean ± SD)
F1	88.2 ± 0.1	87 ± 0.13
F2	78.9 ± 0.13	75.4 ± 0.14
F3	98.2 ± 0.02	95.6 ± 0.16
F4	76.8 ± 0.3	75.2 ± 0.1
F5	89 ± 0.2	82 ± 0.11
F6	68.3 ± 0.23	67.9 ± 0.23
F7	50.8 ± 0.02	45.9 ± 0.21
F8	40.9 ± 0.01	40.8 ± 0.1
F9	45.4 ± 0.2	45.5 ± 0.01
F10	49.7 ± 0.12	49.1 ± 0.2
F11	50.8 ± 0.31	50.1 ± 0.22
F12	62.3 ± 0.03	60.7 ± 0.01
F13	49.7 ± 0.02	48.1 ± 0
F14	70.2 ± 0.02	67.5 ± 0.01
F15	69.3 ± 0.01	68.2 ± 0.28
F16	67.4 ± 0.2	67.1 ± 0.020
F17	85.2 ± 0.1	82.5 ± 0.1
F18	62.3 ± 0.02	62.1 ± 0.2
F19	64.8 ± 0.2	63.5 ± 0.1
F20	76.5 ± 0.1	75.9 ± 0.3

Note: Values are expressed as mean ± standard deviation (SD) (n = 2). Statistical significance was evaluated using one-way ANOVA, with p < 0.05 considered statistically significant.

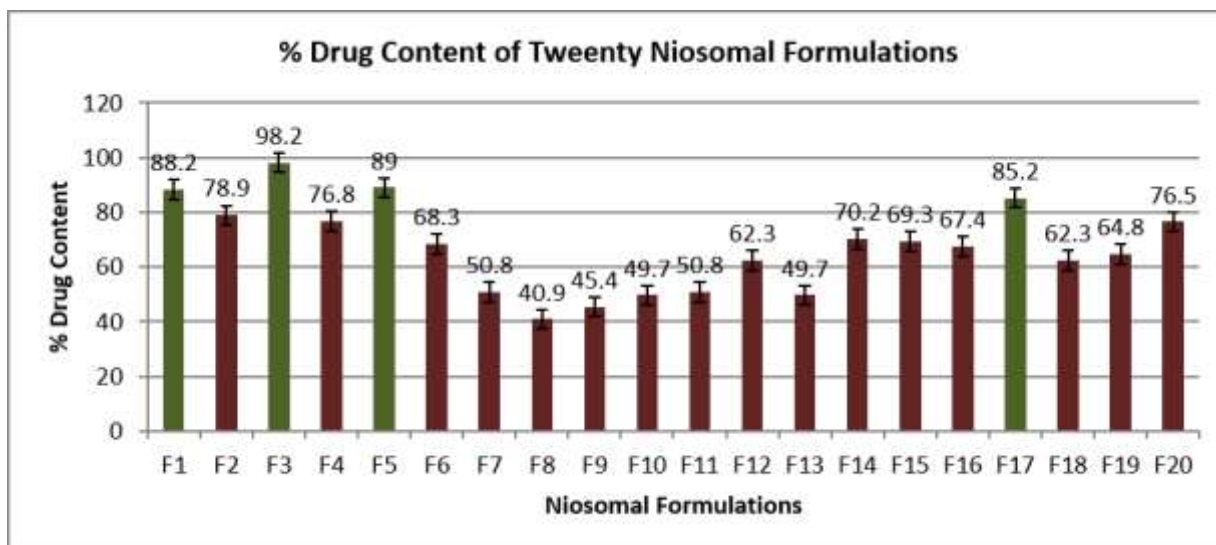


Figure 2. Statistical Comparison of Drug Content for Twenty Formulations (F1-F20).

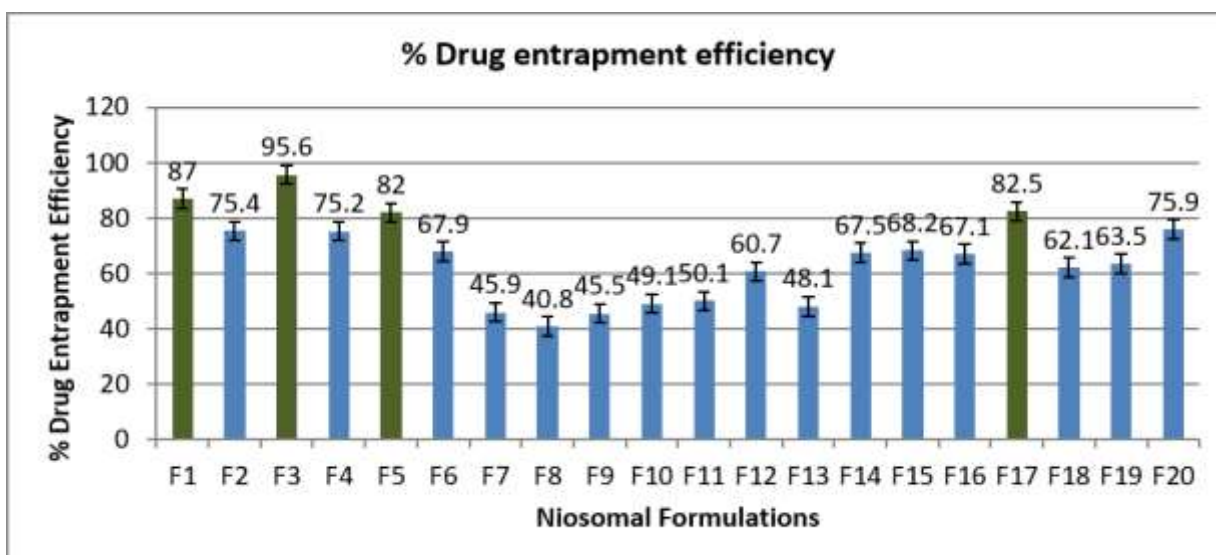


Figure 3. Statistical Comparison of Drug Entrapment Efficiency for Twenty Formulations (F1-F20).

4.4. In Vitro Drug Release Study

In vitro dissolution profiles corresponding to the twenty formulations were carried out for a duration of 3 hours, and the results are shown in Table 3. Tween-based surfactants exhibited superior drug release profiles compared to Span-based surfactants in the ether injection technique. The reason behind such a trend lies in the high hydrophilic-lipophilic balance (HLB) values for the Tween surfactants that facilitate hydration of the membrane, vesicle fluidity, and fast drug diffusion, while the Span surfactants having low HLB values form rigid bilayers and have slow drug diffusion due to high hydrophobicity and low permeability. The formulation of F3 with Tween 20 as the surfactant was found to have the best dissolution profile among the twenty formulations, with 82.26% drug release at 60 minutes and 98.06% drug release at 120 minutes. Furthermore, formulations F1 and F5 were also observed to have reasonable dissolution profiles, with drug release up to 85% at 180 minutes. In contrast, formulation F17 showed comparatively lower release, with 74.22% drug released at 180 minutes. Overall, these results indicate that surfactant type and HLB value significantly influence entrapment and release behavior, where

higher HLB Tween-based systems favor faster release, while lower HLB Span-based systems provide more sustained drug release.

Table 3. Comparative Data for Dissolution Studies of Twenty Niosomal Formulations (F1-F20).

Formulation	30 min	60 min	90 min	120 min	150 min	180 min
F1	64.58	70.47	73.69	78.51	81.46	84.13
F2	37.79	44.49	47.17	50.92	62.17	65.65
F3	79.04	82.26	95.38	98.06	104.49	110.38
F4	38.60	44.22	47.17	50.38	58.15	60.03
F5	48.78	55.74	60.83	67.53	74.76	85.47
F6	14.49	16.37	19.85	21.46	25.21	31.37
F7	7.79	10.21	13.42	15.56	17.44	20.38
F8	1.63	3.51	6.99	9.67	15.03	19.85
F9	3.24	7.26	10.21	12.62	19.58	21.99
F10	4.04	6.19	8.87	10.47	14.49	17.44
F11	2.71	6.72	14.22	19.58	22.26	25.21
F12	4.31	6.19	8.06	10.21	12.88	15.03
F13	9.67	14.76	19.31	28.15	28.96	35.12
F14	8.60	11.54	19.31	23.87	26.01	29.49
F15	16.37	20.38	31.37	35.65	37.53	41.54
F16	3.51	6.72	11.01	14.76	21.99	28.96
F17	13.42	21.19	28.15	46.63	58.42	74.22
F18	4.31	7.26	11.54	16.10	20.92	28.42
F19	4.58	6.19	8.87	10.21	11.81	13.42
F20	1.90	3.78	4.85	8.06	15.56	26.54

Note: In vitro drug release studies were performed in duplicate (n=2) for each formulation. Statistical analysis was conducted using appropriate methods, and differences were considered statistically significant at $p < 0.05$.

4.5. Model Dependent Methods for Kinetics of Drug Release

Model dependent methods were applied for the optimized formulations (F1, F3, F5 and F17) to depict the drug's release kinetics. The parameters including R^2 and k values from different plots (first, second and zero order model, Higuchi and Hixson-Crowell dissolution model) for optimized formulations were presented in Table 4. According to the kinetics plots, the optimized formulation F1 suited the model of Higuchi, F3 the model of zero-order, F5 the model of first-order, and F17 the model of Hixson-Crowell.

Table 4. Kinetics Release Data for Optimized Formulations (F1, F3, F5 And F17).

Model	Statistics	Optimized Formulations			
		F1	F3	F5	F17
Zero order	R^2	0.9834	0.9987	0.9861	0.9777
	K	0.0143	0.0239	0.0262	0.0459
First order	R^2	0.9731	0.96	0.9963	0.9839
	K	0.0017	0.0023	0.0036	0.0115
Second order	R^2	0.9600	0.9476	0.9907	0.8949
	K	0.0002	0.0002	0.0005	0.0035
Hixson-Crowell	R^2	0.9769	0.9635	0.9947	0.9921
	K	0.0012	0.0017	0.0023	0.006
Higuchi	R^2	0.9966	0.9643	0.9504	0.9317
	K	0.2752	0.4547	0.4895	0.8548

4.6. Stability Studies

To assess stability, the optimally designed formulations (F1, F2, F5, and F17) were stored at room temperature and at 40 °C for 30 days. This storage duration was selected as a preliminary short-term stability study to evaluate the initial physicochemical robustness of the formulations under normal and accelerated conditions. Following this period, the drug content and drug entrapment efficiency in the samples were analyzed and compared with the initial data obtained from freshly prepared samples (Table 5).

Table 5. Comparative stability studies data for fresh samples with 1 month stored optimized samples (F1, F3, F5 and F17) at room temperature and refrigerator (4⁰c).

Formulation	% Drug content			% Drug entrapment efficiency		
	Initial data (Mean)	Data after 30 days (Mean)		Initial data (Mean)	Data after 30 days (Mean)	
		Room temp.	4 ⁰ c temp.		Room temp.	4 ⁰ c temp.
F1	88.2	87.2	88	87	86.1	86.8
F3	98	97	98	95	94	94.5
F5	89	88.5	88	82	81.5	81.9
F17	85.2	84.5	85	82.5	81.3	82

Note: Values are expressed as mean (n = 2).

4.7. Overall Discussion

The study revealed that the percentage of drug content and entrapment efficiency were almost similar to the initial values of the fresh samples obtained one month earlier, indicating minimal degradation or leakage of the drug. The results were not significantly affected by storage temperature, suggesting acceptable short-term stability of the formulations under the tested conditions. However, longer-duration stability studies would be required to fully establish long-term stability and regulatory compliance.

5. Conclusion and Further Studies

5.1. Conclusion

The study demonstrated that niosomes formulated with Tween 20 exhibited higher efficacy in drug delivery compared to those made with Tween 80, Span 20, and Span 60. Additionally, the in-vitro evaluation of diclofenac sodium-loaded niosomes was formulation-dependent and showed potential to enhance the permeability of diclofenac over time, offering a new avenue for a well-controlled drug delivery system. According to the evaluation criteria, formulation F3, which was developed by the ether injection technique, had a drug percentage of 98% and a drug entrapment efficiency of 95%. These results suggest better stability. It is seen from the in-vitro release pattern that formulation F3 releases about 98% of the drug in 2 hours, performing better than other formulations. Also, its drug release mechanism shows a zero-order profile with R² value 0.9987.

5.2. Further Studies

This study presents an effective development and in vitro evaluation of diclofenac sodium niosomes; but for further validation of pharmaceutical properties and clinical application, some other experiments have to be done. The following actions could be recommended:

- 1) Characterization of physicochemical parameters including the size of particles, polydispersity index (PDI), zeta potential, and morphology.
- 2) Stability testing at long-term storage using the conditions recommended by ICH.
- 3) Determination of amount of chloroform residues in order to make sure that all of the solvent was removed.
- 4) In vitro permeation testing and pharmacokinetics evaluation in vivo.
- 5) Anti-inflammatory activity and safety estimation in animal models.
- 6) Estimation of possibilities for the scale up and reproducibility of batch.
- 7) Comparison of optimized formulation with niosomes produced by another method.
- 8) Conducting of clinical trials.

Declarations

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

Competing Interests Statement

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' Contributions

Md. Mahbubol Alam, Fahamida Binta Anower and Md. Fardin Islam took part in literature review, conceptualization, methodology, investigation, analysis, and manuscript writing, editing and reviewed the manuscript. Tasin Ahmed Fahim, Md Minhajul Alam, Mahmuda Hassan, and Mayesha Farzana were analyzed data. Fahamida Binta Anower was 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.

Institutional Review Board Statement

Not applicable.

Ethical Approval

Not applicable for this study.

Informed Consent

Not applicable for this study.

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Declaration of Artificial Intelligence

The authors declare that artificial intelligence was not used in the writing, analysis and preparation of this manuscript.

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