Asian Journal of Basic Science & Research
Volume 8, Issue 3, Pages 74-88, July-September 2026

Development of Diclofenac Sodium-Loaded Niosomes by Modified Ether Injection Method Using Tween 80 and Cholesterol for Drug Content, Entrapment Efficiency, Sustained Release, Analgesic, and Anti-Inflammatory Evaluation

Md. Mahbubol Alam[1,2,*], Md. Asaduzzaman Noor[3], Md. Shahinul Islam[3], Nuhan Ahamed[3], Razia Sultana Rami[3], Antu Mozumder[3] & Fahamida Binta Anower[2,3]

[1]*Department of Pharmacy, Faculty of Science and Engineering, University of Information Technology and Sciences (UITS), Dhaka-1212, Bangladesh.* [2]*Department of Pharmacy, Faculty of Life and Earth Sciences, Jagannath University (JnU), Dhaka-1100, Bangladesh.* [3]*Department of Pharmacy, Faculty of Science, Engineering and Technology, Bangladesh University (BU), Dhaka-1207, Bangladesh.*
Corresponding Author (Md. Mahbubol Alam) Email: mahbubhasan089@gmail.com[*]

DOI: https://doi.org/10.38177/ajbsr.2026.8305

Article Received: 27 May 2026 *Article Accepted: 28 July 2026* *Article Published: 14 August 2026*

ABSTRACT

Background: Diclofenac sodium is one of the most common non-steroidal anti-inflammatory drugs (NSAIDs) prescribed to treat pain and inflammation; however, it exhibits gastrointestinal irritation, short biological half-life, and frequent dosing. Recently, niosomal drug delivery system has been proposed as an innovative carrier to enhance drug stability, prolonged release of drug and improvement of drug efficacy.

Objective: This work focuses on development and evaluation of diclofenac sodium loaded niosomes prepared via Modified Ether Injection method employing the surfactant (Tween 80) and cholesterol. Physicochemical properties, sustained release of the drug, analgesic and anti-inflammatory activities of niosomal formulations are determined in the current study.

Methods: Five different diclofenac sodium niosomes (F1-F5) were prepared by changing concentrations of surfactant at constant diclofenac sodium and cholesterol concentrations. The prepared formulations were tested for drug content, entrapment efficiency, and drug release in vitro by means of UV-Visible spectrophotometry and dissolution studies. Analgesic activity was evaluated through acetic acid-induced writhing test, while anti-inflammatory activity was measured through the carrageenan induced paw edema in the Swiss albino mice.

Results: All formulations showed sustained drug release during 120 minutes. Among those tested formulations, F3 possessed the highest values of drug content (95.2%), entrapment efficiency (87.6%) and cumulative drug release (69.29%). In pharmacological evaluations, F3 revealed the highest analgesic activity (80.00% of writhing inhibition) and the best anti-inflammatory activity (66.10% of paw edema inhibition), almost equal to standard diclofenac sodium formulation. It can be concluded that the optimal value of surfactant to cholesterol ratio was found to have a substantial impact on vesicles' properties and drug efficacy.

Conclusion: Diclofenac sodium loaded niosomes developed via the Modified Ether Injection method demonstrate favorable physicochemical properties, sustained drug release, and considerable analgesic and anti-inflammatory activities. The optimal formulation (F3) showed the best performance among the rest, proving the niosomal encapsulation to be a promising sustained release drug delivery system for improvement of therapeutic efficacy of diclofenac sodium.

Keywords: Analgesic; Anti-inflammatory; Cholesterol; Diclofenac Sodium; Drug content; Drug Release; Entrapment Efficiency; Ether Injection Method; NSAIDs; Niosomes; Sustained Release; Tween 80; Vesicular Drug Delivery.

1.0. Introduction

The novel drug delivery systems (NDDS) have greatly advanced the pharmaceutical science field, where the efficiency of traditional drugs has been improved through the optimization of bioavailability, controlled drug release, reduced frequency of dosing, and patient compliance. One of the most promising nanocarriers among other vesicular carriers are niosomes owing to their high stability, biocompatibility, cost-effective nature, and the possibility to incorporate hydrophilic as well as lipophilic drugs. Niosomes are nano-sized bilayer vesicles that consist of non-ionic surfactants and cholesterol that contribute to the increase in the stability of the drug, increase in the systemic circulation time, improvement of bioavailability, sustained drug release, and minimalization of side effects from drugs [1].

Diclofenac sodium is a commonly used NSAID drug that is used for pain relief, inflammation, rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, and musculoskeletal disorders treatment. Although this drug shows great

effectiveness, there are some issues related to the use of its conventional formulation such as irritation of the gastrointestinal tract, rapid elimination from the body, rather short biological half-life, and frequent dosing. Thus, development of the drug delivery system that will be able to ensure the sustained release of the drug during a long period of time becomes an important goal of research [2-3].

A number of studies have focused on the use of niosomal systems for the encapsulation of NSAIDs since such drug delivery systems are associated with better drug loading capacity, stability of the drug molecules, membrane permeability, and sustained release of the drug. The physicochemical behavior of the niosomes and the pharmaceutical effectiveness of the system depend primarily on the nature and amount of non-ionic surfactant used, the cholesterol amount and the technique used for their preparation [4-5]. In general, tween 80 is commonly used as a surfactant because of the ease with which it forms the vesicles, while cholesterol helps in stabilizing the membrane structure, reducing drug leakage and vesicle damage. Among several methods for preparing the niosomes, Modified Ether Injection (MEI) technique is a cost-effective, simple and reliable technique that has the potential of producing vesicles with satisfactory drug loading and sustained release property [6-7].

Despite several reports of successful development of niosomal drug delivery systems, there is paucity of literature regarding the development of niosomes of diclofenac sodium by MEI technique along with optimizing the concentration of Tween 80 and studying the drug content, drug entrapment efficiency, sustained release profile, analgesic and anti-inflammatory effects. Therefore, it is essential to conduct research to optimize the various formulation variables and study their effect on the pharmaceutical and pharmacological parameters.

Therefore, the purpose of this study was to formulate the diclofenac sodium loaded niosomes with the help of MEI technique by using Tween 80 and cholesterol as the major components. The developed formulations were characterized through determination of the drug content and drug entrapment efficiency and then evaluated in vitro for drug release studies. Moreover, the formulations were evaluated for their analgesic and anti-inflammatory activity.

1.1. Study Objectives

In this study, the main goal was to prepare diclofenac sodium-loaded niosomes by utilizing the Modified Ether Injection technique and to analyse their physicochemical characteristics, in vitro release, analgesic effect, and anti-inflammatory action to judge the efficiency of the delivery system as being superior to others. The objectives of the current research work were to:

1) Prepare diclofenac sodium loaded niosomes by using Modified Ether Injection (MEI) technique where non-ionic surfactant used was tween 80 and cholesterol was used as membrane stabilizer.
2) Formulate different niosomes by changing concentration of non-ionic surfactant (Tween 80) at fixed concentration of drug and cholesterol.
3) Study the physiochemical characteristics of the formulated niosomes by estimating the drug content and drug entrapment efficiency.
4) Study the in vitro release profile of the formulated niosomes to check its sustained release behavior.

5) Study the analgesic activity of the formulated niosomes using acetic acid induced writhing test in Swiss albino mice.

6) Study the anti-inflammatory activity of the formulated niosomes using carrageenan induced paw edema test in Swiss albino mice.

7) Determine the optimized niosome formulations which gives highest percentage drug release, drug entrapment efficiency and analgesic and anti-inflammatory activities.

8) Find out the suitability of diclofenac sodium loaded niosomes as sustained release drug delivery system which could enhance the therapeutic effect of the drug without causing side effects of frequent administration.

2.0. Literature Review

Novel Drug Delivery Systems (NDDS) represent an efficient approach to enhance the pharmacological activity of existing drugs by means of improving the bioavailability, increasing the period of drug release, decreasing the dosing frequency, and reducing the toxicity of the drugs. There are different vesicular carriers used for controlled delivery of the drugs; however, niosomes have been extensively studied due to their high stability, inexpensiveness, easy synthesis, and capability of loading hydrophilic and lipophilic drugs. Niosomes are microscopic vesicles containing a bilayer composed of non-ionic surfactants in presence of cholesterol, where the aqueous core contains hydrophilic drugs, whereas the bilayer includes lipophilic drugs. The structural features provide for sustained drug release, increased drug stability, permeability, and targeted delivery. Recent reviews also confirm the high physicochemical stability of niosomes as compared with liposomes, which makes niosomes more promising for application for oral, transdermal, ophthalmic, pulmonary, and targeted delivery of the drugs [8-10].

Diclofenac sodium is one of the most popular non-steroidal anti-inflammatory drugs (NSAIDs) due to the strong analgesic, anti-inflammatory, and antipyretic action. However, conventional forms of diclofenac have some drawbacks, including irritation of the gastrointestinal tract, short elimination half-life, rapid clearance from the body, and necessity for repeated administration of the drug. All these factors reduce the patient adherence and increase the risk of side effects. Therefore, controlled nanocarrier delivery systems are used in order to increase the efficacy of the drug [11].

Within the category of vesicular drug delivery, niosomes have exhibited immense potential to enhance the pharmacokinetic and pharmacodynamic properties of NSAIDs. Cholesterol incorporated into the surfactant bilayer increases the stiffness of membranes and reduces drug leakage, but non-ionic surfactants, such as Tween 80, have been found to assist in creating efficient vesicles and loading drugs. Various researches suggest that optimizing the surfactant-to-cholesterol ratio can play a vital role in determining vesicle size, entrapment efficacy, membrane permeability, and sustained release profile. Recent review papers have also reported that, in general, Tween-based systems tend to exhibit higher drug encapsulation efficiencies and extended-release times than various traditional formulations [9-10,12].

Several methods are being used to prepare niosomes, such as thin film hydration, reverse phase evaporation, microfluidization, bubble technique, proniosome method, and ether injection. However, the Modified Ether

Injection Method (MEIM) is currently receiving increased attention since it yields unilamellar and relatively homogeneous vesicles with enhanced drug encapsulation and release profile. The MEIM is easy, cost-effective, and convenient technique for formulation studies at the laboratory level. Advances in the field of formulation science and technology also imply that formulation methods influence various aspects of vesicles' performances including vesicle morphology and size [8-9,11].

Studies have further developed the application of niosomes in the field of pharmacy beyond simple sustained release systems. Niosomal systems have been effectively employed in various pharmaceutical applications like cancer therapy, vaccine delivery, gene delivery, topical applications, and site-specific drug delivery. Due to their unique capacity to increase drug permeation, decrease toxicity of drugs in systemic circulation, and control drug release, niosomal systems are potential nanocarriers in the field of modern medicine. Besides, there are great prospects of site-specific drug delivery through stimuli responsive and surface modified niosomes [10,13-14].

However, there is scarce literature on the preparation of diclofenac sodium loaded niosomes through the Modified Ether Injection method using Tween 80 along with optimizing the concentration of the surfactant followed by estimation of drug content, entrapment efficiency, sustained drug release, and pharmacological activities of the prepared formulations. Thus, the current research work was focused on the formulation of diclofenac-loaded niosomes employing Tween 80 and cholesterol, followed by the characterization and sustained release study of the formulations.

3.0. Materials and Methods

3.1. Materials

Diclofenac Sodium was selected as the model drug for the formulation of niosomes. Tween 80 was utilized as the non-ionic surfactant, whereas cholesterol served as the membrane stabilization agent. The use of chloroform and diethyl ether was made in the preparation of organic solvents. Acetic acid and carrageenan were chosen for carrying out analgesic and anti-inflammatory investigations. All chemicals and reagents employed were of analytical grades [15]. The apparatus used in this study consisted of UV-Visible Spectrophotometer (UV-T60, UK), pH meter, electronic balance (Shimadzu, Japan), sonicator, and dissolution tester. Glassware such as volumetric flasks, beakers, funnels, and pipettes were used in the preparation of formulations and analysis.

3.2. Experimental Animals

Eight-week-old Swiss albino mice (24-30g) 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). 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 [15].

3.3. Preparation of Diclofenac Loaded Niosomes

Diclofenac-loaded niosomes were prepared by the Modified Ether Injection Method. Required quantities of Diclofenac Sodium, Tween 80, and cholesterol were accurately weighed and dissolved in a mixture of chloroform

and diethyl ether. The organic phase containing the surfactant mixture was slowly injected through a syringe at a flow rate of 1 mL/min while constantly stirring (600-800 rpm) into a preheated aqueous phase maintained at approximately 60°C [16]. As the organic solvent vaporized, vesicular structures were formed spontaneously resulting in the formation of niosomal suspensions. Different formulations were prepared by varying the concentration ratio of Tween 80 while keeping the amount of drug and cholesterol constant. The prepared niosomal dispersions were then sonicated to reduce vesicle size and obtain a uniform distribution [17-18]. Five formulations (F-1 to F-5) were formulated by changing the concentration of surfactant. The concentration of Diclofenac sodium and cholesterol remained the same; however, the concentration of Tween 80 was gradually increased to see the impact on drug release rate. Each formulation was coded as indication in Table 1.

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

Formulation Code	Drug (mg) Diclofenac	Tween 80 mL	Cholesterol (mL)	Drug: Surfactant: Cholesterol ratio	Chloroform (mL)	Total Volume (mL)
F-1	200	200	200	1:1:1	20	600
F-2	200	300	200	1:1.5:1	20	700
F-3	200	400	200	1:2:1	20	800
F-4	200	500	200	1:2.5:1	20	900
F-5	200	600	200	1:3:1	20	1000

3.4. Standard calibration graph for diclofenac sodium

A measured amount of Diclofenac Sodium was accurately weighed using an electronic balance and dissolved in 0.1 N HCl to prepare a stock solution. From this stock solution, a series of working standard solutions with different concentrations (10, 20, 30, 40, 50, 70, 90 and 115 mg/mL) were prepared by appropriate serial dilution using distilled water. All dilutions were performed in 10 mL volumetric flasks to ensure accurate final volume adjustment. The absorbance of each solution was measured at 276 nm using a UV–Visible spectrophotometer [2,15].

3.5. 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) [16-18]. Then each of the five formulations was analyzed for absorbance at 276 nm through UV–visible spectrophotometric technique and the percentage of the drug content was determined [2].

3.6. Entrapment Efficiency

The unentrapped drug was separated from the niosomal suspension by centrifugation method. To determine entrapment efficiency, all five formulations underwent centrifugation at 3000 rpm for 45 minutes to separate the

unentrapped 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 [16-18]. 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].

3.7. In-vitro Drug Release Study

The pattern of in vitro release of a batch of five diclofenac-based niosomal formulations was studied using the technique of membrane-driven diffusion for 2 hours. Membrane containing each niosome formulation was used as the donor phase and contained 20 mg equivalent concentration of diclofenac. 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 [19]. 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 [16]. The filtrates were obtained from each sampled solution by passing them through Whatman filter paper and diluted [17]. Spectrophotometric measurements were carried out at λmax 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.

3.8. Analgesic Activity Study

Analgesic activity was evaluated by the acetic acid-induced writhing method in mice. The analgesic activity of the samples was also studied using acetic acid-induced writhing model in mice [20]. Test samples and vehicle were administered orally 30 mins before intraperitoneal administration of 1% acetic acid but Diclofenac-Na was administered intraperitoneal 15 mins, the mice were observed for specific contraction of body referred to as “writhing” for the next 10 mins [21]. The percent of analgesic activity is calculated by using following formula:

$$\text{Analgesic Activity (\%)} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100 \qquad (1)$$

3.9. Anti-inflammatory Activity Study

Anti-inflammatory activity was evaluated by carrageenan-induced paw edema method. Paw edema was induced by the injection of 0.05 ml of a 1.0% carrageenan suspension in the sub plantar region of the left hind paw. An equivalent volume of saline solution was injected in the same region of the right hind paw. The paw volume was determined before any treatment and measured at 1, 2, 3, 4, h after carrageenan injection with a slide calipers. The extracts (500 mg/kg) were administered 120 min before carrageenan injection. diclofenac (100 mg/kg) was administered as positive control for anti-inflammatory activity 60 min before challenging [21].

$$\text{Anti-inflammatory Activity (\%)} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100 \qquad (2)$$

4.0. Results and Discussion

4.1. Standard Calibration Curve of Diclofenac Sodium

Standard calibration curve of diclofenac sodium was constructed and the obtained calibration curve had the regression equation of y = 0.0205x – 0.0782 and correlation coefficient (R^2 = 0.9986). This confirms the adherence of the Beer–Lambert law within the chosen concentration range. The curve, shown in Figure 1, was utilized for additional drug assessment because it demonstrated strong linearity.

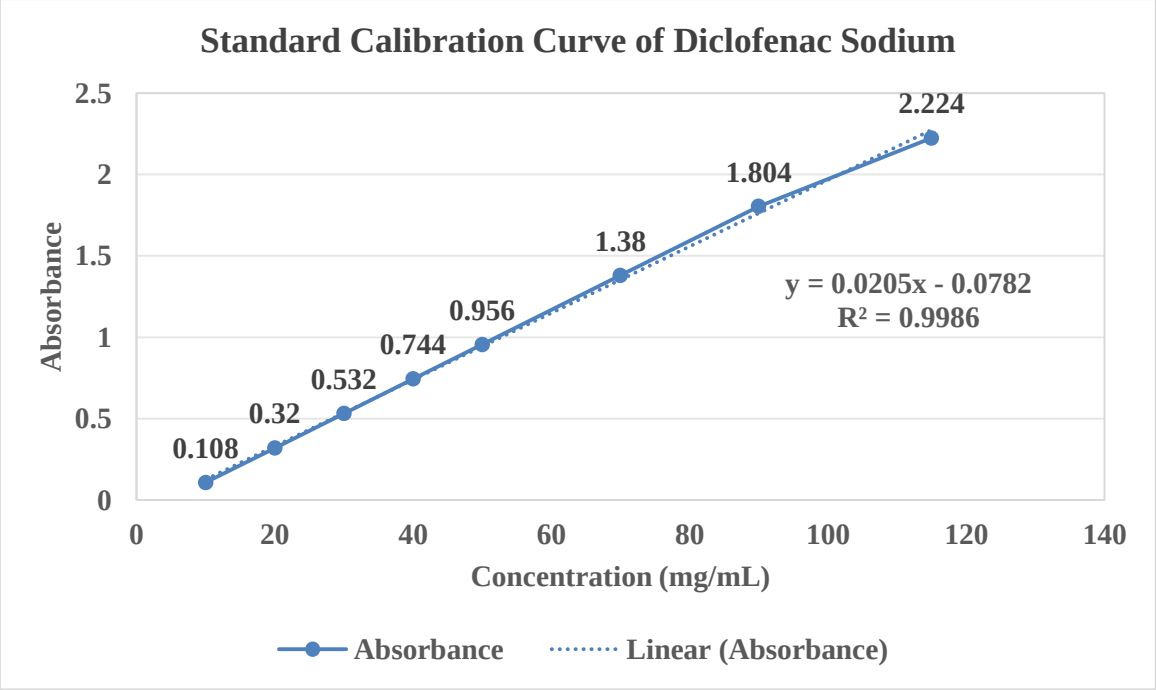

Figure 1. Standard Calibration Curve of Diclofenac Sodium.

4.2. Drug Content and Entrapment Efficiency

Table 2 and Figure 2 summarize the results of the drug content and entrapment efficiency for the five niosomal formulations. Drug content ranged between 79.0 ± 0.22% and 95.2 ± 0.12%, while the entrapment efficiency was found to be in the range of 72.2 ± 0.12% to 87.6 ± 0.17%. Formulation F3 had the highest drug content (95.2 ± 0.12%) and entrapment efficiency (87.6 ± 0.17%), which implies efficient loading of diclofenac sodium into the niosomes. On the other hand, F4 and F5 had relatively low entrapment efficiencies, implying that higher concentration of surfactants can negatively affect the stability of vesicles and lead to the drug leakage. Therefore, these results confirm that surfactant-to-cholesterol ratio is crucial to achieve vesicle stability and high drug content. It can be concluded that the best ratio is 1:2:1 drug: Tween 80: cholesterol (F3).

Table 2. Drug content and entrapment efficiency of diclofenac sodium-loaded niosomal formulations (mean ± SD, n = 3).

Formulation	% Drug content (Mean ± SD)	% Drug entrapment efficiency (Mean ± SD)
F1	83.2 ± 0.11	78 ± 0.15
F2	80.9 ± 0.1	73.2 ± 0.12

F3	95.2 ± 0.12	87.6 ± 0.17
F4	79.8 ± 0.31	72.2 ± 0.12
F5	79 ± 0.22	72.8 ± 0.13

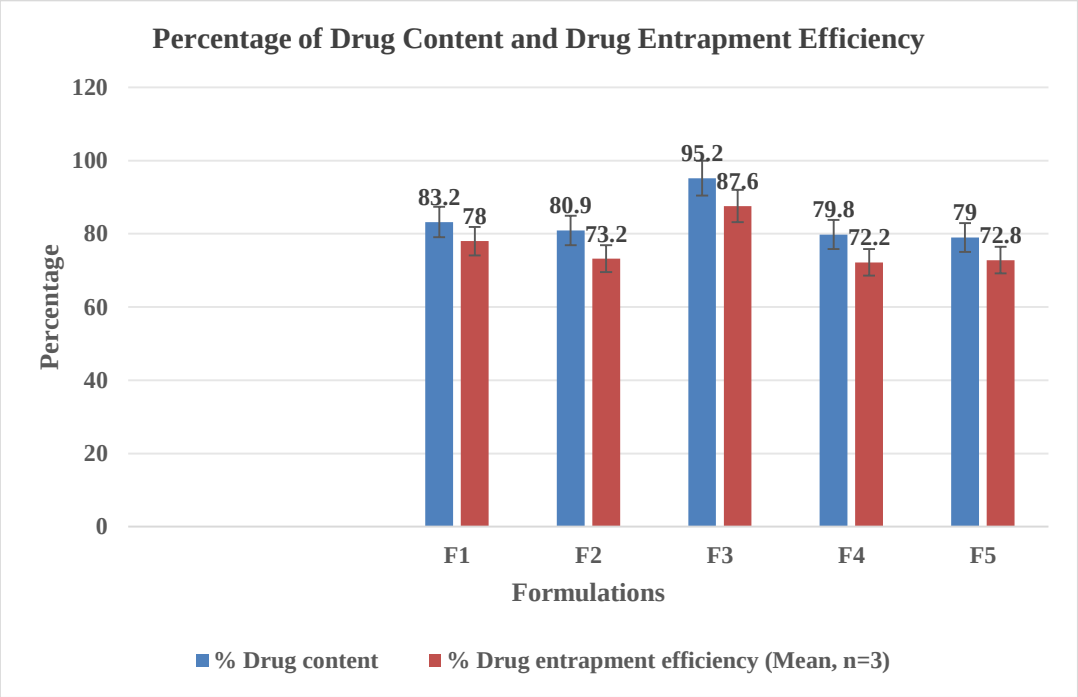

Figure 2. Comparison of drug content and entrapment efficiency of diclofenac sodium-loaded niosomal formulations.

4.3. In Vitro Drug Release Study

The in vitro drug release curves of diclofenac sodium loaded niosomes are given in Table 3 and Figure 3. All formulations released drugs sustainably within 120 minutes, indicating the ability of the niosome system to provide controlled drug delivery. At 120 minutes, the cumulative drug release was:

F3 (69.29%) > F5 (68.74%) > F2 (67.63%) > F4 (58.80%) > F1 (52.17%)

In comparison with other formulations, F3 had maximum cumulative drug release along with a gradual release rate indicating an optimum ratio of drug entrapment and membrane permeability. In case of F1, the lowest release rate could be because of lower membrane fluidity due to lower concentration of the surfactant. On the contrary, low release rate of F4 formulation might be because of high concentration of surfactant leading to improper vesicle formation. Therefore, it is evident from the study that the concentration of Tween 80 significantly affects drug diffusion and sustained release of diclofenac-loaded niosomes.

Table 3. In vitro cumulative drug release profiles of diclofenac sodium-loaded niosomal formulations.

Time (Min)	**F 1**	**F 2**	**F 3**	**F 4**	**F 5**
15	4.14	8.55	5.79	16.84	6.9
30	9.11	14.07	12.42	24.01	10.76
45	16.84	20.7	15.73	27.33	13.52

60	21.8	25.06	21.8	31.19	21.81
75	25.67	33.4	29.53	35.06	34.51
90	30.09	44.44	48.86	46.1	44.45
105	44.44	56.59	61.01	53.28	63.22
120	52.17	67.63	69.29	58.8	68.74

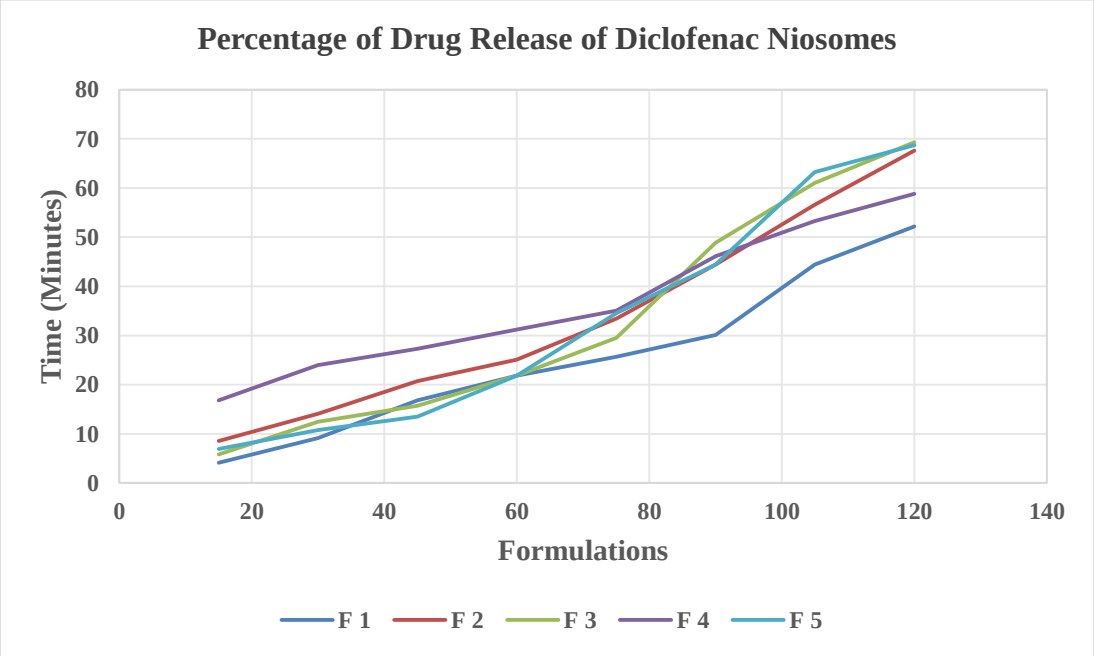

Figure 3. Comparative data for dissolution studies of five Diclofenac niosomal formulations.

4.4. Analgesic Activity

The analgesic efficacy of developed formulations was assessed using writhing test induced by acetic acid and the results, calculated using equation 1, have been provided in Table 4 and Figure 4. Control group had the highest number of writhes (45), whereas the inhibition in standard group was 86.67%. Out of the niosomal formulations, F3 had maximum analgesic effect (80.00%) followed by F1 and F2 (77.78%), F4 (75.56%), and F5 (71.12%). The improved analgesic activity of F3 might be due to high drug loading, high entrapment efficiency, and sustained release of drug ensuring prolonged availability of diclofenac sodium at the target site.

Table 4. Analgesic activity of diclofenac sodium-loaded niosomal formulations evaluated by the acetic acid-induced writhing test.

Group	**Number of Writhing**	**% Writhing Inhibition**
Control	45	0
Standard	06	86.67
F1	10	77.78
F2	10	77.78
F3	09	80.00
F4	11	75.56
F5	13	71.12

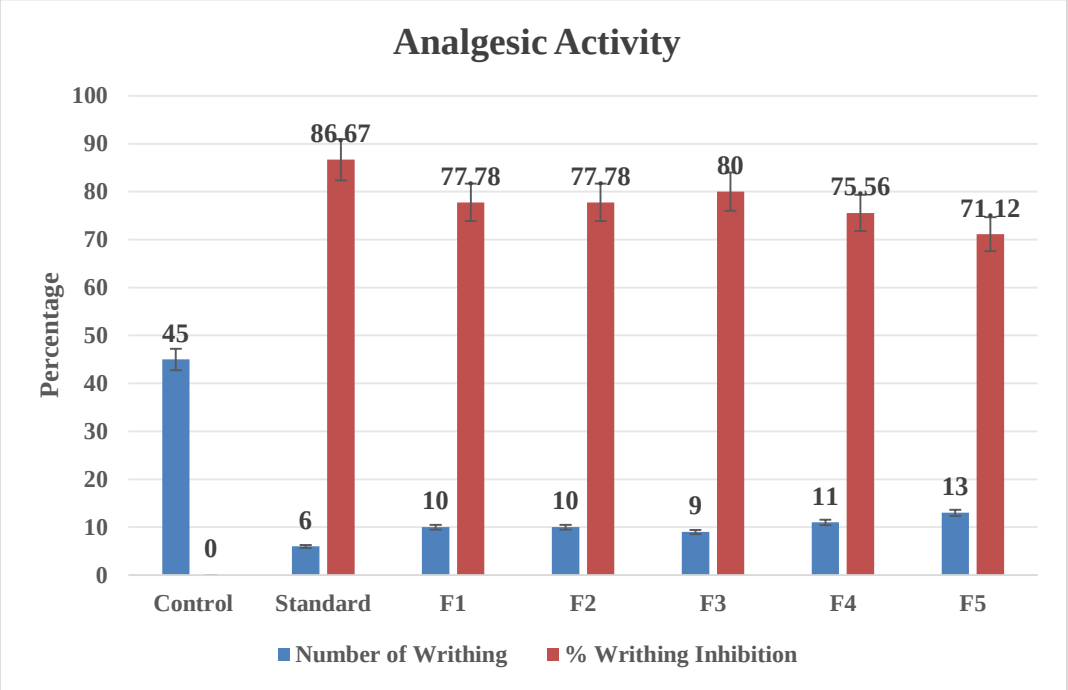

Figure 4. Analgesic activity of diclofenac sodium-loaded niosomal formulations showing the number of writhes and percentage inhibition in the acetic acid-induced writhing model.

4.5. Anti-Inflammatory Activity

Anti-inflammatory activity was determined based on the carrageenan-induced paw edema test, and the results, calculated using equation 2, were provided in Table 5 and Figure 5. The highest inhibition of paw edema was observed in the control group (diclofenac sodium) with 80.48% of inhibition. Of the prepared formulations, F3 showed the strongest anti-inflammatory activity with 66.10% of inhibition, F2 with 44.45%, F1 with 41.17%, F4 with 37.50%, and F5 with 25.92%. The superior anti-inflammatory activity of F3 can be attributed to the optimized vesicular composition, efficient drug encapsulation, and sustained release ability of the formulation, which can contribute to prolonged drug presence at the inflamed tissue. Thus, these findings confirm the impact of the concentration of surfactant on niosomes' pharmacological performance.

Table 5. Anti-inflammatory activity of diclofenac sodium-loaded niosomal formulations evaluated by the carrageenan-induced paw edema model.

Group	0 min	60 min	120 min	180 min	240 min	% decrease of paw volume
Control	5.10	4.95	4.91	4.80	4.70	
Standard	4.95	3.93	3.70	3.50	2.9	80.48
F1	3.93	3.71	3.62	3.45	3.25	41.17
F2	4.10	3.85	3.68	3.51	3.38	44.45
F3	4.23	3.98	3.66	3.34	3.10	66.10
F4	3.86	3.75	3.62	3.53	3.22	37.50
F5	4.05	3.83	3.71	3.65	3.51	25.92

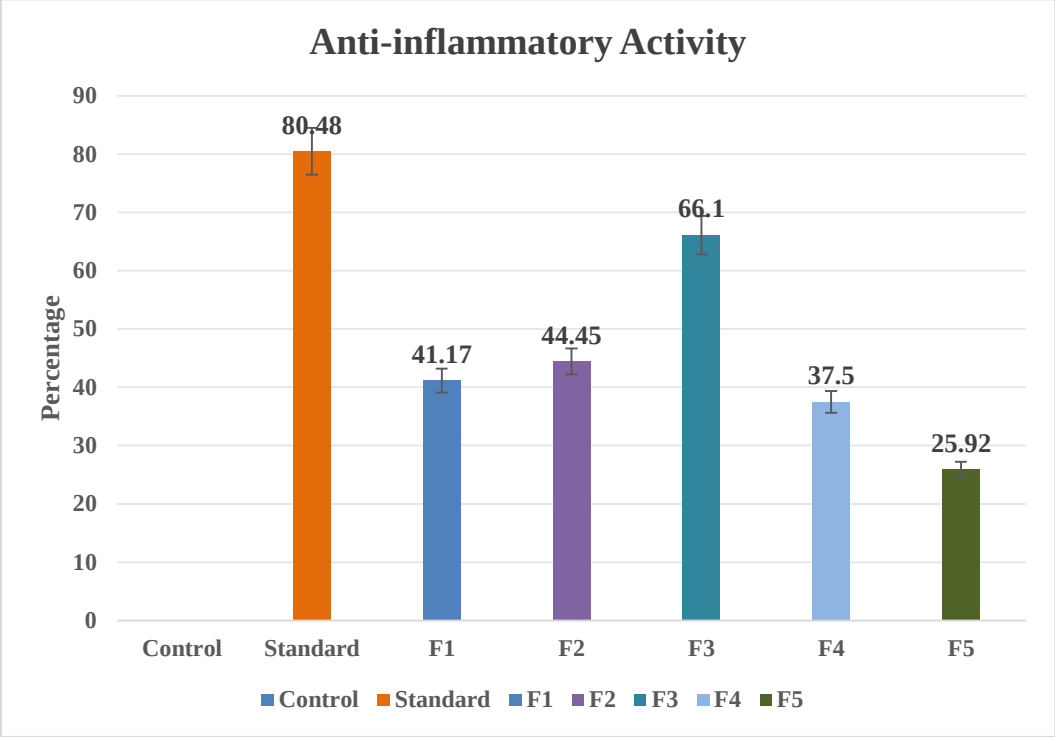

Figure 5. Anti-inflammatory activity of diclofenac sodium-loaded niosomal formulations showing the percentage inhibition of carrageenan-induced paw edema.

4.6. Overall Discussion

In conclusion, it is possible to note that the present study proved the effectiveness of Modified Ether Injection method in obtaining diclofenac sodium-loaded niosomes with sustained release properties. The alteration of the concentration of Tween 80 has significantly influenced the level of drug encapsulation, release, and pharmacological performance. Of all five formulations, F3 had the optimized pharmacological performance; thus, it has shown the highest values of drug content, entrapment efficiency, cumulative release, analgesic and anti-inflammatory activities. All these findings suggest that the ratio of drug:Tween 80: cholesterol 1:2:1 allows achieving the best balance between membrane stability and permeability, thus improving the therapeutic performance. Therefore, the developed niosomes loaded with diclofenac can be considered as promising drug delivery system with sustained release ability.

5.0. Conclusion and Future Perspectives

Diclofenac sodium-loaded niosomes were effectively prepared utilizing the MEI technique where Tween 80 was used as the non-ionic surfactant and cholesterol was added as the stabilizer. The experiments involved the preparation of five different formulations in order to determine how the concentration of the surfactant affected their properties. Diclofenac sodium-loaded niosomes showed good drug content, entrapment efficiency and drug release profiles which prove their efficiency as a vesicular drug delivery system.

The best characteristics among all of the niosomes were obtained for AF-3 (the formulation containing the drug: Tween 80: cholesterol ratio equal to 1:2:1). F3 had the highest content (95.2%) of the drug, entrapment efficiency (87.6%) and cumulative drug release (69.29% during 120 min). In addition, the optimal performance was revealed

by its good analgesic effect (80.00% writhing inhibition) and anti-inflammatory effect (66.10% inhibition of paw edema) which was close to the effect of the standard diclofenac sodium with sustained release of the drug. It means that proper adjustment of the surfactant concentration is of great importance when improving vesicle and drug release properties, as well as the therapeutic effect.

Thus, it can be concluded that the MEI-based diclofenac sodium-loaded niosomes are a prospective sustained-release drug delivery system which may increase the therapeutic effect, decrease the frequency of administration and improve the patients' compliance to treatment.

This encouraging result from the current study serves as a basis for future research to enhance and develop niosomal diclofenac system for clinical use. This future research should include:

1. Physicochemical characterization at an advanced level (particle size, zeta potential, SEM/TEM, and FTIR).
2. Stability assessment using various storage conditions.
3. Release kinetics modeling and drug release mechanism.
4. Pharmacokinetics, biodistribution, and chronic toxicity studies.
5. Scale up process using QbD and DoE approaches.

Declarations

Source of Funding

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 and Md. Asaduzzaman Noor took part in literature review, conceptualization, methodology, investigation, analysis, and manuscript writing, editing and reviewed the manuscript. Md. Shahinul Islam, Nuhan Ahamed and Fahamida Binta Anower analyzed data. Razia Sultana Rami, and Antu Mozumder were 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

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.

Informed Consent

Not applicable for this study.

Acknowledgement

The research facilities and support provided by the Department of Pharmacy, Bangladesh University, Dhaka, Bangladesh, are gratefully acknowledged.

Declaration of Artificial Intelligence

No AI tools were for this study.

References

[1] Faheem, A.M., & Abdelkader, D.H. (2020). Novel drug delivery systems. In Engineering Drug Delivery Systems, Pages 1–16, Elsevier. https://doi.org/10.1016/b978-0-08-102548-2.00001-9.

[2] Yen Thi Hai, T., Giang Ngoc, T., Anh Lan, H., & Giang Thi Thu, V. (2020). Niosomes loaded with diclofenac for transdermal administration: Physico-chemical characterization, ex vivo and in vivo skin permeation studies. Journal of Applied Pharmaceutical Science. https://doi.org/10.7324/japs.2020.101207.

[3] Uchegbu, I.F., & Florence, A.T. (1995). Non-ionic surfactant vesicles (niosomes): Physical and pharmaceutical chemistry. Advances in Colloid and Interface Science, 58(1): 1–55. https://doi.org/10.1016/0001-8686(95)00242-i.

[4] Mucklow, J.C. (2000). Martindale: The complete drug reference. British Journal of Clinical Pharmacology, 49(6): 613–613. https://doi.org/10.1046/j.1365-2125.2000.00206.x.

[5] Moghassemi, S., & Hadjizadeh, A. (2014). Nano-niosomes as nanoscale drug delivery systems: An illustrated review. Journal of Controlled Release, 185: 22–36. https://doi.org/10.1016/j.jconrel.2014.04.015.

[6] Baillie, A.J., Coombs, G.H., Dolan, T.F., & Laurie, J. (1986). Non-ionic surfactant vesicles, niosomes, as a delivery system for the anti-leishmanial drug, sodium stibogluconate. Journal of Pharmacy and Pharmacology, 38(7): 502–505. https://doi.org/10.1111/j.2042-7158.1986.tb04623.x.

[7] Baillie, A.J., Florence, A.T., Hume, L.R., Muirhead, G.T., & Rogerson, A. (1985). The preparation and properties of niosomes—non-ionic surfactant vesicles. Journal of Pharmacy and Pharmacology, 37(12): 863–868. https://doi.org/10.1111/j.2042-7158.1985.tb04990.x.

[8] Bautista-Solano, A.A., Dávila-Ortiz, G., Perea-Flores, M. de J., & Martínez-Ayala, A.L. (2025). A comprehensive review of niosomes: Composition, structure, formation, characterization, and applications in bioactive molecule delivery systems. Molecules, 30(17): 3467. https://doi.org/10.3390/molecules30173467.

[9] Mawazi, S.M., Ge, Y., & Widodo, R.T. (2025). Niosome preparation techniques and structure—an illustrated review. Pharmaceutics, 17(1): 67. https://doi.org/10.3390/pharmaceutics17010067.

[10] Ababei-Bobu, A., Profire, B.-Ștefania, Iacob, A.-T., Chirliu, O.-M., Lupașcu, F.G., & Profire, L. (2025). Niosomes as vesicular carriers: From formulation strategies to stimuli-responsive innovative modulations for targeted drug delivery. Pharmaceutics, 17(11): 1473. https://doi.org/10.3390/pharmaceutics17111473.

[11] Vallala, R., & Daravath, B. (2026). Niosomes in drug delivery: A comprehensive review and therapeutic perspectives. Next Nanotechnology, 9: 100440. https://doi.org/10.1016/j.nxnano.2026.100440.

[12] Padarthi, P.K., V., K., Challa, R.R., Vallamkonda, B., Grandhe, N., Dogiparthi, L.K., & Kaliyaperumal, R. (2025). Non-ionic surfactant vesicles (niosomes): Structure, functions, classification and its advances in enhanced drug delivery. Recent Advances in Drug Delivery and Formulation, 19(2): 87–104. https://doi.org/10.2174/0126673878329822411261034 04.

[13] Vardhan, H., Khan, A., Jain, A., & Singhai, A.K. (2025). Niosomes in drug delivery: Current researches in niosomes. Asian Journal of Research in Pharmaceutical Sciences, Pages 44–56. https://doi.org/10.52711/2231-5659.2025.00007.

[14] Thilagam, N.B.S., Karthik, V.P., Gnanasambandan, R., & Sowmya, C. (2026). A comprehensive review on strategies of topical niosomes and their synergistic effect for enhanced therapeutic outcomes. Pharmaceutical Nanotechnology, 14(1): 29–44. https://doi.org/10.2174/0122117385345369241212071947.

[15] Md, S., Alhakamy, N.A., Aldawsari, H.M., Kotta, S., Ahmad, J., Akhter, S., Shoaib Alam, M., Khan, M.A., Awan, Z., & Sivakumar, P.M. (2020). Improved analgesic and anti-inflammatory effect of diclofenac sodium by topical nanoemulgel: Formulation development—in vitro and in vivo studies. Journal of Chemistry, 2020: 1–10. https://doi.org/10.1155/2020/4071818.

[16] Alam, Md.M., Ullah, A., Kazi, Md.W., Binta Anower, F., Sultana, Most.A., Islam, Md.J., Azad, A.K., & Mia, Md.S. (2025). Design and in-vitro evaluation of ether-injection–derived Span-20 niosomes as nanocarriers for atorvastatin. Asian Journal of Applied Science and Technology, 77(86): 96–104. https://doi.org/10.38177/ajast.2025.9408.

[17] Alam, Md.M., Mia, Md.S., Akhter, F., Binta Anower, F., Islam, O., Sultana, M.A., & Binta Kader, S. (2025). Ether injection-based formulation and in-vitro characterization of atorvastatin-encapsulated niosomes: A smart nanocarrier for enhanced drug delivery. Mediterranean Journal of Basic and Applied Sciences, 09(04): 01–07. https://doi.org/10.46382/mjbas.2025.9401.

[18] Maksuda Akter Maya, Mohaiminul Islam, Antu Mozumder, Diba Rani Saha, Nowrin Rashid Ratri, Fahamida Binta Anower, & Md.Mahbubol Alam (2026). Formulation and optimization of Tween 80 cholesterol-based atorvastatin-loaded niosomes prepared by the ether injection method: In-vitro assessment of drug content, entrapment efficiency, dissolution characteristics, and stability. Asian Journal of Basic Science & Research, 08(02): 181–194. https://doi.org/10.38177/ajbsr.2026.8216.

[19] Akhter, F., Alam, Md.M., Binta Anower, F., Sultana, M.A., Ahmed, Md.T., Khan, S., & Sonchita, A.R. (2025). Comparative in-vitro quality evaluation of six commercially available brands of metformin tablets

marketed in Dhaka, Bangladesh. Mediterranean Journal of Basic and Applied Sciences, 09(04): 43–51. https://doi.org/10.46382/mjbas.2025.9405.

[20] Winter, C.A., Risley, E.A., & Nuss, G.W. (1962). Carrageenin-induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. Experimental Biology and Medicine, 111(3): 544–547. https://doi.org/10.3181/00379727-111-27849.

[21] Fahamida Binta Anower, Lamia Akther Enni, Alamgir Hossain, Sadia Afrin Sorna, Shourav Nandi, Mahbuba Alam, & Md.Mahbubol Alam (2026). In vivo pharmacological evaluation of the therapeutic potential of Ageratum conyzoides methanolic leaf extract: Analgesic, anti-inflammatory, and antidiabetic activities. Asian Journal of Basic Science & Research, 08(02): 155–165. https://doi.org/10.38177/ajbsr.2026.8214.