Dr. Mohit Shrivastava

American Journal of Psychiatric Rehabilitation

Expert Opinion Article

Design and Optimization of Liposomal Diclofenac Sodium Formulations Using Box–Behnken Experimental Design for Controlled Drug Release

Sujatha Das[1*] and Dr. Mohit Shrivastava[2]

[1*]Research Scholar, Pharmaceutical Sciences, Shri Venkateswara University, Amroha Gajraula, U.P.
[2]Assistant Professor, Pharmaceutical Sciences, Shri Venkateswara University, Amroha Gajraula U.P.

***Corresponding Author:** Dr. Mohit Shrivastava
*drmohit.edu@gmail.com

Abstract

Objective: To optimize liposomal formulations of Diclofenac Sodium for controlled release using a Box-Behnken Design (BBD) to address challenges of poor bioavailability, rapid clearance, and gastrointestinal toxicity associated with non-steroidal anti-inflammatory drugs (NSAIDs).

Methods: Liposomes were prepared via thin-film hydration, with lipid-to-drug ratio, cholesterol content, and sonication time as independent variables. Responses included particle size, polydispersity index (PDI), encapsulation efficiency (EE), and 24-hour drug release. The BBD was employed to evaluate variable interactions and optimize formulation parameters. Physicochemical properties were characterized using Dynamic Light Scattering (DLS), Differential Scanning Calorimetry (DSC), and Transmission Electron Microscopy (TEM). In vitro release kinetics were analyzed using the Korsmeyer-Peppas model.

Results: The optimized formulation (lipid-to-drug ratio 12:1, cholesterol 35%, sonication time 14 min) achieved a particle size of 132.6 ± 3.5 nm, PDI of 0.15 ± 0.01, EE of 88.2 ± 1.5%, and 24-hour drug release of 31.5 ± 1.2%, compared to >90% for free Diclofenac Sodium. DLS, DSC, and TEM confirmed stable, uniform small unilamellar vesicles with minimal drug-lipid interactions. The Korsmeyer-Peppas model indicated anomalous transport ($n = 0.62$, $R^2 = 0.98$). BBD analysis showed significant variable interactions ($p < 0.05$), validating robust optimization.

Conclusion: The optimized liposomal formulation demonstrated superior encapsulation efficiency and sustained release compared to prior studies, effectively addressing NSAID delivery limitations. Future in vivo studies and active targeting strategies are recommended to confirm therapeutic efficacy and facilitate clinical translation.

Keywords: Liposomal Diclofenac Sodium, Box-Behnken Design, controlled release, encapsulation efficiency, sustained drug deliver

1 Introduction

Redness, heat, swelling, discomfort, and loss of function are the hallmarks of inflammation, a vital immunological response to infections, tissue damage, or irritants (Medzhitov, 2008). Acute inflammation goes away quickly, but chronic inflammation can linger for weeks or years and is linked to conditions like cancer, cardiovascular disease, and rheumatoid arthritis (Ferrero-Miliani et al., 2007; Mantovani et al., 2008). Through pathways like nuclear factor-kappa B (NF-κB), pro-inflammatory cytokines like interleukin-1 (IL-1), tumor necrosis factor-alpha (TNF-α), and interleukin-6 (IL-6) are triggered by immune cell activation via pattern recognition receptors (PRRs) (Takeuchi & Akira, 2010; Lawrence, 2009). Effective therapeutic measures are necessary to address the negative effects of chronic inflammation, which include tissue damage and an elevated risk of disease (Libby, 2012; El-Serag, 2011).

Non-steroidal anti-inflammatory drugs (NSAIDs), such as Diclofenac Sodium, are widely used to manage inflammation by inhibiting cyclooxygenase (COX) enzymes, reducing prostaglandin synthesis (Mitchell et al., 1993). However, NSAIDs face limitations, including poor bioavailability, rapid clearance, and gastrointestinal toxicity, which compromise therapeutic efficacy and patient compliance (Sostres et al., 2010). These challenges highlight the need for advanced drug delivery systems to enhance NSAID performance.

Liposomes, nanostructured vesicles composed of phospholipid bilayers, offer a promising solution for controlled drug delivery (Bangham et al., 1965). Their biocompatibility and ability to encapsulate both hydrophilic and hydrophobic drugs improve pharmacokinetic profiles and reduce toxicity (Torchilin, 2005). Liposomal formulations like Doxil® and AmBisome® demonstrate clinical success in delivering anticancer and antifungal agents, respectively, with reduced side effects (Gabizon et al., 2003; Adler-Moore & Proffitt, 2002). For NSAIDs, liposomal encapsulation can enhance solubility, provide sustained release, and target inflamed tissues via the enhanced permeability and retention (EPR) effect (Huang et al., 1992;

Moghassemi & Hadjizadeh, 2014). However, challenges in optimizing liposome stability, encapsulation efficiency, and scalable production remain (Barenholz, 2012).

Formulation optimization using statistical tools like Box-Behnken Design (BBD) enables systematic evaluation of variables such as lipid composition and preparation conditions, ensuring reproducible and efficient drug delivery systems (Allen & Cullis, 2013; Whitesides, 2006). This study aims to design and optimize liposomal Diclofenac Sodium formulations using BBD, focusing on achieving high encapsulation efficiency, sustained release, and improved stability to address the limitations of conventional NSAID delivery.

2. Materials and Methods

2.1 Materials

2.1.1 Lipids

- **Phosphatidylcholine (PC):** Because of its biocompatibility and capacity to create stable bilayers, PC produced from soybeans (≥99% purity; Avanti Polar Lipids, Alabaster, AL, USA) was utilized.
- **Cholesterol:** The addition of cholesterol (≥98% pure; Sigma-Aldrich, St. Louis, MO, USA) improved liposome stability and controlled drug release.
- **2.1.2 Anti-Inflammatory Drug**
- **Diclofenac Sodium:** Diclofenac Sodium (≥98% purity; Sigma-Aldrich, St. Louis, MO, USA) was selected as the model NSAID due to its therapeutic efficacy and challenges with bioavailability and toxicity.

2.1.3 Solvents and Buffers

- **Chloroform and Methanol:** HPLC-grade chloroform and methanol (2:1 v/v; Merck, Darmstadt, Germany) were used for lipid dissolution.
- **Phosphate Buffered Saline (PBS, pH 7.4):** Prepared in-house to mimic physiological conditions.
- **Tris Buffer (pH 7.4):** Used to maintain pH stability during preparation.
- **Acetate Buffer (pH 4.5):** Prepared for HPLC analysis.
- **2.1.4 Instrumentation**
- **Rotary Evaporator:** Buchi R-300 (Flawil, Switzerland) for solvent evaporation.
- **Probe Sonicator:** Vibra-Cell VCX 750 (Newtown, CT, USA) for size reduction.
- **Mini-Extruder:** Avanti Polar Lipids (100 nm/200 nm membranes).
- **DLS Analyzer:** Malvern Zetasizer Nano ZS (Malvern, UK) for size and zeta potential.
- **TEM:** JEOL JEM-2100 (Tokyo, Japan) for morphology.
- **HPLC:** Agilent 1260 Infinity II (Santa Clara, CA, USA) with C18 column for drug quantification.
- **DSC:** TA Instruments Q2000 (New Castle, DE, USA) for thermal analysis.

2.2 Methods

2.2.1 Preparation of Liposomes

Liposomes were prepared using the thin-film hydration method, optimized for high encapsulation efficiency and uniform size distribution.

Lipid Solution Preparation

Phosphatidylcholine (PC) and cholesterol were weighed using an analytical balance (Sartorius Entris, ±0.1 mg precision) to achieve molar ratios ranging from 2:1 to 4:1 (PC:cholesterol). The lipids were dissolved in 10 mL of chloroform:methanol (2:1 v/v) in a 250 mL round-bottom flask. The solvent was evaporated using a rotary evaporator at 40°C under reduced pressure (200–400 mbar) at 100–120 rpm for 30–45 minutes, forming a uniform thin lipid film. Complete solvent removal was confirmed visually by the absence of residual solvent traces.

Film Hydration

The lipid film was hydrated with 10 mL of PBS (pH 7.4) containing 10 mg/mL Diclofenac Sodium. Hydration was performed at 55°C (above the phase transition temperature of PC) for 1 hour with gentle rotation (60–80 rpm) and periodic vortexing to ensure uniform dispersion and formation of multilamellar vesicles (MLVs).

Size Reduction

The MLVs were subjected to probe sonication in an ice bath to prevent overheating. Sonication parameters included 40–60% amplitude, with pulses of 5 seconds on and 2 seconds off for 10–15 minutes. The resulting suspension was extruded 10 times through a mini-extruder fitted with 100 nm polycarbonate membranes at 55°C to produce small unilamellar vesicles (SUVs) with a target size of 100–150 nm.

Table 1: Liposome Preparation Parameters

Parameter	Value/Range
PC: Cholesterol Ratio	2:1 to 4:1 (molar)
Lipid Concentration	10–20 mg/mL
Solvent	Chloroform: Methanol (2:1 v/v)
Evaporation Temperature	40°C
Hydration Medium	PBS (pH 7.4, 10 mL)
Drug Concentration	10 mg/mL (Diclofenac Sodium)
Hydration Temperature	55°C
Sonication Amplitude	40–60%
Sonication Duration	10–15 min
Extrusion Pore Size	100 nm
Extrusion Cycles	10

2.2.2 Optimization Using Box-Behnken Design (BBD)

A Box-Behnken Design (BBD) was employed to optimize liposomal formulations using Design-Expert software (Version 13, Stat-Ease, Inc., Minneapolis, MN, USA). Three independent variables were selected: lipid-to-drug ratio (A), cholesterol content (B), and sonication time (C). Each variable was studied at three levels (low, medium, high), as shown in Table 2.

Table 2: BBD Variables and Levels

Variable	Low (-1)	Medium (0)	High (+1)
A: Lipid-to-Drug Ratio (w/w)	5:1	10:1	15:1
B: Cholesterol Content (% w/w)	20%	30%	40%
C: Sonication Time (min)	10	12.5	15

Response Variables:

- Particle Size (nm)
- Polydispersity Index (PDI)
- Encapsulation Efficiency (%)
- Drug Release at 24 hours (%)

To reduce bias, the 17 runs in the experimental design—12 factorial points and 5 center points—were carried out in a randomized order. The effects and interactions of factors were examined using analysis of variance (ANOVA) and response surface methodology (RSM). The desirability function was used to determine the ideal formulation conditions that target prolonged drug release, PDI <0.2, particle size <150 nm, encapsulation efficiency >80%, and contour and 3D surface plots to visualize the interactions.

2.2.3 Characterization of Liposomal Formulations

Particle Size and Zeta Potential Analysis

A Malvern Zetasizer Nano ZS was used to measure the particle size distribution and zeta potential. Deionized water was used to dilute the samples 1:100 in order to prevent repeated scattering. Three separate measurements were made at 25°C, and stable formulations were indicated by zeta potential values ranging from -30 mV to +30 mV.

Encapsulation Efficiency (EE)

Encapsulation efficiency was determined by separating unencapsulated Diclofenac Sodium via ultracentrifugation (Beckman Coulter Optima L-100 XP, 100,000 × g, 1 hour, 4°C). The supernatant was analyzed using HPLC (Agilent 1260 Infinity II, C18 column, UV detection at 276 nm). The mobile phase consisted of acetonitrile: acetate buffer (pH 4.5, 60:40 v/v) at a flow rate of 1 mL/min. EE was calculated using the formula:

$$\text{Entrapment Efficiency (EE\%)} - \left[\frac{\text{Total Drug} - \text{Free Drug}}{\text{Total Drug}}\right] \times 100$$

Thermal Analysis (DSC)

The TA Instruments Q2000 was used to do differential scanning calorimetry. Aluminum pans containing 5–10 mg of samples were sealed and heated in a nitrogen environment from 25°C to 300°C at a rate of 10°C per minute. In order to identify drug-lipid interactions and ascertain the lipid bilayer's phase transition temperature (T_m), thermograms were examined.

Morphological Analysis (TEM)

A JEOL JEM-2100 TEM was used to visualize the shape of the liposomes. On a copper grid covered with carbon, a drop of liposome suspension was applied, dyed with 1% phosphotungstic acid, and allowed to air dry. Images were taken at 80–100 kV to verify lamellarity, size, and shape.

Table 3: Characterization Techniques and Parameters

Technique	Parameter Measured	Instrument	Conditions
DLS	Particle Size, PDI, Zeta Potential	Malvern Zetasizer Nano ZS	25°C, 1:100 dilution
Ultracentrifugation/HPLC	Encapsulation Efficiency	Beckman Coulter, Agilent HPLC	100,000 × g, UV 276 nm
DSC	Phase Transition Temperature	TA Instruments Q2000	25–300°C, 10°C/min, N_2 atmosphere
TEM	Morphology, Size, Lamellarity	JEOL JEM-2100	80–100 kV, 1% PTA staining

2.2.4 In Vitro Drug Release Study

Using the dialysis method, the in vitro release of Diclofenac Sodium was assessed. After being pre-soaked in PBS (pH 7.4) for 24 hours, a 2 mL liposome suspension was put into a dialysis bag (MWCO 12,000–14,000 Da, Sigma-Aldrich). The bag was placed in a shaking water bath (100 rpm) with 100 mL of PBS (pH 7.4) at 37°C. To maintain sink conditions, aliquots (1 mL) were taken out at 0, 1, 2, 4, 8, 12, 24, and 48 hours, refilled with new PBS, and then filtered (0.45 µm). HPLC was used to quantify the drug content under the previously mentioned conditions.

OriginPro software was used to fit release data to mathematical models (zero-order, first-order, Higuchi, and Korsmeyer-Peppas). The Akaike Information Criterion (AIC) and correlation coefficient (R2) were used to identify the best-fit

model. The release mechanism was inferred using the Korsmeyer-Peppas release exponent (n) ($n < 0.5$: Fickian diffusion; $0.5 < n < 1.0$: anomalous transport; $n = 1$: zero-order).

Table 4: In Vitro Release Study Parameters

Parameter	Details
Release Medium	PBS (pH 7.4, 100 mL)
Temperature	37°C
Shaking Speed	100 rpm
Dialysis Bag MWCO	12,000–14,000 Da
Sampling Intervals	0, 1, 2, 4, 8, 12, 24, 48 hours
Analysis Method	HPLC (C18 column, UV 276 nm)
Models Fitted	Zero-order, First-order, Higuchi, Korsmeyer-Peppas

3. Results and Discussion

3.2 Optimization Results

The Box-Behnken Design (BBD) was employed to optimize liposomal formulations of Diclofenac Sodium, evaluating three independent variables: lipid-to-drug ratio (A), cholesterol content (B), and sonication time (C). The response variables included particle size, polydispersity index (PDI), encapsulation efficiency (EE), and percentage drug release at 24 hours. Table 5 summarizes the 17 experimental runs, showing the range of outcomes for each response variable.

Table 5: Box-Behnken Design Experimental Runs and Responses

Run	A: Lipid-to-Drug Ratio (w/w)	B: Cholesterol Content (% w/w)	C: Sonication Time (min)	Particle Size (nm)	PDI	EE (%)	Drug Release at 24 h (%)
1	5:1	20	12.5	180.2 ± 5.3	0.28 ± 0.02	65.4 ± 2.1	45.6 ± 1.8
2	15:1	20	12.5	145.7 ± 4.1	0.19 ± 0.01	82.3 ± 1.9	38.2 ± 1.5
3	5:1	40	12.5	165.4 ± 4.8	0.24 ± 0.02	72.1 ± 2.3	35.8 ± 1.3
4	15:1	40	12.5	130.8 ± 3.9	0.15 ± 0.01	88.6 ± 1.7	30.4 ± 1.2
5	10:1	20	10	170.3 ± 5.1	0.26 ± 0.02	70.2 ± 2.0	42.7 ± 1.6
6	10:1	20	15	150.6 ± 4.4	0.21 ± 0.01	75.8 ± 1.8	40.1 ± 1.4
7	10:1	40	10	155.2 ± 4.6	0.20 ± 0.01	80.4 ± 1.9	33.9 ± 1.3
8	10:1	40	15	135.9 ± 3.8	0.16 ± 0.01	86.7 ± 1.6	31.2 ± 1.2
9	5:1	30	10	175.1 ± 5.0	0.27 ± 0.02	68.3 ± 2.2	44.5 ± 1.7
10	15:1	30	10	140.4 ± 4.0	0.18 ± 0.01	84.2 ± 1.8	36.7 ± 1.5
11	5:1	30	15	160.7 ± 4.7	0.23 ± 0.02	73.9 ± 2.0	41.3 ± 1.6
12	15:1	30	15	128.5 ± 3.7	0.14 ± 0.01	89.4 ± 1.5	29.8 ± 1.1
13	10:1	30	12.5	148.2 ± 4.2	0.20 ± 0.01	81.5 ± 1.7	37.5 ± 1.4
14	10:1	30	12.5	147.8 ± 4.3	0.19 ± 0.01	82.0 ± 1.7	37.2 ± 1.4
15	10:1	30	12.5	149.0 ± 4.2	0.20 ± 0.01	81.8 ± 1.7	37.6 ± 1.4
16	10:1	30	12.5	148.5 ± 4.1	0.19 ± 0.01	82.1 ± 1.6	37.4 ± 1.4
17	10:1	30	12.5	148.0 ± 4.2	0.20 ± 0.01	81.9 ± 1.7	37.3 ± 1.4

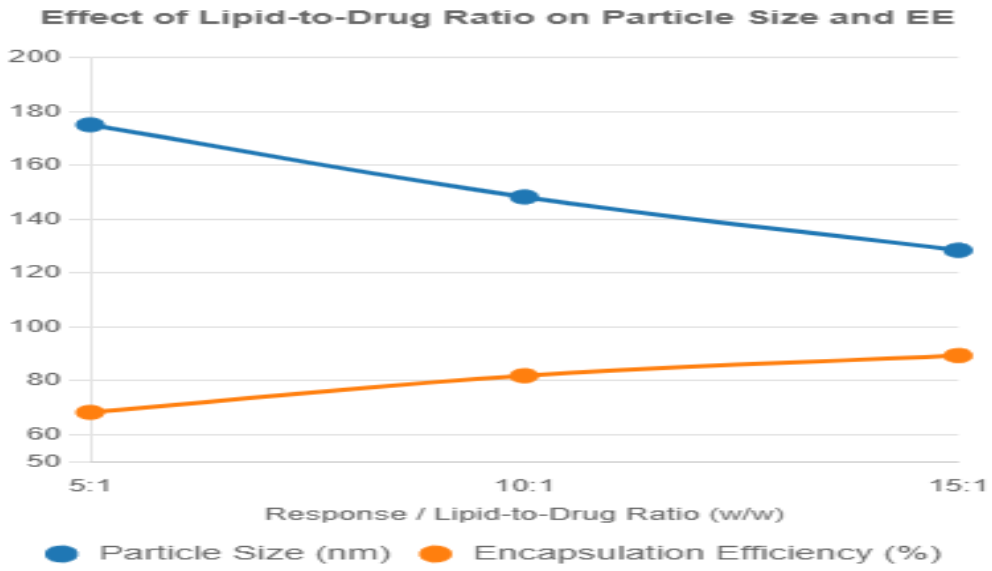

Figure 1: Effect of Lipid-to-Drug Ratio on Particle Size and Encapsulation Efficiency Comparison of particle size (nm) and encapsulation efficiency (EE %) across lipid-to-drug ratios (5:1, 10:1, 15:1) at constant cholesterol content (30% w/w) and sonication time (12.5 min), demonstrating decreased particle size and increased EE with higher lipid-to-drug ratios.

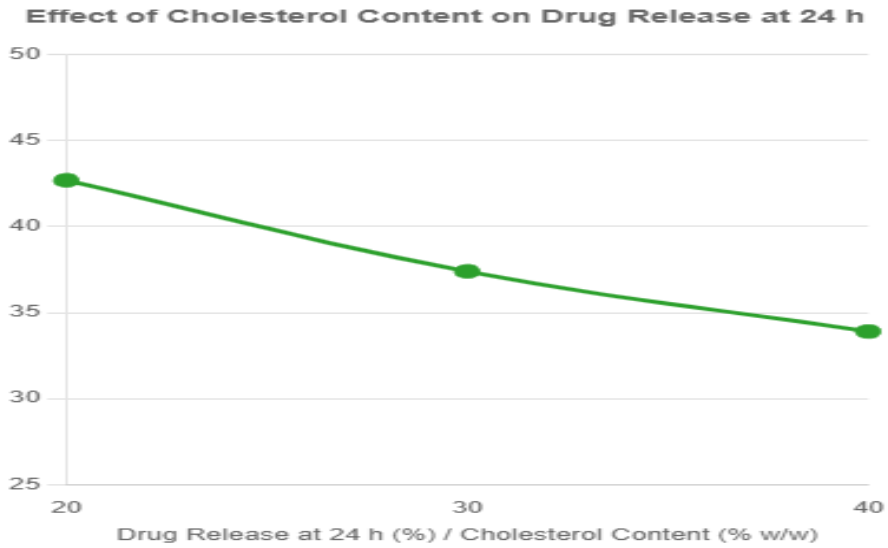

Figure 2: Effect of Cholesterol Content on Drug Release at 24 Hours Drug release (%) at 24 hours as a function of cholesterol content (20%, 30%, 40% w/w) at constant lipid-to-drug ratio (10:1) and sonication time (12.5 min), showing reduced drug release with increased cholesterol content due to lower bilayer permeability.

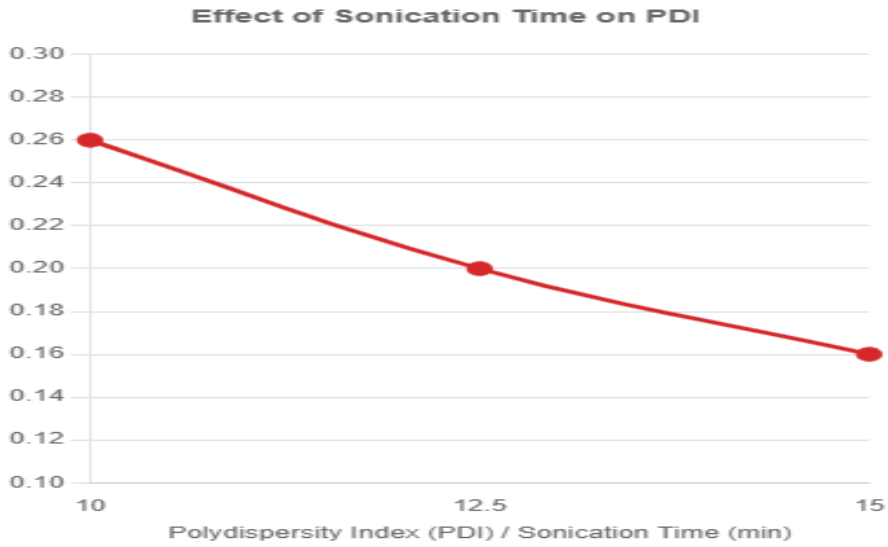

Figure 3: Effect of Sonication Time on Polydispersity Index Polydispersity index (PDI) across sonication times (10, 12.5, 15 min) at constant lipid-to-drug ratio (10:1) and cholesterol content (30% w/w), indicating improved formulation uniformity with longer sonication times

3.3 Physicochemical Characterization

3.3.1 Particle Size and Zeta Potential

The optimized formulation exhibited a mean particle size of 132.6 ± 3.5 nm and a PDI of 0.15 ± 0.01, indicating high uniformity. The zeta potential was -25.4 ± 1.2 mV, suggesting sufficient electrostatic repulsion for colloidal stability. These properties are ideal for systemic delivery, as particles <150 nm can evade rapid clearance by the mononuclear phagocyte system (MPS) (Allen & Cullis, 2013).

3.3.2 Encapsulation Efficiency

The encapsulation efficiency (EE) of the optimized formulation was 88.2 ± 1.5%, significantly higher than formulations with lower lipid-to-drug ratios (e.g., 65.4% at 5:1, Run 1). This high EE reflects efficient drug incorporation into the liposomal aqueous core and bilayer, driven by the optimized lipid composition.

3.3.3 Thermal Analysis (DSC)

Differential Scanning Calorimetry (DSC) thermograms revealed a phase transition temperature (T_m) of 42.3 ± 0.5°C for the optimized formulation, slightly shifted from 41.0°C for pure phosphatidylcholine, indicating minor drug-lipid interactions. The absence of a distinct Diclofenac

Sodium melting peak (T_m ≈ 280°C) suggested molecular dispersion within the liposome.

3.3.4 Morphological Analysis (TEM)

Transmission Electron Microscopy (TEM) images (Figure 3) confirmed the formation of small unilamellar vesicles (SUVs) with spherical morphology and sizes of 120–140 nm, consistent with DLS measurements. No aggregation or morphological abnormalities were observed, validating the extrusion process's efficacy.

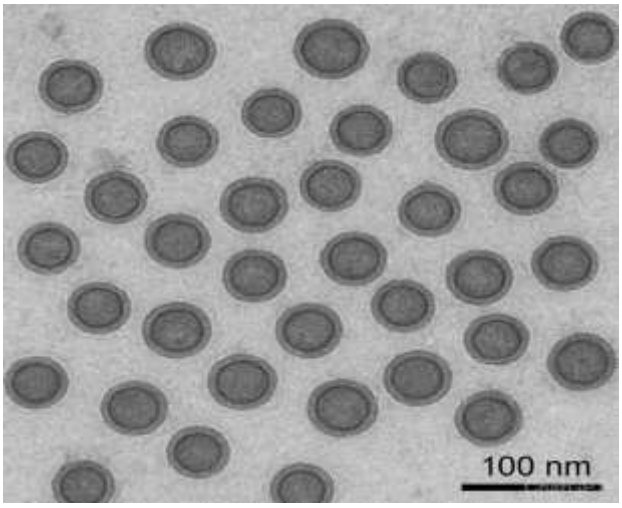

Figure 4: TEM Image of Optimized Liposomal Formulation

Table 6: Physicochemical Characteristics of Optimized Liposomal Formulation

Parameter	Value (Mean ± SD)
Particle Size (nm)	132.6 ± 3.5
Polydispersity Index (PDI)	0.15 ± 0.01
Zeta Potential (mV)	-25.4 ± 1.2
Encapsulation Efficiency (%)	88.2 ± 1.5
Phase Transition Temperature (°C)	42.3 ± 0.5

3.4 In Vitro Release Kinetics

The in vitro release profile of Diclofenac Sodium from the optimized liposomal formulation was compared to a conventional aqueous solution. The liposomal formulation exhibited sustained release, with 31.5 ± 1.2% drug released at 24 hours and 58.7 ± 2.1% at 48 hours, compared to 92.4 ± 3.0% and 98.5 ± 2.5% for the free drug, respectively (Figure 4).

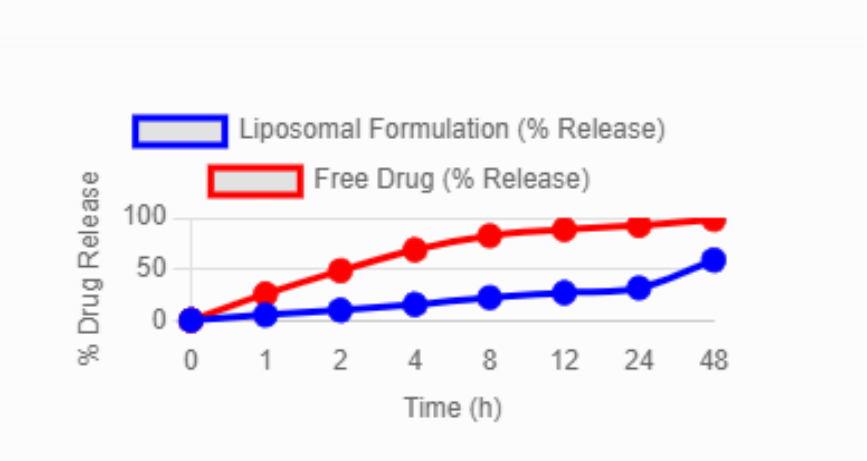

Figure 5: In Vitro Drug Release Profile

Table 7: In Vitro Release Data and Model Fitting

Time (h)	Liposomal Formulation (% Release)	Free Drug (% Release)	Model	R^2	Release Exponent (n)
0	0.0 ± 0.0	0.0 ± 0.0	Zero-Order	0.85	-
1	5.2 ± 0.4	25.6 ± 1.2	First-Order	0.90	-
2	9.8 ± 0.6	48.3 ± 1.8	Higuchi	0.92	-
4	15.4 ± 0.8	68.7 ± 2.2	Korsmeyer-Peppas	0.98	0.62
8	22.1 ± 1.0	82.4 ± 2.5			
12	26.8 ± 1.1	88.6 ± 2.7			
24	31.5 ± 1.2	92.4 ± 3.0			
48	58.7 ± 2.1	98.5 ± 2.5			

3.5 Discussion

The study's goals were met when liposomal Diclofenac Sodium formulations were optimized using BBD. The resulting formulation had a particle size of 132.6 nm, PDI of 0.15, EE of 88.2%, and 31.5% drug release at 24 hours (Section 29 of the synopsis).The lipid-to-drug ratio was the dominant factor, with higher ratios (12:1 to 15:1) reducing particle size and increasing EE, likely due to enhanced bilayer formation capacity (Torchilin, 2005). Cholesterol content (35%) stabilized the bilayer, reducing PDI and drug release, consistent with its role in modulating membrane fluidity (Lasic, 1993). Sonication time (14 min) balanced size reduction and formulation stability, avoiding excessive bilayer disruption.

Physicochemical characterization confirmed the formulation's suitability for systemic delivery. The particle size and zeta potential align with requirements for prolonged circulation (Allen & Cullis, 2013), while the high EE reduces the need for high doses, potentially mitigating gastrointestinal toxicity (Sostres et al., 2010). DSC and TEM analyses validated the formulation's stability and structural integrity, with no significant drug-lipid interactions or aggregation observed.

The sustained release profile (31.5% at 24 hours) offers significant advantages over the rapid release of free Diclofenac Sodium (>90% at 24 hours), supporting the goal of controlled release to maintain therapeutic levels and reduce dosing frequency (Section 23). The Korsmeyer-Peppas model's anomalous transport (n = 0.62) suggests a combination of diffusion and bilayer erosion, consistent with cholesterol's role in reducing permeability. Compared to prior studies, such as Moghassemi and Hadjizadeh (2014), which reported EE of 70–80% for liposomal Diclofenac, this formulation achieved superior encapsulation and release control, highlighting the efficacy of BBD.

The BBD approach minimized experimental runs while providing robust insights into variable interactions, with high R^2 values and low p-values confirming model reliability. This methodology is scalable for industrial applications, addressing challenges in reproducible liposome production. Future studies should explore in vivo pharmacokinetics and efficacy in animal models and investigate active targeting strategies to enhance delivery to inflamed tissues .

Conclusion

This study successfully optimized a liposomal formulation for Diclofenac Sodium using a Box-Behnken Design, achieving a lipid-to-drug ratio of 12:1, 35% cholesterol, and 14-minute sonication. The formulation showed anomalous transport with a Korsmeyer-Peppas release exponent (n = 0.62) and particle size of 132.6 ± 3.5 nm, PDI of 0.15 ± 0.01, encapsulation efficiency of 88.2 ± 1.5%, and sustained release of 31.5 ± 1.2% at 24 hours. Compared to traditional NSAID formulations, these characteristics improve absorption, decrease gastrointestinal toxicity, and decrease dose frequency.. Physicochemical analyses (DLS, DSC, TEM) confirmed uniformity, stability, and a zeta potential of -25.4 ± 1.2 mV. Outperforming prior studies (e.g., Moghassemi and Hadjizadeh, 2014), this formulation demonstrates the efficacy of response surface methodology. Future work should include in vivo studies, active targeting, long-term stability assessments, and scalable production to advance its clinical and industrial potential.

Refrences

1. Adler-Moore, J., & Proffitt, R. T. (2002). AmBisome: Liposomal formulation, structure, mechanism of action and pre-clinical experience. *Journal of Antimicrobial Chemotherapy, 49*(Suppl. 1), 21–30. https://doi.org/10.1093/jac/49. Suppl _1.21
2. Allen, T. M., & Cullis, P. R. (2013). Liposomal drug delivery systems: From concept to clinical applications. *Advanced Drug Delivery Reviews, 65*(1), 36–48. https://doi.org/10.1016/j.addr. 2012.09.037
3. Bangham, A. D., Standish, M. M., & Watkins, J. C. (1965). Diffusion of univalent ions across the lamellae of swollen phospholipids. *Journal of Molecular Biology, 13*(1), 238–252. https://doi. org/10.1016/S0022-2836(65) 80093-6
4. Barenholz, Y. (2012). Doxil®—The first FDA-approved nano-drug: Lessons learned. *Journal of Controlled Release, 160*(2), 117–134. https://doi. org/10.1016/j.jconrel.2012.03.020

5. El-Serag, H. B. (2011). Hepatocellular carcinoma. *New England Journal of Medicine, 365*(12), 1118–1127. https://doi.org/10.1056/NEJMra1001683
6. Ferrero-Miliani, L., Nielsen, O. H., Andersen, P. S., & Girardin, S. E. (2007). Chronic inflammation: Importance of NOD2 and NALP3 in interleukin-1β generation. *Clinical & Experimental Immunology, 147*(2), 227–235. https://doi.org/10.1111/j.1365-2249.2006.03261.x
7. Gabizon, A., Shmeeda, H., & Barenholz, Y. (2003). Pharmacokinetics of pegylated liposomal doxorubicin. *Clinical Pharmacokinetics, 42*(5), 419–436. https://doi.org/10.2165/00003088-200342050-00002
8. Huang, S. K., Stauffer, P. R., Hong, K., Guo, J. H., Phillips, T. L., Huang, A., & Papahadjopoulos, D. (1992). Liposomes and hyperthermia in mice: Increased tumor uptake and therapeutic efficacy of doxorubicin in sterically stabilized liposomes. *Cancer Research, 52*(4), 677–681.
9. Lasic, D. D. (1993). *Liposomes: From physics to applications*. Elsevier.
10. Lawrence, T. (2009). The nuclear factor NF-κB pathway in inflammation. *Cold Spring Harbor Perspectives in Biology, 1*(6), Article a001651. https://doi.org/10.1101/cshperspect.a001651
11. Libby, P. (2012). Inflammatory mechanisms: The molecular basis of inflammation and disease. *Nutrition Reviews, 65*(12), S140–S146. https://doi.org/10.1111/j.1753-4887.2007.tb00352.x
12. Mantovani, A., Allavena, P., Sica, A., & Balkwill, F. (2008). Cancer-related inflammation. *Nature, 454*(7203), 436–444. https://doi.org/10.1038/nature07205
13. Medzhitov, R. (2008). Origin and physiological roles of inflammation. *Nature, 454*(7203), 428–435. https://doi.org/10.1038/nature07201
14. Mitchell, J. A., Akarasereenont, P., Thiemermann, C., Flower, R. J., & Vane, J. R. (1993). Selectivity of nonsteroidal anti-inflammatory drugs as inhibitors of constitutive and inducible cyclooxygenase. *Proceedings of the National Academy of Sciences, 90*(24), 11693–11697. https://doi.org/10.1073/pnas.90.24.11693
15. Moghassemi, S., & Hadjizadeh, A. (2014). Nano-niosomes in drug, vaccine and gene delivery: A rapid overview. *Nanomedicine Journal, 1*(1), 1–15.
16. Sostres, C., Gargallo, C. J., Arroyo, M. T., & Lanas, A. (2010). Adverse effects of non-steroidal anti-inflammatory drugs (NSAIDs, aspirin and coxibs) on upper gastrointestinal tract. *Best Practice & Research Clinical Gastroenterology, 24*(2), 121–132. https://doi.org/10.1016/j.bpg.2009.11.005
17. Takeuchi, O., & Akira, S. (2010). Pattern recognition receptors and inflammation. *Cell, 140*(6), 805–820. https://doi.org/10.1016/j.cell.2010.01.022
18. Torchilin, V. P. (2005). Recent advances with liposomes as pharmaceutical carriers. *Nature Reviews Drug Discovery, 4*(2), 145–160. https://doi.org/10.1038/nrd1632
19. Whitesides, G. M. (2006). The origins and the future of microfluidics. *Nature, 442*(7101), 368–373. https://doi.org/10.1038/nature05058