RESEARCH PAPER

DESIGN AND DEVELOPMENT OF NANOSTRUCTURED LIPID CARRIERS BASED ANTI-INFLAMMATORY GEL FOR TOPICAL DRUG DELIVERY SYSTEM

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ABSTRACT

Objective: The present study aimed to formulate a transdermal delivery system using a optimize Meloxicam-loaded NLCs to enhance its topical delivery and minimize systemic side effects.

Methods: NLCs were prepared using a modified hot high-speed homogenization technique and optimized via a 3^2 factorial design with response surface methodology using Design Expert® software. The solid lipid-to-liquid lipid ratio (X_1) and surfactant concentration (X_2) were selected as independent variables, while particle size (Y_1) and entrapment efficiency (Y_2) served as dependent responses. Based on the 3^2 factorial design, nine different formulations were developed and statistically evaluated. Characterization of the NLCs included analysis of particle size, zeta potential, polydispersity index (PDI), and drug entrapment efficiency. The optimized NLCs were incorporated into gel formulations for transdermal application, serving as a drug reservoir system. The prepared gels were evaluated for physical appearance, pH, viscosity, spreadability, homogeneity, in vitro drug diffusion and invivo study.

Results: NLCs formulation comprising 2% of lipid (70:30) and surfactant (2%) was confirmed as a optimized batch having minimum values for particle size (334.0±1.41nm) with PDI value 0.319±0.11. The zeta-potential was found to be -48.3±1.9, indicating good physical stability. The optimized batch also shows good percentage entrapment efficiency (EE) of 91.57 ± 1.1%. The pharmacokinetic parameters of the optimized Meloxicam transdermal system were significantly improved compared with the pure drug.

Conclusion: The overall results of the present study clearly indicated promising potentials of NLC-based transdermal delivery system to deliver the Meloxicam for the extended period of time in a controlled manner.

Key words: Meloxicam, High speed homogenization, Entrapment efficiency, in-vitro diffusion studies

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INTRODUCTION

Nanostructured Lipid Carriers (NLCs) are progressive drug delivery systems that use a combination of solid and liquid lipids to form a core matrix, providing a more stable, next level option to Solid Lipid Nanoparticles . Nanostructured Lipid Carriers offer numerous advantages, including better drug solubility, greater stability, controlled drug release, and improved permeability for drugs to reach various tissues. These adaptable nanocarriers are used in pharmaceuticals and cosmetics for oral, parenteral, transdermal and ocular drug delivery, curing conditions varying from cancer to infections [1]. Nanostructured Lipid Carriers permitting for higher drug entrapment efficiency and constant release compared to solid lipid nanoparticles . Studies proven Nanostructured Lipid Carriers's efficacy in topical applications, minimalizing side effects by transporting drugs directly to the skin and enhancing the bioavailability. Meloxicam is a optimistic non-steroidal anti-inflammatory drug (NSAID) for acute and chronic pain prevention and to reduce the symptoms of rheumatoid arthritis. Due to its low water solubility, the clinical use of meloxicam is less permitted. Orally administered Meloxicam based pharmaceuticals may have adverse effects on the gastrointestinal tract and even reduce the lifespan of patients with rheumatoid arthritis[2]. Transdermally delivered Meloxicam ensures consistent plasma levels from a single dose, prevents significant gastrointestinal side effects, and enhances local analgesia . The best strategy for enhancing drug penetration into the skin is to choose suitable vehicles with the optimum

characteristics. Nanostructured lipid carriers facilitate the penetration of active substances into the deeper layers of the skin. The lipid carriers advantage from easily incorporating lipophilic substances that are poorly soluble in most solvents . These preparations designate a decrease in pore size, improved entrapment efficiency, and the release of active substances due to an increased lipophilic phase. Thus, nanostructured lipid-based formulations may constitute a viable option for the cutaneous release of Meloxicam [3]. Hence, in the present study, the possibility of Nanostructured Lipid Carriers as a novel carrier system for topical application of Meloxicam, with regard to the prolong of the release and bypass the hepatic first-pass effect was checked. Thus, the possibility of delivering Meloxicam through the skin for local inflammation at low doses is advantageous [4-5]. The role played by the oily phase component of the NLC was also adjudicated by comparing the in vitro release. To increase

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the therapeutic efficacy , Meloxicam loaded Nanostructured Lipid Carriers were incorporated within the gel matrix or drug reservoir which was expected to deliver the drug for the extended period of time. A cellulose acetate-based rate-controlling membrane was incorporated to modulate drug release from the NLC-loaded system and to achieve a zero-order release profile suitable for transdermal delivery. The membrane acts as an additional diffusion barrier, enabling precise control over drug permeation independent of the formulation matrix.

MATERIALS AND METHODS

Meloxicam **was** obtained as a gift sample from Alkem Laboratories Ltd., Mumbai. Stearic acid, Kolliwax GMS, Compritol 888, and Precirol ATO5, Migleol, Oliec Acid, Capric triglyceride, and Castor oil were purchased from Sigma Al drich, Mumbai. Other required chemicals were of analytical grade procured from S.D Fine Chemicals, Mumbai, India. The **in vivo** study was conducted according to CPCSEA guidelines after obtaining approval from the Institutional Animal Ethics Committee (IAEC), Nirmala College of Pharmacy (Approval No. 13/PHD/IAEC/NCPA, dated 29-04-2024).

Screening of lipid: For this purpose, the solubility of Meloxicam was determined in different solid lipids, oils, and surfactants. The solubility in solid lipids, i.e., Stearic acid, Kolliwax GMS, Compritol 888, and Precirol ATO5(200mg each) was taken in each test tube, and were melted using water bath kept at 80°C. The drug was added until the saturation was achieved. The remaining amount of the drug was weighed again, and by that way, the quantity required to dissolve drug was found out. 5mL of each liquid lipid [Migleol, Oliec Acid, capric triglyceride, and Castor oil] was taken in separate test tubes. Meloxicam was added in each lipid till it gets saturated [6].

Optimization of Nanostructured Lipid Carriers : The Nanostructured Lipid Carriers were optimized with respect to the percentage of solid lipids and surfactant. For the purpose of optimization 3^2 full factorial design approach was utilized. The solid lipid : liquid lipid ratio (X1) and surfactant concentration (X2) were selected as independent variables while particle size (Y1) and entrapment efficiency (Y2) were dependent variables. The X1 and X2 were varied at 3 different levels thus based on 2 factors and 3 levels, 9 different formulations were prepared according to factorial design and statistical analysis of obtained values of dependent variables was made by design expert [7]. Actual and coded values for formulations of nanostructured lipid carriers were specified in Table no 1. For a better understanding of the two variables for the optimal Meloxicam NLC performance, the models were presented as three-dimensional (3D) response surface graphs . To carry out the above-mentioned study, for each factor (operating variable), the experimental range was selected on the basis of the results of preliminary experiments and the feasibility of preparing the NLC at the extreme values as shown in Table 2.

Preparation of Nanostructured Lipid Carriers: The Nanostructured Lipid Carriers was prepared by a modified method of hot high pressure homogenization technique. Meloxicam was dispersed in the solid lipid phase and kept in a heating water bath at 80°C . The aqueous phase comprised surfactant was dissolved in water. Both the phases were maintained at a temperature above the melting point of the lipid (80°C). At this temperature, the melted hot lipid phase was then dispersed in the hot surfactant phase obtaining a pre-emulsion under mechanical stirring (Remi Instruments Ltd., Mumbai, India) at 800 rpm at 80°C for 30 min. Further, this pre emulsion was ultra sonicated for 15 min to prevent the crystallization of lipids. The o/w emulsion obtained was subsequently cooled down to room temperature with continuous stirring and the lipid was recrystallized to form Nanostructured lipid carrier (NLC) [8]. The obtained NLC dispersions were lyophilized and used for further characterization studies. The formulation of different ingredients with the composition was listed in the Table 5.

Characterization and evaluation of Meloxicam loaded Nanostructured Lipid Carriers

Particle Size (PS), Zeta Potential (ZP) and Polydispersity Index (PDI): Samples were diluted (1:10) with deionized water to obtain appropriate scattering intensity and to minimize multiple scattering effects. The mean particle size (Z-average), polydispersity index (PDI), and zeta potential were determined by photon correlation spectroscopy (PCS) using a Zetasizer HAS 3000 (Malvern Instruments, Malvern, UK). Measurements were carried out at 25 ± 0.1 °C with a fixed scattering angle of 90°. The refractive index and viscosity of the dispersant (deionized water) were set at 1.330 and 0.8872 cP, respectively. Samples were equilibrated for 120 s prior to analysis. Particle size and PDI were measured using disposable polystyrene cuvettes, while zeta potential was determined using folded capillary cells [9]. All measurements were performed in triplicate, and each measurement consisted of at least 10 runs. Results are expressed as mean ± standard deviation (SD).

Entrapment Efficiency (EE%**):** The entrapment efficiency (EE%) of nanostructured lipid carriers (NLCs) was determined using an ultrafiltration–centrifugation technique to effectively separate the unentrapped (free) drug from the nanoparticulate system. Briefly, an accurately weighed quantity of drug-loaded NLC dispersion (equivalent to 100 mg) was transferred into an ultrafiltration unit (molecular weight cut-off: 10–30 kDa) and centrifuged at 10,000 rpm for 25 min at 25 °C. Under these conditions, the free (unentrapped) drug passed into the filtrate, while the NLCs were retained in the upper compartment. The filtrate was collected, appropriately diluted with methanol, and analyzed for drug content using a UV–Visible spectrophotometer at 362 nm, employing phosphate buffer (pH 7.4) as the blank. Care was taken to ensure that the dilution factor fell within the linearity range of the calibration curve [10].

All experiments were performed in triplicate (n = 3), and the results are expressed as mean ± standard deviation (SD). The entrapment efficiency was calculated as the percentage of drug entrapped within the NLCs relative to the total drug content, according to the following equation:

$$EE\% = \frac{\text{Total drug added} - \text{Free drug added}}{\text{Total drug added}} \times 100$$

Scanning electron microscopy (SEM)

The morphology and surface characteristics of nanostructured lipid carriers (NLCs) were examined using scanning electron microscopy (SEM). A small quantity of lyophilized sample was gently dispersed onto a double-sided adhesive carbon tape affixed to an aluminum stub.

The samples were sputter-coated with a thin layer of gold (approximately 10 nm thickness) under an argon atmosphere using a sputter coating unit to improve surface conductivity and image quality. The coated samples were then placed in the SEM chamber and analyzed under high vacuum conditions [11].

Preparation of rate controlling membrane

Cellulose acetate films were prepared using the solvent evaporation method. A 2% w/w polymer solution was formulated by dissolving cellulose acetate in 25 mL of ethyl acetate. Dibutyl phthalate was incorporated as a plasticizer at a concentration of 40% w/w relative to the polymer content. From this solution, 20 mL was carefully poured into a 9.4 cm diameter Petri plate positioned on a horizontal, flat surface. To control the evaporation rate of the solvent, a funnel was inverted and placed over the Petri plate. The films were allowed to dry at ambient conditions for 24 hours. Once dried, the resulting cellulose acetate films were carefully removed and stored in a desiccator until further use [12].

Preparation of Nanostructured lipid carriers loaded gels

An accurately weighed quantity of 0.5 g of Carbopol 934 was dispersed in 5 mL of distilled water and allowed to swell

overnight at room temperature. The swollen Carbopol dispersion was then stirred using a magnetic stirrer at 800 rpm for 60 minutes to ensure uniform hydration. Subsequently, the preformulated Meloxicam-loaded nanostructured lipid carriers (NLCs) along with preservatives methylparaben and propylparaben were incorporated into the hydrated polymer base. This mixture was further stirred at 600 rpm for 1 hour to ensure homogeneous distribution of all components. The pH of the formulation was adjusted to 7.4 using 0.5% triethanolamine. The resulting gel was then transferred into a measuring cylinder, and the final volume was adjusted to 10 mL with distilled water. The prepared NLC formulations (F1–F9) were incorporated into Carbopol-based gels and labeled as MG1–MG9. To achieve a more sustained drug release profile, additional gel formulations (MG10, MG11, and MG12) were prepared by incorporating 0.5 g each of Methyl Cellulose, Sodium Carboxymethyl Cellulose, and Hydroxypropyl Methylcellulose, respectively, in place of Carbopol [13].

Evaluation of Drug Reservoir Gels

The drug reservoir gels were evaluated for the Drug content, P^H, viscosity extrudability, and Spreadability [14].

Design of membrane moderated transdermal therapeutic system

A circular silicone rubber ring with an internal diameter of 2.5 cm and a wall thickness of

3 mm was used as the drug reservoir holder. This ring was firmly affixed to an imperforate

backing membrane using an adhesive strip (Johnson & Johnson Ltd., Mumbai), effectively

forming a closed compartment for the drug reservoir.A gel formulation containing 15 mg of

Meloxicam was placed into the compartment, serving as the drug reservoir.

A celluloseacetat membrane of known thickness was then affixed to the top of the ring using a

suitable adhesive. This membrane acts as the rate-controlling barrier, allowing the diffusion of

the drug in a controlled manner. Finally, a double-sided adhesive strip was attached along the

rim of the ring, above the cellulose acetate membrane. This facilitates application to the skin

and ensures adhesion of the system during use [15].

In vitro diffusion study

The release of Meloxicam from membrane-moderated therapeutic systems was studied using a Franz diffusion cell. The receptor compartment was filled with 15 mL phosphate buffer (pH 7.4) and maintained at 32 ± 0.5 °C with continuous stirring at 50 rpm. A dialysis membrane was mounted between the donor and receptor compartments. The formulation was placed in the donor compartment over the membrane surface. At predetermined time intervals over 24 hours, 1 mL samples were withdrawn from the receptor compartment and immediately replaced with an equal volume of fresh buffer to maintain sink conditions. The collected samples were analyzed using a UV spectrophotometer at 362 nm to determine the amount of Meloxicam released.[16]

Pharmacokinetic Study: The pharmacokinetic performance of pure Meloxicam and the optimized Meloxicam loaded membrane-moderated transdermal systems was evaluated in healthy New Zealand white rabbits using a randomized two-period, two-treatment crossover study design. Twelve healthy rabbits (10 ± 2 weeks old) weighing 3.0 ± 0.2 kg were included in the study. The animals were randomly allocated into two groups (n = 6) and acclimatized under standard laboratory conditions. Prior to dosing, the rabbits were fasted overnight with unrestricted access to water to minimize variability in drug absorption. All animals were handled carefully throughout the study to reduce stress [17].

The study consisted of two treatment periods separated by a washout interval of two weeks to eliminate any residual drug from the previous treatment. In the first treatment period, one group received pure Meloxicam while the other received the optimized FDT formulation. Following the washout period, the treatments were crossed over between the two groups. Both formulations were administered orally by gastric intubation. The animal dose was calculated from the recommended human therapeutic dose using body weight conversion according to the following equation [18]:

Human dose of Meloxicam = 7.5mg.

$$\text{Animal dose} = \frac{\text{Human dose} \times \text{Animal weight}}{\text{Human weight}}$$

$$= 7.5 \times 3/70 = 0.3214\text{mg} = 0.4\text{mg}$$

Estimation of Meloxicam in Plasma by LC–MS/MS

Plasma concentrations of Meloxicam were determined using a validated UPLC–MS/MS method with Piroxicam as the internal standard (IS). Chromatographic analysis was performed using a Waters UPLC system coupled with an API 2000 triple quadrupole mass spectrometer equipped with a heated nebulizer operating in positive electrospray ionization mode. Separation was achieved on an Agilent Zorbax Eclipse XDB C18 column (4.6 × 150 mm, 5 µm) maintained at 35°C. The mobile phase consisted of 5 mM ammonium formate and acetonitrile (30:70, v/v), delivered at a flow rate of 1.0 mL/min with an injection volume of 15 µL. The total run time was 2 min, and both Meloxicam and Piroxicam were detected within 0.8–1.5 min using multiple reaction monitoring (MRM) transitions of m/z 352.1→115.1 for Meloxicam and m/z 332.2→121.0 for Piroxicam.

Stock solutions of Meloxicam and Piroxicam (1 mg/mL) were prepared in methanol and stored at 2–8°C. Working standard solutions were prepared by serial dilution with 60% acetonitrile in water. Calibration standards ranging from 10 to 2000 ng/mL were prepared by spiking blank rabbit plasma with appropriate volumes of the working standard solutions. Plasma samples (200 µL) were mixed with 20 µL of the internal standard solution and extracted using Phenomenex Strata-X solid-phase extraction cartridges. The cartridges were conditioned with methanol and water, washed with water, and the analytes were eluted with methanol. The eluates were evaporated under a gentle stream of nitrogen at 50°C, and the dried residues were reconstituted with 300 µL of the mobile phase. After vortex mixing, 15 µL of each sample was injected into the UPLC–MS/MS system [19-20] .

Chromatographic data were acquired and processed using Analyst® software version 1.4.2 (Applied Biosystems, Canada). Quantification was based on the peak area ratio of Meloxicam to the internal standard using a weighted ($1/x^2$) linear regression model. The calibration curve showed good linearity over the concentration range of 10–2000 ng/mL with the regression equation $\mathbf{y = 0.00232x - 0.00133}$, which was used for the determination of Meloxicam concentrations in rabbit plasma.

Determination of Pharmacokinetic Parameters

Pharmacokinetic parameters, including maximum plasma concentration (C_max), time to reach maximum plasma concentration (T_{max}), area under the plasma concentration–time curve (AUC), absorption rate constant (K_a), elimination rate constant (K_{el}), and elimination half-life ($t_{1/2}$), were determined using standard non-compartmental analysis. The pharmacokinetic profiles of the optimized formulation were compared with those of the pure drug to assess the improvement in oral absorption and systemic bioavailability [21].

RESULTS AND DISCUSSION

One of the most important factors that affect the design and development of nanostructured lipid carriers is the type of lipids and surfactants to be used . One of the key factors in designing nanostructured lipid carriers is the selection of appropriate lipids, as drug solubility in the lipid matrix directly impacts encapsulation efficiency. During the preformulation phase, solid

and liquid lipids were screened for their ability to solubilize Meloxicam. Meloxicam solubility was highest in Kolliwax GMS (0.34 mg/mg), followed by Stearic Acid, Precirol ATO5, and Compritol 888ATO. Thus, Kolliwax GMS was selected as the solid lipid.Among liquid lipids, Oleic Acid showed the highest solubility (10.2 mg/mL), followed by Castor Oil, Miglyol, and Capric Triglyceride. Therefore, Oleic Acid was chosen as the liquid lipid. Results of solubility study of Meloxicam in different solid lipids and in different liquid lipids are shown in figure 1 &2 respectively.

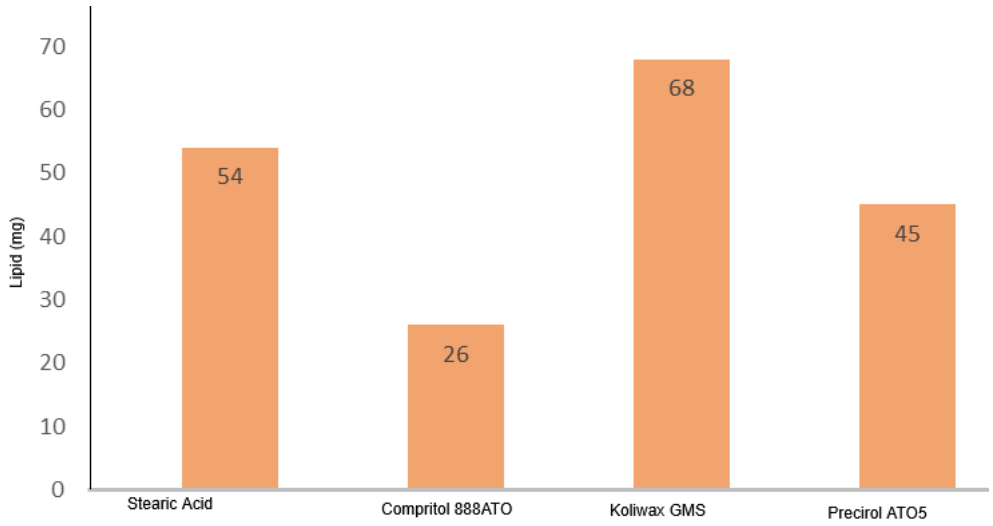

Figure 1: Solubility of Meloxicam in Solid Lipids

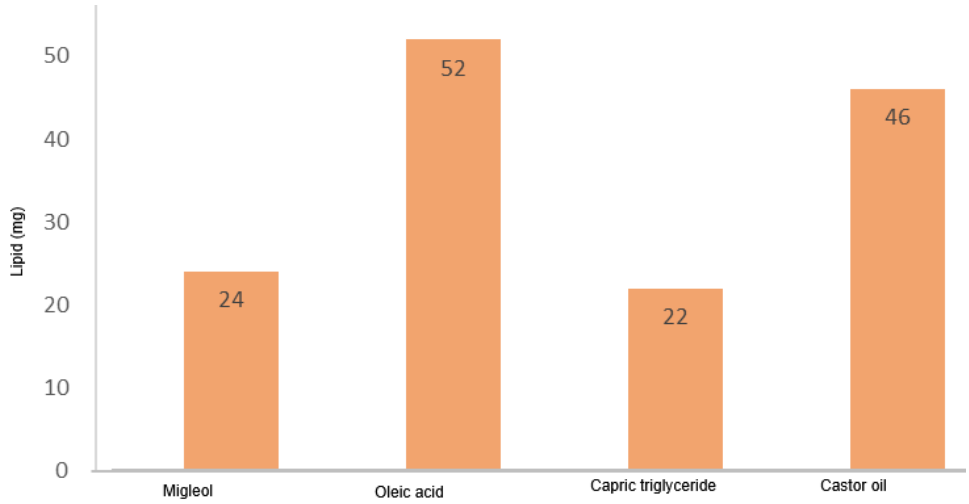

Figure 2: Solubility of Meloxicam in Liquid Lipids

Thus kolliwax GMS and oleic acid were selected as solid and liquid lipid respectively. Nanostructured lipid carriers prepared with Tween 20, Tween 80, and Pluronic F68 were evaluated for particle size. **Tween 20** produced the smallest particles and was selected as the surfactant.

Table 1: Actual and coded values for formulations of nanostructured lipid carriers

Factors	Levels	Levels	Levels
	-1	0	+1
X1 (Solid lipid : Liquid Lipid) (% w/w)	90:10	80:20	70:30
X2 (Concentration of emulsifier) (%)	1	1.5	2

Table 02: Different concentrations of solid and liquid lipids and surfactant used in optimization study of nanostructured lipid carriers

Formulation code	SL:LL (% w/v) (X1)	Surfactant (% w/v) (X2)
F1	-1	-1
F2	0	-1
F3	+1	-1
F4	-1	0
F5	0	0
F6	+1	0
F7	-1	+1
F8	0	+1
F9	+1	+1

Table 3: Summary of results of regression analysis for responses Y1 and Y2

Models	R2 value	Adjusted R2	Predicted R2	Standard deviation (SD)	Percentage of Coefficient of Variation (CV)%
Response (Y1) quadratic	0.9992	0.9987	0.9952	3.991	4.78
Response (Y2) quadratic	0.9997	0.9994	0.9974	18.81	45.04

Actual values: X1 -1 = 1%, 0 = 1.5%, +1 = 2%, X2 -1 = 1%, 0 = 1.5%, +1 = 2%

NLCs – Nanostructured Lipid Carriers, SL: Solid lipid, LL: Liquid lipid.

Table 4: Analysis of Variance of Calculated Model for Responses

Result of the analysis of variance	Entrapment efficiency (%)	Particle size (nm)
Regression	Regression	Regression
Some of square	207.006	4599.9
Degrees of freedom (df)	5	5
Mean squares	41.40	919.98
F-value	1814.84	4074.61
P	0.000	0.000
Inference	Significance	Significance
Lack of fit tests	Lack of fit tests	Lack of fit tests
Some of square	0.087	1.12
Degrees of freedom (df)	3	3
Mean squares	0.0290	1.12
F-value	1.59	3.25
P	0.324	0.142
Inference	Non Significance	Non Significance
Residual	Residual	Residual
Some of square	0.160	1.58
Degrees of freedom (df)	7	7
Mean squares	0.0228	0.23
Standard deviation (SD)	3.991	18.81

Table 5: Composition of Meloxicam loaded Nanostructured Lipid Carriers formulations

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
Meloxicam (mg/ml)	10	10	10	10	10	10	10	10	10
Kolliwax GMS and Oleic acid in 70:30 ratio (%)	1	1.5	2	1	1.5	2	1	1.5	2
Tween 80 (%)	1	1	1	1.5	1.5	1.5	2	2	2

Upon optimizing the nanostructured lipid carrier (NLC) formulation, several parameters warrant further investigation, including the solid lipid-to-liquid lipid ratio, surfactant and drug concentrations, as well as the total lipid content. These variables significantly influence critical physicochemical properties such as particle size, polydispersity index (PDI), zeta potential, and entrapment efficiency.

In the lipid phase, the solid-to-liquid lipid ratio typically varies among 90:10, 80:20, and 70:30 (w/w). Different combinations and concentrations of solid and liquid lipids can alter the degree of order within the lipid matrix, thereby affecting the available space for drug encapsulation. Structural variations and the quantity of liquid lipid used during formulation influence both drug loading capacity and its stability within the lipid matrix.

Table 6 : Results of Entrapment efficiency , Average Particle size, Polydispersity index and Zeta Potential of NLCs

S.No	Batch Code	Entrapment Efficency (%)	Average Particle size (nm±S.D)	Poly dispersity Index (X±SD)	Zeta Potential (mV±SD)
1	F1	81.41 ± 1.2	376.1±2.18	0.374±0.12	-31.8±1.8
2	F2	84.56 ± 1.3	363.4±1.21	0.368±0.14	-34.7±1.2
3	F3	88.67 ± 1.4	351.5±2.16	0.351±0.15	-37.3±1.7
4	F4	77.34 ± 0.9	399.3±2.84	0.362±0.16	-40.8±1.4
5	F5	80.44 ± 0.7	387.2±2.62	0.350±0.11	-44.4±1.5
6	F6	84.34 ± 0.5	375.3±1.21	0.337±0.13	-45.3±1.4
7	F7	85.37 ± 1.4	366.2±2.11	0.349±0.17	-42.9±1.6
8	F8	88.43 ± 1.3	345.5±2.18	0.329±0.13	-47.1±1.7
9	F9	91.57 ± 1.1	334.0±1.41	0.319±0.11	-48.3±1.9

Experimental trials involving different solid-to-liquid lipid ratios (90:10, 80:20, and 70:30) revealed a notable impact on the particle size of the resulting NLCs. Specifically, a higher solid lipid content corresponded to an increase in particle size 388 nm, 340 nm, and 246 nm, respectively. This phenomenon may be attributed to the challenges in melting and dispersion of higher amounts of solid lipid, potentially leading to agglomerate formation during production. Additionally, during the cooling and solidification phase, excess solid lipid may promote fusion or aggregation, resulting in larger particles with broader size distributions.

The solid-to-liquid lipid ratio also influenced the entrapment efficiency (EE) of the NLCs. The trial results indicated that as the proportion of solid lipid increased, the EE decreased: 64.80% for 90:10, 73.17% for 80:20, and 82.60% for 70:30. This can be explained by the enhanced flexibility imparted by the liquid lipid component to the lipid matrix, which allows for improved accommodation and retention of the active drug substance.

Based on these findings, a solid-to-liquid lipid ratio of 70:30 (w/w) was identified as optimal and selected for subsequent formulation studies.

The particle size of the prepared nanostructured lipid carriers (NLCs) was found to be more significantly influenced by the concentration of surfactant compared to the solid lipid to liquid lipid ratio within the studied formulation domain. Particle size varied from 334.0 ± 1.41 nm to 399.3 ± 2.84 nm, as presented in Table 4. A reduction in particle size was observed with increasing surfactant concentration, indicating an inverse relationship between these variables.

Statistical analysis revealed that a **quadratic model** best fit the experimental data. Polynomial equations describing the main effects were generated using the **ANOVA** function of the employed software. The model F-values for particle size and entrapment efficiency were 35.97 and 12.59, respectively, suggesting that the models were statistically significant for both responses.

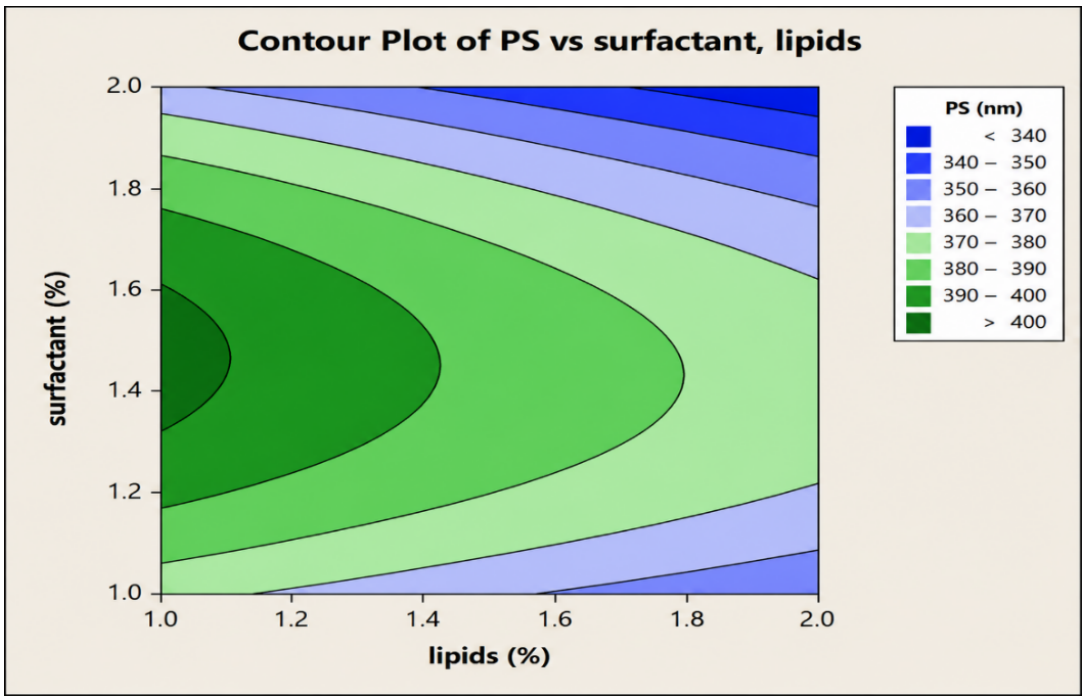

Figure 3: Contour plots of particle sizes of Meloxicam loaded Nanostructured Lipid Carriers

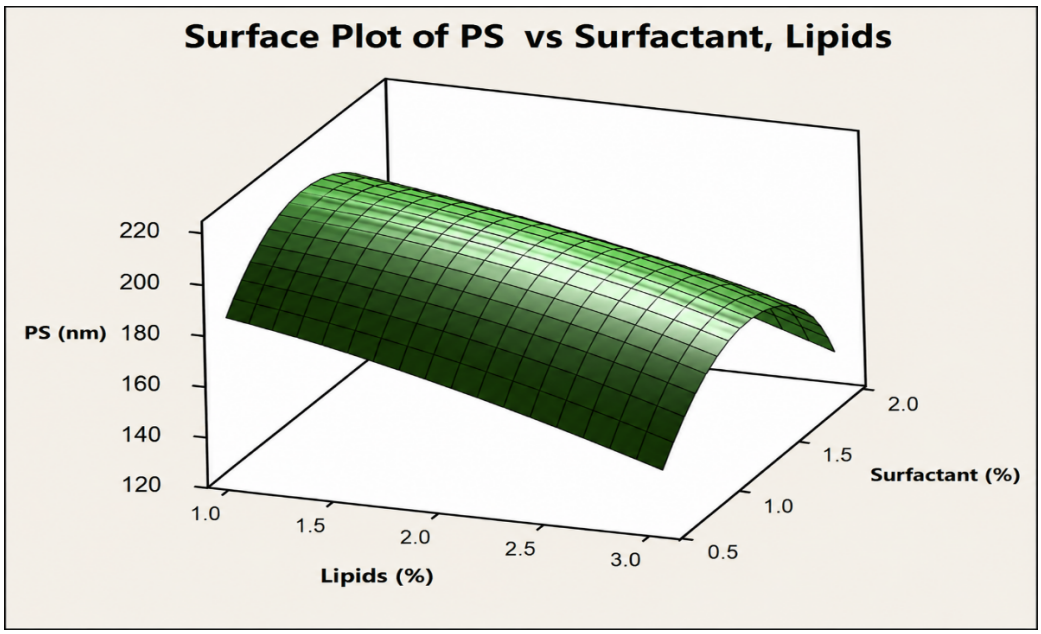

Figure 4: Surface plots of particle sizes of Meloxicam loaded Nanostructured Lipid Carriers

Contour plots and response surface analysis plots were constructed to visualize the effects of independent variables on the responses and are illustrated in Figures 3 and 4, respectively.

The quadratic equation for particle size (Y1) was:

Y1=87.138−13.483(X1)−7.5(X2)+1.067(X12)−31.683(X22)−1.425(X1X2)

In this equation, the negative coefficient for surfactant concentration indicates an inverse relationship with particle size, demonstrating that increasing surfactant concentration decreases the particle size of NLCs. This reduction may be attributed to improved stabilization of lipid droplets and lowering of interfacial tension during formulation. The positive coefficients represent direct relationships between the corresponding variables and particle size.

Although the experimental particle size ranged between 334 and 399 nm, the intercept value in the polynomial equation (87.138) appears much lower because the model was developed using coded values of the independent variables rather than actual formulation values. In response surface methodology, factors such as surfactant concentration (X_1) and lipid ratio (X_2) are transformed into coded levels (−1, 0, +1) prior to regression analysis. Therefore, the constant term does not directly represent the actual particle size in nanometers. Instead, the final predicted response is obtained only after substituting the coded factor levels into the equation. Hence, the regression model should be interpreted as a mathematical representation of factor interactions and trends rather than as direct experimental values.

The quadratic model for entrapment efficiency (Y2) was:

Y2=80.5597+3.4117(X1)+1.7900(X2)+0.2712(X12)+5.9262(X22)−0.2675(X1X2)

This equation suggests a direct correlation between surfactant concentration and entrapment efficiency, with a more pronounced effect of X1(surfactant) than X2 (lipid ratio). The enhancement in entrapment efficiency can be attributed to the surfactant's ability to increase drug partitioning within the lipid matrix, as supported by the data in Table 4. The maximum entrapment efficiency (EE) of 91.57 ± 1.1% was observed in formulation F9, where both the liquid lipid and surfactant concentrations were at their highest levels. Contour plots and surface response analysis plots for EE are shown in Figures 5 and 6, further confirming these findings.

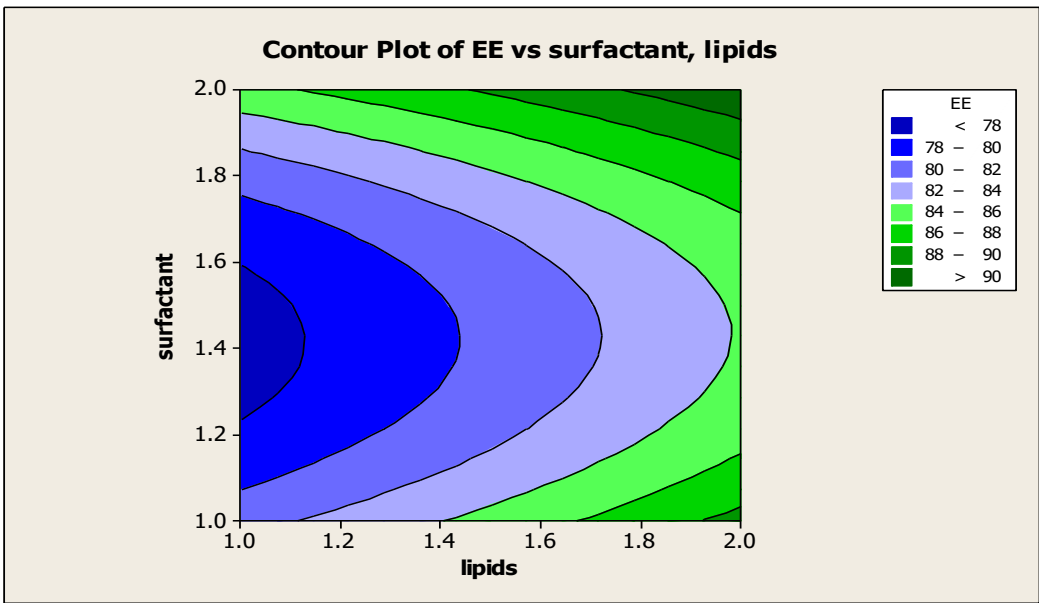

Figure 5: Contour plots of entrapment efficiency of Meloxicam loaded Nanostructured Lipid Carriers

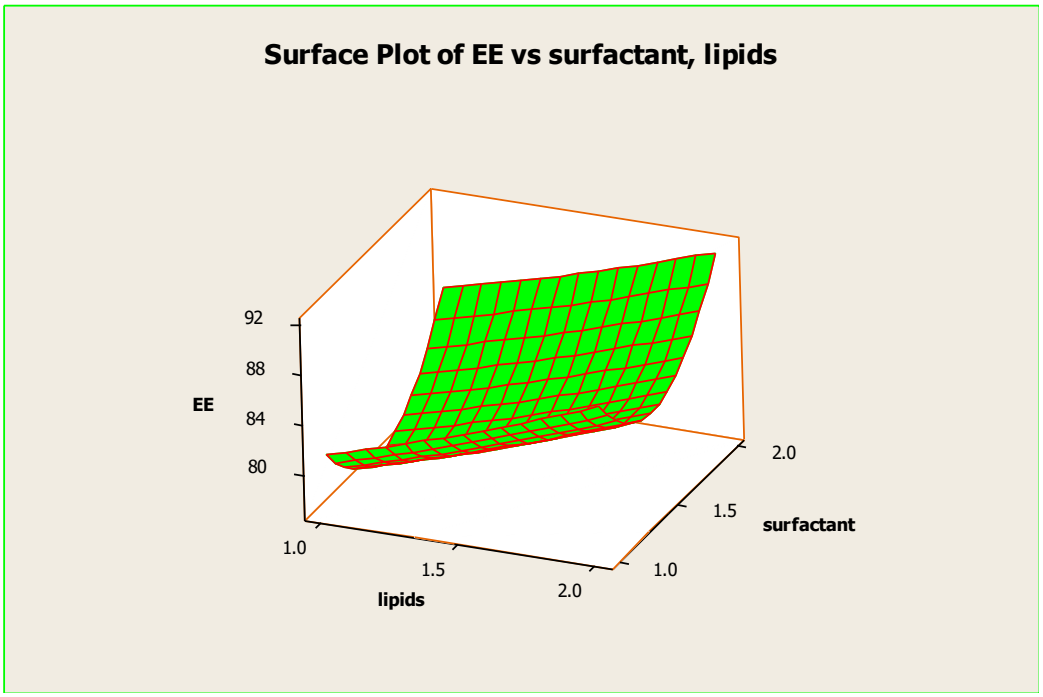

Figure 6: Surface plots of entrapment efficiency of Meloxicam loaded Nanostructured Lipid Carriers

The morphology of the nanostructured lipid carriers (NLCs) was analyzed using a scanning electron microscope (SEM). The SEM analysis revealed that the NLCs were spherical in shape with well-defined boundaries and good structural integrity, as illustrated in Figure 7.

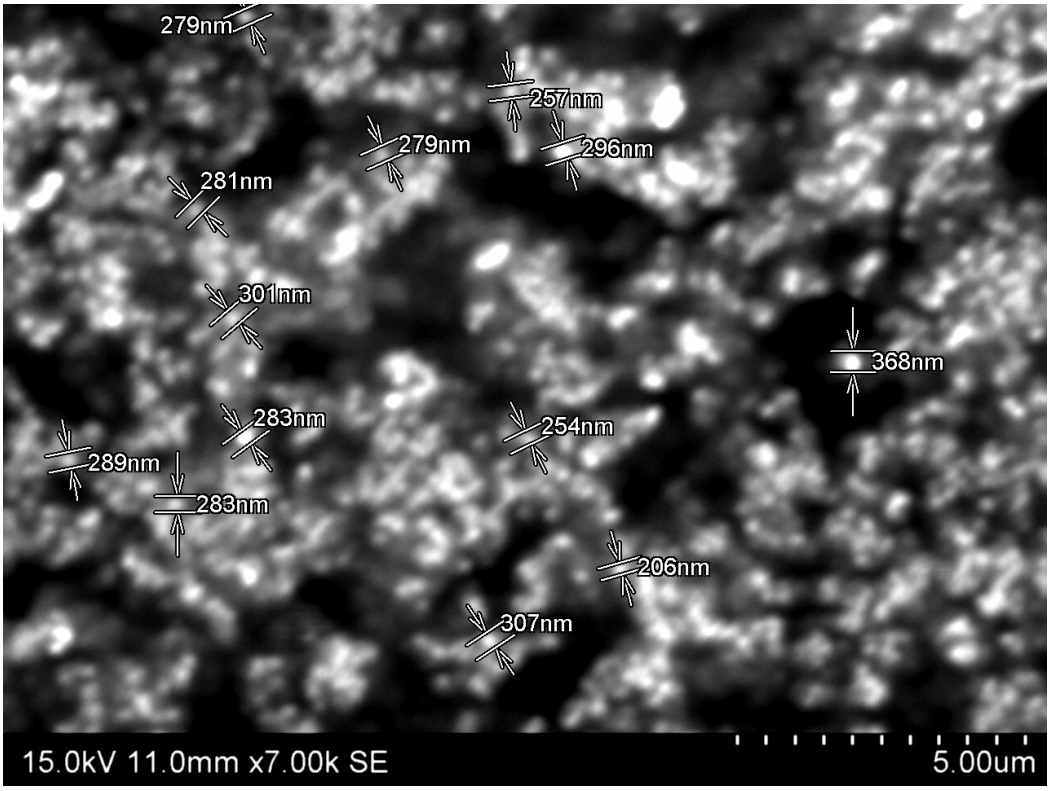

Figure7: SEM Photograph showing of Meloxicam loaded Nanostructured Lipid Carriers

The NLC-based reservoir gels were evaluated for various physicochemical parameters including drug content, pH, viscosity, extrudability, and spreadability. The results of these evaluations are presented in Table 7.

Table 7: Compositions of Meloxicam loaded Nanostructured Lipid Carriers containing Carbopol gels

S.No	Batch Code	Quantity of Meloxicam loaded Nanostructured Lipid Carriers (mg)	Quantity of Carbopol gels (up to)
1	MG1	4094.59	10 gm
2	MG2	3378.66	10 gm
3	MG3	2819.44	10 gm
4	MG4	3694.07	10 gm
5	MG5	3107.90	10 gm
6	MG6	2634.57	10 gm
7	MG7	2928.42	10 gm
8	MG8	2512.72	10 gm
9	MG9	2184.12	10 gm

The observed values indicate that the gels possess desirable characteristics suitable for topical applications. To achieve controlled and sustained drug delivery suitable for transdermal application, a cellulose acetate-based rate-controlling membrane was incorporated into the formulation design. Although nanostructured lipid carriers (NLCs) provide inherent controlled release properties, the addition of a polymeric membrane offers an additional level of modulation over drug diffusion. The membrane functions as a diffusion barrier, regulating the release of drug from the NLC matrix and minimizing burst release effects. This dual-control system (NLC matrix + polymeric membrane) enables a more predictable and near zero-order release profile, which is desirable for transdermal therapeutic systems. Furthermore, the membrane ensures consistent drug permeation across the skin by maintaining a controlled concentration gradient over an extended period.

The membrane functions as a diffusion barrier, regulating the release of drug from the NLC matrix and minimizing burst release effects. This dual-control system (NLC matrix + polymeric membrane) enables a more predictable and near zero-order release profile, which is desirable for transdermal therapeutic systems. Furthermore, the membrane ensures consistent drug permeation across the skin by maintaining a controlled concentration gradient over an extended period. For transdermal application, the optimized NLC dispersion was incorporated into a gel based matrix and further integrated with a cellulose acetate rate-controlling membrane. This dual-controlled system (NLC matrix and polymeric membrane) enabled modulation of drug release, minimizing initial burst release and promoting sustained drug diffusion.In-vitro drug diffusion studies were conducted on the membrane moderated transdermal pathches and the corresponding diffusion kinetics are summarized in Table 8.

Table 8: Composition of gels prepared with different polymers containing Meloxicam loaded Nanostructured Lipid Carriers with Kolliwax GMS and Oleic acid in 70:30 ratio

Ingredients (mg)	MG10	MG11	MG12
Meloxicam loaded Nanostructured Lipid Carriers	2184.12	2184.12	2184.12
Methyl cellulose	500	-	-
Sodium carboxy methyl cellulose	-	500	-
Hydroxy propyl methyl cellulose	-	-	500
Methyl paraben	100	100	100
Propyl paraben	50	50	50
Glycerine (ml)	1	1	1
Distilled water (ml) up to	10	10	10

Table 9: Physico chemical properties of the gels prepared with different polymers containing Meloxicam loaded Nanostructured Lipid Carriers with Kolliwax GMS and Oleic acid in 70:30 ratio

S.No	Batch Code	% Drug Content	pH	Viscosity (Cps)	Spreadability (g.cm/sec)	Extrudability
1	MG1	99.37±0.13	7.21±0.07	3626±12	31.32±1.24	87.13±0.04
2	MG2	99.48±0.17	7.32±0.04	3691±15	31.88±1.56	87.11±0.05
3	MG3	99.16±0.14	7.39±0.06	3713±13	32.24±1.16	87.71±0.07
4	MG4	99.22±0.21	7.43±0.07	3786±17	32.43±1.29	88.91±0.04
5	MG5	99.33±0.14	7.26±0.03	3794±14	31.33±1.47	87.11±0.06
6	MG6	99.36±0.17	7.34±0.05	3792±22	32.38±1.36	88.70±0.02
7	MG7	99.62±0.13	7.41±0.04	3794±11	32.65±2.42	89.34±0.05
8	MG8	99.41±0.15	7.45±0.06	3856±15	33.46±2.07	90.13±0.09
9	MG9	99.56±0.23	7.29±0.01	3793±16	33.64±1.71	88.13±0.07
10	MG10	99.18±0.07	7.38±0.02	3846±13	33.43±2.42	89.41±0.07
11	MG11	99.22±0.16	7.42±0.03	3879±21	33. 56±2.16	91.32±0.05
12	MG12	99.29±0.13	7.47±0.05	3986±13	34.67±2.34	92.43±0.07

Table10: Invitro Drug diffusion Kinetic data of transdermal patches containing the gels with Meloxicam loaded nanostructured lipid carriers

Formulation	Correlation Coefficient Values	Correlation Coefficient Values	Correlation Coefficient Values	Correlation Coefficient Values	Diffusion Rate Constant (mg/hr)	Exponential Coefficient (n)	T 50	T 90
Formulation	**Zero Order**	**First Order**	**Higuchi Model**	**Peppas Model**	**Diffusion Rate Constant (mg/hr)**	**Exponential Coefficient (n)**	**T 50**	**T 90**
MG1	0.9907	0.8212	0.9562	0.9957	0.96	0.7386	7.81	14.06
MG2	0.9896	0.8207	0.9574	0.9972	0.87	0.7509	8.62	15.69
MG3	0.9926	0.8354	0.9507	0.9965	0.76	0.7644	9.86	17.76
MG4	0.9903	0.8451	0.9563	0.9972	0.86	0.7551	8.72	15.69
MG5	0.9919	0.8208	0.9519	0.9962	0.77	0.7556	9.74	17.53
MG6	0.9946	0.7427	0.9457	0.9964	0.69	0.7879	10.86	19.56
MG7	0.9918	0.8359	0.9257	0.9967	0.81	0.7593	9.23	16.66
MG8	0.9951	0.7893	0.9455	0.9969	0.72	0.8019	10.41	18.75
MG9	0.9997	0.7802	0.9265	0.9999	0.65	0.9698	11.54	20.77
MG10	0.9917	0.8294	0.9525	0.9964	0.76	0.7552	9.86	17.76
MG11	0.9952	0.8059	0.9441	0.9965	0.69	0.7977	10.87	19.57
MG12	0.9997	0.7314	0.9268	0.9999	0.62	0.9731	12.09	21.77

Table 11: Plasma Concentrations of Meloxicam following oral administration of Pure Meloxicam and optimized transdermal system

Time (h)	Plasma concentration (ng/ml) (Mean s.d)	Plasma concentration (ng/ml) (Mean s.d)
Time (h)	**Pure drug**	**Meloxicam transdermal system**
0	0	0
0.5	07.52 1.87	21.22 1.37
1	12.361.75	36.451.77
1.5	13.521.53	45.211.55
2	17.621.15	56.231.22
3	19.741.66	67.631.14
4	21.821.36	78.131.66
5	37.631.44	88.191.68
6	44.921.25	99.321.86
8	49.771.42	122.161.73
10	54.911.23	151.431.24
12	47.731.25	144.85 1.35
14	39.631.35	127.601.42
16	31.971.54	112.301.62
18	28.851.33	98.801.19
20	20.931.22	86.901.66
24	13.281.37	67.201.46

The correlation coefficient values (r) suggested that the drug release followed zero-order kinetics. The in vitro drug release data were best fitted to the Korsmeyer–Peppas model, with diffusional exponent (n) values ranging from 0.7386 to 0.8019. These values indicate a non-Fickian (anomalous) transport mechanism, suggesting that drug release is governed by a combination of diffusion through the lipid–polymer matrix and relaxation (or erosion) of the polymeric network. In the present study, the release experiment was performed using a Franz diffusion cell with a dialysis membrane, which does not represent an ideal geometric matrix system. The presence of the membrane introduces an additional diffusional barrier, and the formulation may undergo structural changes such as swelling or matrix reorganization during the release process.

Therefore, the obtained n values should be considered as indicative of overall release behavior rather than absolute mechanistic constants. The release mechanism is more appropriately described as membrane-mediated anomalous transport involving both diffusion and matrix relaxation processes under the experimental conditions employed.

Drug diffusion studies from Carbopol-based gels over a 24-hour period demonstrated that increasing the surfactant concentration in the formulations resulted in a reduction in drug diffusion. This was attributed to the formation of rigid mixed micelles at higher surfactant levels, which hindered the release of the drug. In addition to Carbopol, various gel formulations were developed using alternative polymers such as methylcellulose, sodium carboxymethylcellulose, and hydroxypropyl methylcellulose, with the aim of achieving prolonged drug release. Among these, the hydroxypropyl methylcellulose based gels demonstrated sustained drug diffusion over a 24-hour period.

The pharmacokinetic study showed that the **Meloxicam transdermal system** provided improved and prolonged drug absorption compared with the pure drug. The transdermal formulation achieved a **maximum plasma concentration (C_{max})**

of 151.43 ng/mL at **10 h (Tmax)**, whereas the pure drug reached a $\mathbf{C_{max}}$ **of 54.91 ng/mL** at the same time.

The $\mathbf{AUC_{0-24}}$ of the transdermal system (**2480.12 ng·h/mL**) was approximately **3.2 times higher** than that of the pure drug (**771.56 ng·h/mL**), indicating enhanced bioavailability. The **elimination rate constant (Kel)** was lower (**0.0642 h^{-1}**) for the transdermal formulation than for the pure drug (**0.1098 h^{-1}**), resulting in a longer **elimination half-life ($t_{1/2}$) of 10.80 h** compared with **6.31 h** for the pure drug.

The plasma concentration remained higher throughout the 24-hour study, indicating sustained drug release and prolonged therapeutic effect. These results demonstrate that the optimized Meloxicam transdermal system improves systemic drug exposure and extends the duration of drug action compared with the pure drug. At **24 h**, the transdermal system maintained a plasma concentration of **67.20 ng/mL**, whereas the pure drug decreased to **13.28 ng/mL**, confirming prolonged drug availability.

Conclusion : The optimized nanostructured lipid carrier (NLC) formulation consisted of 2% total lipid (Kolliwax GMS:oleic acid, 70:30) and 2% surfactant (Tween 80). This formulation exhibited a mean particle size of 334.0 ± 1.41 nm with a polydispersity index (PDI) of 0.319 ± 0.11, indicating a relatively narrow size distribution suitable for stable nanosystems. The zeta potential value of −48.3 ± 1.9 mV suggested excellent physical stability, attributable to strong electrostatic repulsion between particles, thereby minimizing aggregation.The formulation also demonstrated a high entrapment efficiency (EE) of 91.57 ± 1.1%, confirming efficient incorporation of Meloxicam within the lipid matrix. The high EE can be attributed to the compatibility of the drug with the lipid components and the imperfect crystalline structure of the NLC system, which facilitates greater drug accommodation.Overall, the developed NLC-based transdermal system demonstrated significant potential for prolonged and controlled delivery of Meloxicam, which may enhance therapeutic efficacy and improve patient compliance by reducing dosing frequency. The optimized Meloxicam transdermal system exhibited higher bioavailability, slower elimination, and prolonged plasma drug levels, demonstrating its potential as an effective sustained-release transdermal delivery system.

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AUTHORS' CONTRIBUTIONS

All the authors contributed equally in making the manuscript ready for submission.

CONFLICTS OF INTEREST

Authors declare no conflicts of interest

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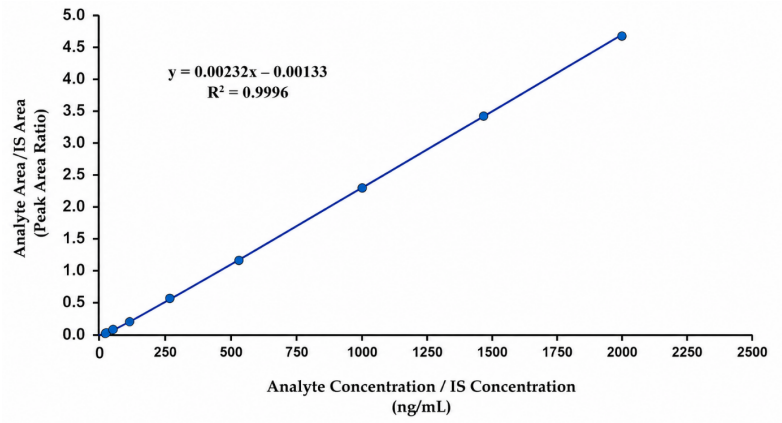

Figure 7: Calibration Curve for Estimation of Meloxicam in Plasma

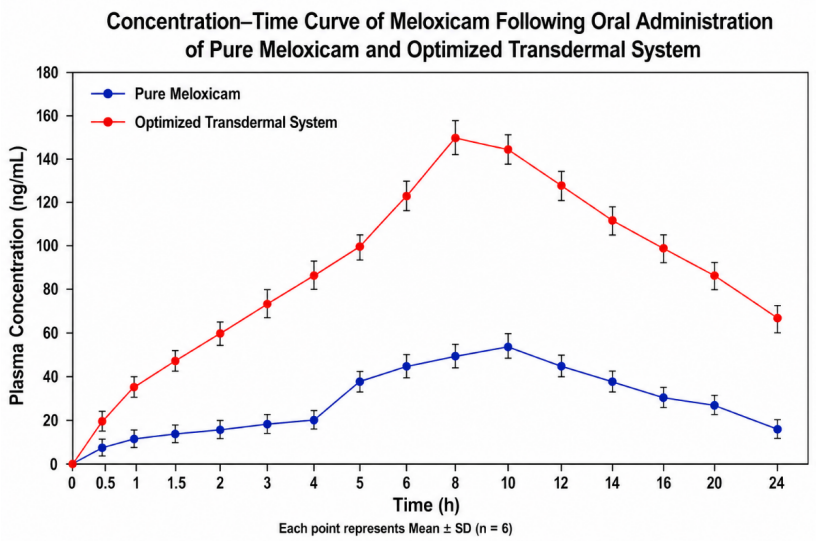

Figure 8: Concentration-Time Curve of Meloxicam following oral administration of Pure Meloxicam and optimized transdermal system

Table 7: Statistical Treatment of Pharmacokinetic Parameters (Mean S.D.) of following oral administration of Pure Meloxicam and optimized transdermal system

Pharmacokinetic parameter	Pure Drug	Optimized transdermal system	Calculated value of 't'
Cmax (ng/ml)	54.91 1.23	151.431.24	135.2***
t1/2 (h)	6.31 0.31	10.8 0.13	17.9***
Kel (h-1)	0.109 0.002	0.064 0.001	18.6***
AUC0-24 (ng h/ml)	771.56 1.11	2751.54. 1.27	79.4***
Null hypothesis (Ho): There is no significant difference between the pharmacokinetic parameters of Meloxicam obtained with pure drug and optimized transdermal system.Table value of 't' with 10 DF at the 0.001 level is 4.587.	Null hypothesis (Ho): There is no significant difference between the pharmacokinetic parameters of Meloxicam obtained with pure drug and optimized transdermal system.Table value of 't' with 10 DF at the 0.001 level is 4.587.	Null hypothesis (Ho): There is no significant difference between the pharmacokinetic parameters of Meloxicam obtained with pure drug and optimized transdermal system.Table value of 't' with 10 DF at the 0.001 level is 4.587.	Null hypothesis (Ho): There is no significant difference between the pharmacokinetic parameters of Meloxicam obtained with pure drug and optimized transdermal system.Table value of 't' with 10 DF at the 0.001 level is 4.587.
Result: Ho is not accepted as the calculated 't' value more than the table Value of't' with 10 DF at 0.001 levels of significance. It was therefore concluded that there was significant difference between the pharmacokinetic parameters of obtained with pure drug and optimized transdermal system.	Result: Ho is not accepted as the calculated 't' value more than the table Value of't' with 10 DF at 0.001 levels of significance. It was therefore concluded that there was significant difference between the pharmacokinetic parameters of obtained with pure drug and optimized transdermal system.	Result: Ho is not accepted as the calculated 't' value more than the table Value of't' with 10 DF at 0.001 levels of significance. It was therefore concluded that there was significant difference between the pharmacokinetic parameters of obtained with pure drug and optimized transdermal system.	Result: Ho is not accepted as the calculated 't' value more than the table Value of't' with 10 DF at 0.001 levels of significance. It was therefore concluded that there was significant difference between the pharmacokinetic parameters of obtained with pure drug and optimized transdermal system.