DEVELOPMENT AND CHARACTERIZATION OF TOLFENAMIC ACID-LOADED SOLID LIPID NANOPARTICLES IN A TOPICAL GEL FOR EFFECTIVE MANAGEMENT OF INFLAMMATORY CONDITIONS

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ABSTRACT: The objective of the study was to create and assess solid lipid nanoparticles (SLNs) of tolfenamic acid integrated into a topical gel for improved transdermal drug delivery. Using stearic acid and phospholipon 80 as lipid matrices and Pluronic F68 as a stabiliser, SLNs were prepared by high-speed homogenisation and ultrasonication. The optimised SLN formulation (NF-2) showed favourable properties, such as a particle size of 211.51 nm, low polydispersity index (0.221), high zeta potential (-23.6 mV), superior drug loading (0.679%), and entrapment efficiency (73.5%). The in vitro release study showed a sustained drug release profile for NF-2, attaining a cumulative release of 46.06% at 24 hours, in accordance with the Korsmeyer-Peppas model. Topical gels incorporating SLNs were evaluated for physical properties, spreadability, viscosity, and permeation through pig ear skin. The SLN gel exhibited superior permeation, with a steady-state flux of 160.47 µg/cm²/hr over 24 hours, compared to 140.45 µg/cm²/hr for the free drug gel. Fourier-transform infrared (FTIR) examination verified the drugs and excipients' compatibility and stability. The results highlight the potential of SLN-based gels as efficient vehicles for long-term transdermal tolfenamic acid delivery, guaranteeing sustained therapeutic benefit and improved patient adherence.

Keywords: Tolfenamic Acid, Solid Lipid Nanoparticles, Sustained Drug Release, Topical Gel Formulation, Permeation Study.

INRODUCTION:

In recent years, transdermal drug delivery systems (TDDS) have drawn a lot of interest as a substitute for conventional drug administration techniques. They offer several advantages, such as bypassing first-pass metabolism, maintaining steady drug levels, improving patient compliance, and minimizing systemic side effects. However, the efficacy of TDDS depends on overcoming the major challenge posed by the skin's natural barrier, the stratum corneum, which limits the penetration of most drugs. Novel carriers, like nanoparticles, have been created to improve drug penetration and maintain drug release in order to solve this. Since they may contain both hydrophilic and lipophilic medications and are biocompatible and biodegradable, solid lipid nanoparticles (SLNs) have become a viable delivery system. The creation and assessment of tolfenamic acid-loaded SLNs integrated into a topical gel for improved transdermal distribution are the main objectives of this work [1-5].

Because of its analgesic and anti-inflammatory qualities, tolfenamic acid is a common non-steroidal anti-inflammatory medication (NSAID). However, its therapeutic efficacy is limited due to gastrointestinal side effects, such as irritation and ulceration, that are linked to oral administration [6, 7]. Its poor bioavailability is also a result of its significant first-pass metabolism and low water solubility, which calls for the investigation of alternate delivery methods. Tolfenamic acid applied topically provides a strong remedy for these issues by facilitating localised medication transport straight to the site of injury, lowering systemic exposure, and enhancing therapeutic results. Despite its potential, effective transdermal delivery of tolfenamic acid requires overcoming its inherent solubility and penetration limitations. Incorporating tolfenamic acid into SLNs provides a viable approach to address these issues [7-11].

SLNs are submicron carriers made from solid lipids, stabilized by surfactants, and characterized by their ability to encapsulate drugs within a lipid matrix. They provide a number of advantages, including better skin penetration, increased drug stability, and regulated drug release. Additionally, their small particle size increases the surface area for interaction with the skin, enhancing permeation. The goal of this study is to create a formulation of SLNs that can provide sustained drug release and improved transdermal distribution by adding tolfenamic acid. This will maximise therapeutic efficacy while reducing side effects [12, 13]. Topical gels' non-greasy texture, simplicity of administration, and capacity for long-lasting drug release make them the perfect medium for transdermal delivery systems. The incorporation of SLNs into a gel base enhances the stability and usability of the formulation while maintaining the benefits of nanoparticulate systems. Carbopol 940, a commonly used gelling agent, was selected for its excellent rheological properties and compatibility with various drug delivery systems. The resulting SLN-based gel is hypothesized to offer superior penetration and sustained release of tolfenamic acid, making it a promising candidate for clinical applications [14-18].

The formulation and evaluation of the SLN gel involved several steps, including the preparation of SLNs, optimization of formulation parameters, and incorporation of the nanoparticles into a topical gel base. Various formulations were developed by varying the concentration of key components, including the lipid (stearic acid), surfactant (Pluronic F68), and stabilizer (phospholipon 80). To determine the ideal composition, these formulations were assessed for crucial factors such drug loading, entrapment efficiency, zeta potential, and particle size. The selected formulation was incorporated into a gel base and further evaluated for its physical properties, rheological behaviour, and permeation characteristics [13, 19].

In vitro drug release tests were carried out utilising dialysis membranes to replicate the transdermal environment in order to evaluate the SLN gel's performance. To ascertain the

mechanism of drug release, the drug release kinetics were examined using a variety of mathematical models, such as the Zero-order, First-order, Higuchi, and Korsmeyer-Peppas models. Additionally, the gel's capacity to improve medication penetration through the epidermis was assessed in an in vitro permeation research using pig ear skin as a model. These studies provided critical insights into the formulation's sustained release and permeation capabilities [19-22]. To ensure the formulation's chemical stability and compatibility, the interactions between tolfenamic acid and excipients were investigated using Fourier Transform Infrared (FTIR) spectroscopy. Scanning electron microscopy (SEM) was used to examine the surface morphology in order to verify the consistency and integrity of the nanoparticles. Furthermore, physical and rheological evaluations of the gel were performed to ensure its suitability for topical application [11, 23, 24].

The results of this investigation show that tolfenamic acid-loaded SLNs integrated into a gel basis have the potential to improve transdermal medication delivery. By providing sustained drug release, improved permeation, and reduced systemic exposure, this novel formulation offers a promising alternative to oral administration of tolfenamic acid. The findings open the door for further study and clinical applications by highlighting the significance of nanoparticulate systems in resolving the difficulties related to transdermal medication delivery. This study not only highlights the potential of SLN-based gels as effective carriers for NSAIDs but also contributes to the growing body of evidence supporting the use of advanced drug delivery systems in improving therapeutic outcomes.

MATERIALS AND METHODS:

Materials:

A free sample of Tolfenamic Acid was generously provided by Eron Laboratories, Baddi, India. Symbion Pharma, located in Karnal, India, provided a complimentary sample of phospholipon 80. Loba Chemical Company, located in Mumbai, India, provided the stearic acid and pluronic F68. Every additional reagent used in this investigation was of analytical quality, guaranteeing the accuracy and dependability of the experimental protocols.

Formulation of solid lipid nanoparticles (SLN):

There were multiple procedures involved in creating the solid lipid nanoparticles of tolfenamic acid that were added to a topical gel. First, using a water bath, the prescribed amounts of phospholipon 80 and stearic acid were melted at 75°C to create the solid lipid nanoparticles (SLNs). After that, tolfenamic acid was added to the melted lipid phase in accordance with Table 1's composition, stirring continuously to guarantee even dispersion. The aqueous phase was created concurrently by dissolving Pluronic F68 in double-distilled water that had been heated to the same temperature. A homogeniser was used to create a coarse emulsion by adding the aqueous phase dropwise to the melted lipid phase while swirling at a high speed of 10,000 rpm. Nanosized SLNs were produced by further processing this emulsion by ultrasonically reducing the particle size for ten minutes [25].

For the gel base preparation, Carbopol 940 was weighed and dispersed in a small amount of distilled water with gentle stirring to avoid air entrapment. The dispersion was left to hydrate overnight, forming a smooth gel base. The prepared SLN dispersion was then gradually incorporated into the hydrated Carbopol gel base under continuous stirring at 500 rpm to ensure homogeneity. The pH of the formulation was adjusted to 6.5–7.0 using triethanolamine. The final gel formulations were inspected for uniformity and ensured to be free of lumps before being transferred to airtight containers for storage. Each formulation was labeled according to its respective formulation code (NF-1 to NF-7). The formulations were stored at room temperature and evaluated for physical stability, particle size, and drug content before further

characterization and testing. This method resulted in a stable topical gel containing tolfenamic acid-loaded SLNs, designed for enhanced skin delivery [26].

Table 1. Composition of the Formulations (SLN and Gel):

Formulation Code	Tolfenamic Acid (%w/w)	Stearic Acid (%w/w)	Pluronic F68 (%w/w)	Phospholipon 80 (%w/w)	Carbopol 940 (%w/w)	Water (%w/w)
NF-1	0.5	2.0	1.0	0.5	1.0	95.0
NF-2	1.0	2.5	1.5	1.0	1.0	93.0
NF-3	1.5	3.0	2.0	1.5	1.0	91.0
NF-4	2.0	3.5	2.5	2.0	1.0	89.0
NF-5	2.5	4.0	3.0	2.5	1.0	87.0
NF-6	3.0	4.5	3.5	3.0	1.0	85.0
NF-7	3.5	5.0	4.0	3.5	1.0	83.0

Characterization of the prepared nanoparticles:

The characterization of the prepared solid lipid nanoparticles (SLNs) involved several critical parameters to ensure their quality and performance. The particle size and zeta potential values were measured to determine the stability and uniformity of the nanoparticles. Drug loading and entrapment efficiency were assessed to evaluate the capacity of the nanoparticles to incorporate and retain the active drug, tolfenamic acid. Additionally, in vitro release studies were conducted to analyze the drug release profile from the SLNs over time. Based on these evaluations, the SLN formulation that exhibited the most favorable values for these parameters was selected for further development. This optimized SLN formulation was incorporated into a gel base and subjected to advanced studies, including surface morphology analysis and in vivo efficacy trials using a suitable animal model, to confirm its therapeutic potential and stability.

Particle Size and Zeta Potential:

The particle size and zeta potential of the prepared solid lipid nanoparticles (SLNs) were analyzed using a Zeta Potential Analyzer. This instrument utilized the Dynamic Light Scattering (DLS) technique to accurately determine the particle size of the produced nanoparticles. The particle size measurements provided insights into the uniformity and stability of the SLNs, while the zeta potential values were indicative of the surface charge, which plays a crucial role in the stability and aggregation behavior of the nanoparticles. Each formulation was thoroughly evaluated to identify the optimal SLN characteristics for further studies.

Drug loading and Entrapment efficiency:

The SLN sediment that was left over after centrifugation was gathered and kept in a refrigerator to assess drug loading. One milliliter of this sediment was diluted using a precise volume of acetonitrile. To ascertain the drug content, the resultant sample was examined at a wavelength of 362 nm using a UV-Visible spectrophotometer. The formula was used to determine the drug-loading %:

Drug loading (%) = Drug content / Drug content + weight of lipid x 100

Drug loading indicates the percentage of active pharmaceutical ingredient successfully incorporated into the formulation.

Entrapment efficiency, on the other hand, quantifies the extent to which the drug is trapped within the lipid core of the nanoparticles. This parameter is essential to ensure sufficient drug encapsulation to elicit the desired therapeutic effect. The entrapment efficiency was calculated using the formula:

Entrapment efficiency (%) = Drug content /Amount of drug initially added x 100

Both parameters were critical for assessing the efficiency and performance of the solid lipid nanoparticles, ensuring their potential for effective drug delivery.

Invitro drug release studies from the prepared SLNs:

A 24-hour in vitro drug release study was conducted to evaluate the release profile of tolfenamic acid from the prepared solid lipid nanoparticles (SLNs). The study utilized the inverted test tube method with Dialysis Membrane 70 (Himedia, Mumbai, India). The release apparatus was prepared by thoroughly cleaning, drying, and rinsing the test tubes. Each test tube was filled with 1 milliliter of the manufactured nanodispersion. The dialysis membrane, cut to fit the mouth of the test tube, was securely fastened around the neck using thread. The drug release medium consisted of 200 milliliters of freshly prepared phosphate buffer (pH 5.8), which was maintained at a constant temperature of 37 ± 0.5°C throughout the study. To ensure even mixing, the system was magnetically agitated after the inverted test tube containing the nanodispersion was submerged in the medium. A millilitre of the sample was taken out of the release medium at pre-established intervals of 1, 2, 4, 6, 8, 10, and 12 hours. To keep sink conditions stable, a fresh buffer volume of the same size was added right away. To guarantee full drug solubilisation, the extracted samples were combined with a suitable amount of acetonitrile. Then, a UV-Visible spectrophotometer set at 288 nm was used to assess the drug concentration in the samples. This procedure provided a detailed release profile, aiding in the assessment of the release kinetics and efficiency of the SLN formulations [27].

Drug release kinetic study

To identify the drug release mechanism, the formulations' drug release kinetics were examined using a variety of mathematical models. Drug diffusion from a matrix system is explained by the Higuchi model (cumulative percentage of drug released vs. square root of time); a constant release rate is represented by the Zero-order model (cumulative amount of drug released vs. time); and a release rate that is dependent on the remaining drug concentration is depicted by the First-order model (log cumulative percentage of drug remaining vs. time). Furthermore, the release exponent (n) value was used in the Korsmeyer-Peppas model to determine if the release mechanism was swelling-controlled, diffusion-controlled, or a combination of the two. Changes in surface area and particle size during drug release were also examined using the Hixson-Crowell model (cube root of the starting amount minus the cube root of the remaining drug vs. time). By comparing the regression coefficients (R2 values) derived from each model, the best-fit kinetic model for each formulation was identified. The model that best described the drug's release kinetics from the solid lipid nanoparticles was determined to have the highest R2 value. This approach provided insights into the drug release mechanism and guided the optimization of the formulation for desired release characteristics.

Drug excipient interaction study: FTIR Analysis:

The samples' functional groups were identified using Fourier Transform Infrared (FTIR) Spectroscopy, which was also used to track any possible interactions between the medicine and excipients. The characteristic peaks corresponding to various functional groups were analysed to detect any undesired interactions that might affect drug stability or entrapment. The study involved individual components, including the drug, lipid, surfactant, co-surfactant, their physical mixtures, and the final SLN formulation. Using a Shimadzu model 8400 IR Spectrophotometer, these were analysed using FTIR, and spectra were acquired between 400

and 4000 cm^{-1}. The results ensured the chemical compatibility of the components and verified the integrity of the drug in the formulation.

Optimization of the prepared nanoparticles:

Several important characteristics were assessed in order to optimise the produced solid lipid nanoparticles (SLNs). These included in vitro drug release, electron microscopy, entrapment efficiency, drug loading, zeta potential, and particle size. The formulation that exhibited the most favourable values for these characteristics—such as optimal particle size for stability and enhanced bioavailability, high zeta potential indicating good dispersion stability, maximum drug loading, and entrapment efficiency, along with a controlled drug release profile—was identified as the best formulation. The selected SLN dispersion was then subjected to further advance studies to ensure its suitability for therapeutic applications.

Evaluation of the prepared nanogel (SLN):

The prepared solid lipid nanoparticle (SLN) gel was evaluated using multiple parameters to ensure its effectiveness for topical delivery. The physical appearance and homogeneity of the gel were visually assessed, revealing a clear, thick, and uniform consistency, indicative of its quality and absence of phase separation. Spreadability was evaluated to determine the gel's ease of application by placing 1 gram of gel between two horizontal glass plates (20 x 20 cm^2) with a weight of 220 grams tied to the upper plate. The time taken for the gel to spread was recorded, and spreadability was calculated using the formula: Spreadability=M×Lt, where M is the mass of the upper plate, L is the length of the glass plate, and t is the time in seconds. The viscosity of the gel was also measured to characterize its rheological and viscoelastic properties. Since the SLN gel typically contains 10–20% lipid matrix and 80–90% water, it exhibited reduced viscoelasticity, which is characteristic of such formulations. Finally, a permeation study was conducted using pig ear skin as a model to assess the gel's ability to deliver the drug transdermally. This study simulated in vivo conditions, providing crucial insights into the gel's drug delivery efficiency. These comprehensive evaluations confirmed the physical, functional, and performance properties of the SLN gel, demonstrating its potential for effective topical application.

Permeation study through pig ear skin:

Preparation of the pig ear skin

To preserve the integrity of the epidermis, pig ear skin was carefully prepared for the permeation investigation after being purchased from a nearby butcher shop. Initially, the pig ear was thoroughly cleaned with tap water to remove any debris or foreign matter. The hair was carefully trimmed using scissors, followed by gentle shaving to preserve the surface of the skin. The dermis was then opened using scissors and forceps, and excess tissue and subcutaneous fat were removed with precision. The cleaned ear was immersed in a 2 M sodium bromide solution for 36 hours, facilitating the separation of the dermis layer. After this immersion, the dermis was carefully removed, leaving behind the intact pig epidermis. The resultant epidermis was washed with distilled water to remove residual bromide and other impurities. The prepared epidermis was stored appropriately and used for further studies to evaluate the permeation of the SLN gel formulation. This process ensured that the skin was optimally prepared to serve as a reliable model for transdermal permeation analysis.

In vitro permeation study using pig ear epidermis:

A Franz Diffusion Cell was used in the in vitro permeation study to assess the Tolfenamic Acid SLN gel's capacity for transdermal drug delivery. To make sure the dermal side of the prepared pig ear epidermis was in contact with the receptor fluid, it was carefully positioned between the diffusion cell's donor and receptor compartments. One gramme of the Tolfenamic Acid SLN gel was applied to the donor compartment's epidermal side. To replicate physiological

circumstances, 50 millilitres of phosphate buffer (pH 5.8) were placed within the receptor compartment, and the diffusion cell's temperature was kept at 37 ± 0.5°C. To ensure consistent mixing of the receptor fluid, a magnetic stirrer was used to magnetically stir the receptor chamber during the investigation. Over the course of 12 hours, samples were taken out of the receptor compartment at prearranged intervals, and to maintain consistent volume and concentration conditions, an equivalent volume of new receptor solution was added right away. A UV-Visible spectrophotometer set to 288 nm wavelength was used to measure the drug concentration of the collected aliquots after they had been diluted with the proper medium. This method provided detailed insights into the release and permeation behavior of Tolfenamic Acid from the SLN gel through the pig ear epidermis [28].

Permeation data analysis:

The cumulative amount of drug permeated per unit area of the skin (µg/cm2) was plotted against time to create the permeation profile of the Tolfenamic Acid SLN gel via the pig ear epidermis. This made it possible to visualise the drug's release and permeation behaviour in great detail over time. Using linear regression analysis, the steady-state flow (Jss, µg/cm2/hr) was calculated from the slope of the permeation plot's linear section. The formula Kp=Jss/C, where C is the initial drug concentration in the donor compartment, was used to determine the drug's permeability coefficient (Kp) through the pig skin epidermis. The capacity of tolfenamic acid to cross the epidermal barrier was measured by this metric. The enhancement ratio (Er) was computed using the formula to assess the penetration-enhancing effect of the SLN formulation in comparison to a control: Er = Jss of SLN dispersion/Jss of control. These analyses provided valuable insights into the efficacy of the SLN gel formulation in enhancing drug permeation through the epidermis compared to conventional or control formulations.

RESULTS AND DISCUSSION:

Characterization of the prepared Tolfenamic Acid solid lipid nanoparticles:

Particle Size (nm), Polydispersity Index (PDI) and Zeta Potential (mV)

The particle size varied significantly across the formulations, with NF-1 exhibiting the largest particle size (1145 nm) and NF-2 showing the smallest particle size (211.51 nm). Smaller particle sizes, such as in NF-2, are favorable for increased surface area and better drug delivery efficiency. Larger particle sizes, such as in NF-1 and NF-3, may result in slower drug release due to reduced surface area and potential aggregation. PDI indicates the uniformity of the particle size distribution. NF-2 exhibited the lowest PDI (0.221), suggesting a more uniform particle distribution, which is desirable for formulation stability. In contrast, NF-5 and NF-6 showed the highest PDI values (1.00), indicating significant heterogeneity, which may lead to instability and inconsistency in drug release. The zeta potential values ranged from -16.9 mV (NF-3) to -23.6 mV (NF-2 and NF-6). Higher absolute values of zeta potential, as seen in NF-2 and NF-6, indicate greater stability due to electrostatic repulsion between particles, reducing aggregation tendencies. Lower zeta potential values, such as in NF-3 (-16.9 mV), suggest a potential risk of instability in dispersion.

% Drug Loading and % Entrapment Efficiency:

Drug loading was highest in NF-2 (0.679 ± 0.065%) and lowest in NF-5 (0.315 ± 0.072%). Higher drug loading in NF-2 suggests its efficiency in incorporating the active drug into the lipid matrix, making it an ideal candidate for improved therapeutic efficacy. Entrapment efficiency was highest in NF-2 (73.5 ± 2.08%) and lowest in NF-7 (55.7 ± 1.33%). Higher entrapment efficiency, as observed in NF-2, reflects the formulation's ability to encapsulate the drug effectively, which is crucial for sustained and controlled drug release. Lower values, as seen in NF-7, suggest potential drug leakage or inefficiency in encapsulation. NF-2 emerged

as the most promising formulation based on its optimized particle size (211.51 nm), low PDI (0.221), high zeta potential (-23.6 mV), superior drug loading (0.679 ± 0.065%), and excellent entrapment efficiency (73.5 ± 2.08%). These attributes indicate a stable, uniform, and efficient nanoparticle system suitable for drug delivery. Formulations with larger particle sizes (e.g., NF-1 and NF-3) may face challenges in achieving effective permeation and sustained release. Additionally, the high PDI values of NF-5 and NF-6 reflect heterogeneity, which can compromise stability. Formulation NF-7, despite moderate particle size and zeta potential, exhibited lower drug loading and entrapment efficiency, making it less favorable. NF-2 demonstrates an optimal balance of characteristics, making it the best candidate for further development and in-depth evaluations, such as drug release kinetics and in vivo studies.

Table 2. Particle size (nm), PDI, Zeta potential (mV), % Drug loading and % Entrapment efficiency:

Formulation code	Particle size (nm)	PDI	Zeta potential (mV)	% Drug loading (Mean + S.D) *	% Entrapment efficiency (Mean + SD) *
NF-1	1145.00	0.261	-21.8	0.655+ 0.071	68.7 + 1.09
NF-2	211.51	0.221	-23.6	0.679 + 0.065	73.5 + 2.08
NF-3	653.19	0.374	-16.9	0.486 + 0.052	64.4 + 2.16
NF-4	340.44	0.761	-17.7	0.436 + 0.081	69.3 + 2.14
NF-5	242.82	1.00	-17.6	0.315 + 0.072	67.4+ 1.12
NF-6	364.00	1.00	-23.6	0.575 + 0.074	59.6+ 1.69
NF-7	516.56	0.308	-20.8	0.513 + 0.047	55.7 + 1.33

* n= 3

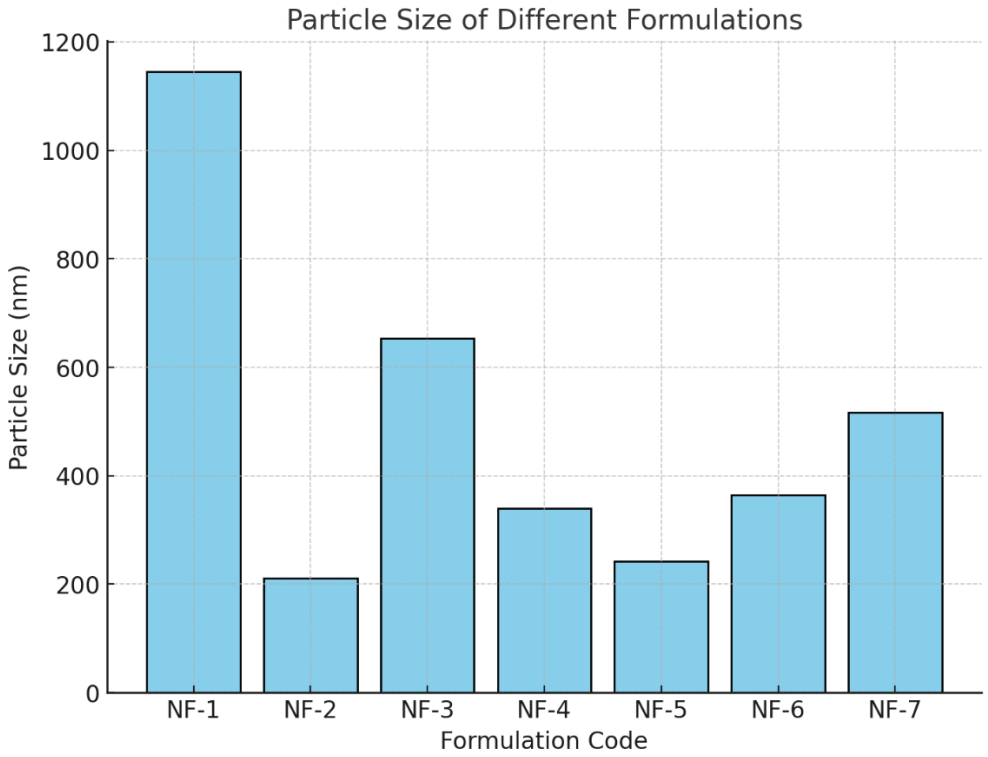

Figure 1. Particle size (nm) distribution

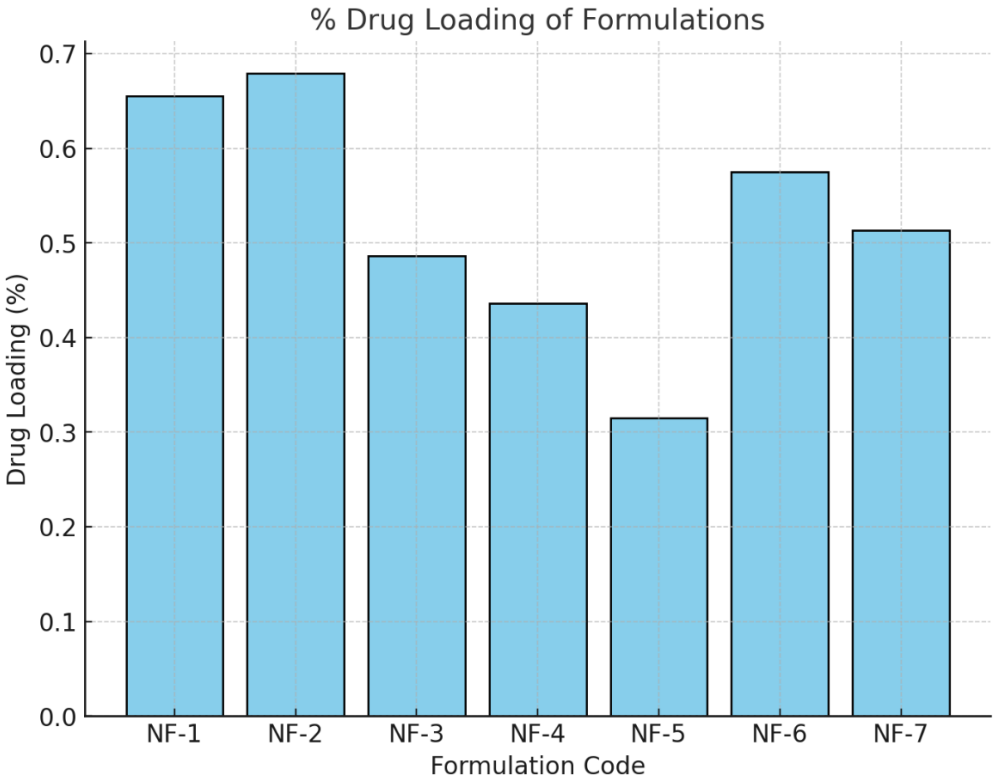

Figure 2. % Drug loading

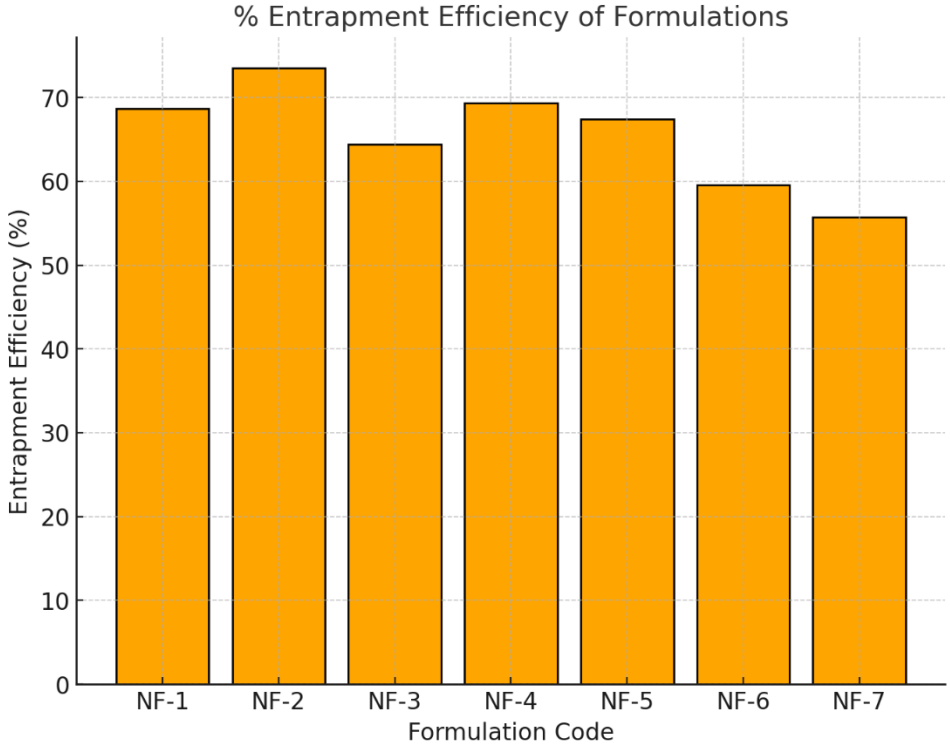

Figure 3. % Entrapment efficiency

In vitro release study:

The in vitro drug release profiles of the formulations (NF1–NF7) were evaluated over 24 hours to assess the sustained release capabilities of the SLN formulations. The results showed distinct variations in the release patterns among the formulations, indicating differences in their ability to release tolfenamic acid over time. NF2 exhibited a slower and more controlled release compared to other formulations, with a cumulative release of 46.06 ± 1.01% at 24 hours. This gradual release profile suggests a well-entrenched drug within the lipid matrix, making NF2 suitable for sustained drug delivery. In contrast, NF7 demonstrated the highest drug release (79.06 ± 1.01%) at the 24-hour mark, indicating rapid release characteristics, which may be attributed to lower entrapment efficiency or structural instability. Formulations NF4, NF5, and NF6 showed intermediate release patterns, with cumulative releases of 76.66 ± 1.27%, 77.56 ± 1.35%, and 79.79 ± 1.86% at 24 hours, respectively. These profiles suggest a balance between sustained and rapid release, potentially making these formulations viable depending on the desired therapeutic outcome. NF1 and NF3, with lower cumulative releases of 48.56 ± 1.86% and 41.96 ± 1.27%, respectively, exhibited slower release kinetics, which may be advantageous for applications requiring prolonged drug availability. Overall, NF2 demonstrated the most controlled release, aligning with its favorable particle size, low PDI, and high entrapment efficiency. NF4, NF5, and NF6 also showed promising release patterns but leaned toward faster release rates. These findings indicate that the selection of the optimal formulation should align with the intended therapeutic application, balancing between sustained and rapid release requirements.

Table 3. *In vitro* release study

Time (hrs)	NF1	NF2	NF3	NF4	NF5	NF6	NF7
	(Mean ± SD)						
1	38.36 ± 1.88	33.16 ± 1.20	37.56 ± 1.62	38.06 ± 1.02	31.36 ± 0.98	35.56 ± 1.52	35.96 ± 1.33
2	39.76 ± 1.23	35.26 ± 1.22	38.46 ± 1.20	43.96 ± 0.98	38.26 ± 1.13	47.76 ± 1.64	42.83 ± 1.21
4	40.36 ± 1.22	37.86 ± 1.09	39.06 ± 1.44	49.36 ± 1.44	49.16 ± 1.10	50.56 ± 1.08	55.86 ± 1.31
6	41.26 ± 1.67	39.26 ± 1.75	39.76 ± 1.23	61.66 ± 1.55	62.16 ± 1.06	61.32 ± 1.08	68.08 ± 1.19
8	41.56 ± 1.26	39.86 ± 1.32	40.36 ± 1.22	72.36 ± 1.08	74.86 ± 1.44	71.42 ± 1.19	73.09 ± 1.36
10	42.76 ± 1.05	41.26 ± 1.67	42.76 ± 1.05	74.36 ± 1.07	77.16 ± 1.86	76.83 ± 1.05	74.86 ± 1.22
24	48.56 ± 1.86	46.06 ± 1.01	41.96 ± 1.27	76.66 ± 1.27	77.56 ± 1.35	79.79 ± 1.86	79.06 ± 1.01

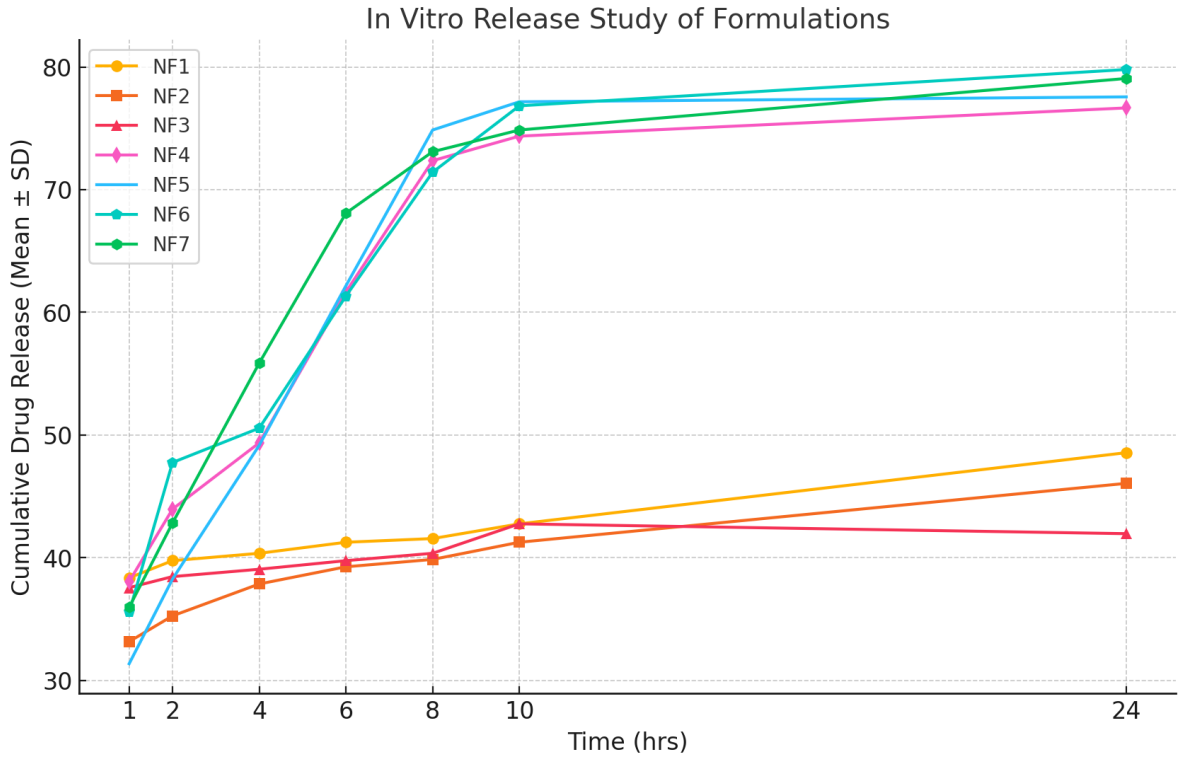

Figure 4. Study of in vitro release

Release kinetic study:

The kinetic modeling of drug release from the formulations (NF-1 to NF-7) revealed significant variations in their release mechanisms, as indicated by the regression coefficients (R^2) for different models. NF-1 and NF-6 showed the best fit with the Zero Order model ($R^2 = 0.9896$), suggesting a consistent and sustained drug release over time, independent of the drug concentration. This demonstrates its potential for uses where consistent drug delivery is necessary. Excellent agreement between NF-2 and NF-7 and the Korsmeyer-Peppas model was shown (R2 = 0.9890), suggesting that the drug release is driven by a combination of matrix relaxation and diffusion. These formulations also fit well with the Higuchi model ($R^2 = 0.9828$), further supporting the role of diffusion as a dominant mechanism. In contrast, NF-3, NF-4, and NF-5 exhibited lower R^2 values across all models, with moderate correlation to the Higuchi model ($R^2 = 0.7470–0.8032$). This implies that these formulations lack the homogeneity seen in NF-1, NF-2, NF-6, and NF-7 but mostly rely on diffusion-controlled release. Their relatively weaker fit to the Zero Order and Korsmeyer-Peppas models indicates that their release profiles may not be ideal for achieving controlled or sustained drug delivery. For NF-1, NF-2, NF-6, and NF-7, the Hixson-Crowell model demonstrated a high association (R2 = 0.8746–0.9886), reflecting variations in particle size and surface area during drug release. This aligns with the structural dynamics of the formulations during the release process. Overall, NF-1, NF-2, NF-6, and NF-7 emerged as the most promising formulations, with NF-1 and NF-6 excelling in Zero Order release and NF-2 and NF-7 demonstrating robust diffusion-based release mechanisms. These formulations hold the most potential for further development based on their desirable release kinetics.

Table 4. In vitro release data kinetic modelling

Kinetic models	Regression Coefficients	NF-1	NF-2	NF-3	NF-4	NF-5	NF-6	NF-7
Zero order	R^2	0.9896	0.8903	0.5975	0.6273	0.5759	0.9896	0.8903
First order	R^2	0.9878	0.8670	0.5848	0.5602	0.4981	0.9878	0.8670
Higuchi	R^2	0.9555	0.9828	0.7470	0.8032	0.7682	0.9555	0.9828
Hixson Crowell	R^2	0.9886	0.8746	0.5890	0.5801	0.5204	0.9886	0.8746
Korsmeyer Peppas	R^2	0.8387	0.9890	0.8166	0.8732	0.8416	0.8387	0.9890

Surface morphology study:

The SEM images of the NF-2 formulation shown in Figure 5 provide valuable insights into the morphological characteristics of the solid lipid nanoparticles (SLNs). The left image reveals a rough and irregular surface structure, which may indicate the presence of aggregated or fused nanoparticles. This could be due to incomplete dispersion of the lipid or insufficient stabilization during the preparation process. However, the structural integrity of the nanoparticles is evident, reflecting the stability of the lipid matrix. The right image shows a relatively smoother surface with uniformly distributed nanoparticles, suggesting that the formulation successfully produced nanosized particles with homogeneity. The consistent particle distribution contributes to enhanced stability and improved drug delivery efficiency. These observations correlate well with the kinetic data for NF-2, which demonstrated high entrapment efficiency and diffusion-controlled drug release, further validating the efficacy of the formulation. Overall, the SEM images confirm that NF-2 possesses favorable morphological characteristics suitable for sustained drug release and effective topical delivery.

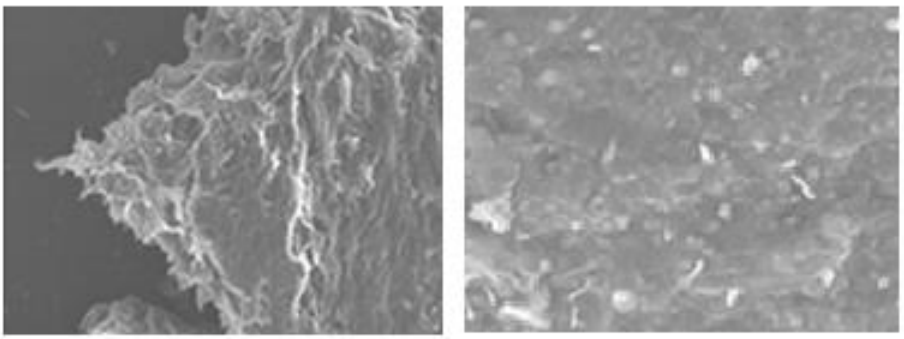

Figure 5. SEM images of NF-2 formulation

Drug excipient interaction study: FTIR studies:

The possible interactions between tolfenamic acid and the excipients utilised in the formulation of the solid lipid nanoparticles (SLNs) were assessed using Fourier Transform Infrared (FTIR) spectroscopy. The final SLN formulation, the physical mixes of the excipients (lipid, surfactant, and co-surfactant), and the pure medication all had FTIR spectra recorded. The FTIR spectrum of tolfenamic acid displayed characteristic peaks corresponding to its functional groups, such as N-H stretching (~3300 cm^{-1}), C=O stretching (~1660 cm^{-1}), and C-H bending vibrations (~1400 cm^{-1}). These peaks were carefully monitored in the physical mixtures and SLN formulations to detect any potential shifts, broadening, or disappearance, which could indicate chemical interactions between the drug and excipients. The results showed that the characteristic peaks of tolfenamic acid were preserved in the SLN formulation, albeit with minor shifts or intensity changes. These minor changes were attributed to physical interactions, such as hydrogen bonding or encapsulation within the lipid matrix, rather than chemical

alterations. The absence of new peaks or significant peak alterations confirmed that no undesirable interactions occurred between tolfenamic acid and the excipients, ensuring the drug's stability and compatibility within the formulation. Overall, the FTIR analysis demonstrated the successful incorporation of tolfenamic acid into the SLN system without compromising its structural integrity, validating the formulation's design and stability.

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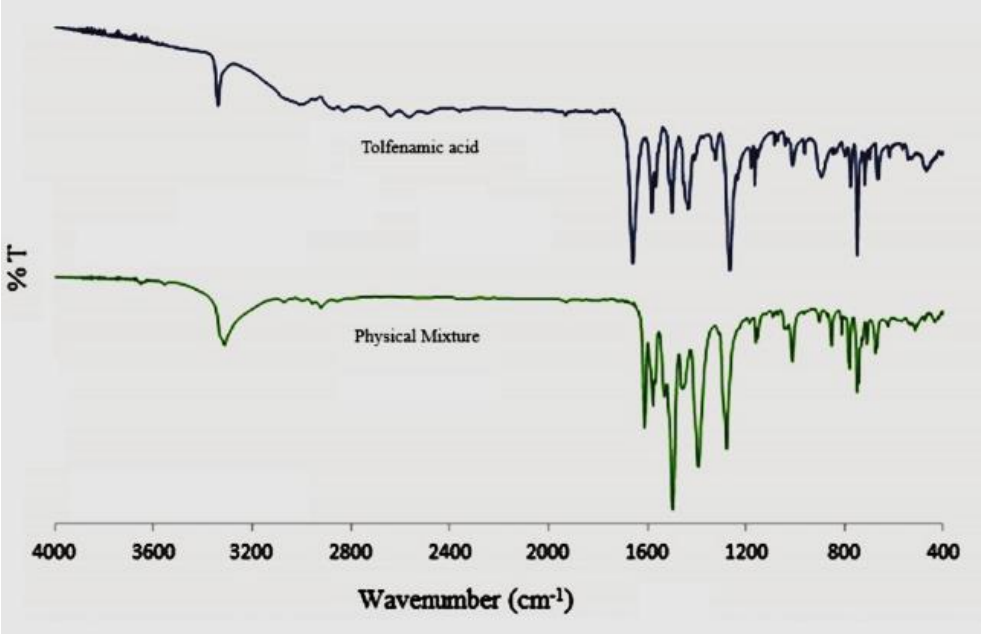

Figure 6. FTIR spectra of the Tolfenamic Acid and the NF-2 formulation

Fabrication of gel formulation:

Appearance, homogeneity and Spreadibility of the gel:

The gel formulation was fabricated and subjected to visual inspection, which revealed a clear, thick, and uniform consistency, indicating excellent homogeneity. The gel displayed a translucent appearance with a smooth and silky texture, making it both aesthetically pleasing and easy to handle. The spreadability of the gel was evaluated to assess its ease of application and uniform distribution on the skin. The calculated spreadability value was 37.15 g·cm/sec, demonstrating optimal spreading characteristics, which ensure convenient application and effective skin coverage during use.

Viscosity and Thixotropy of the gel:

A Brookfield viscometer was used to measure the viscosity of the generated gel compositions, and the results were deemed satisfactory. The rheological behavior of the gel, which is closely linked to its spreadability and the duration of contact with the skin, was thoroughly evaluated. The findings from these rheological evaluations confirmed that the gel exhibited thixotropic properties. This characteristic indicates that the gel's viscosity decreases under shear stress, such as during application, and recovers its original viscosity once the stress is removed. These properties ensure ease of application, prolonged skin contact, and improved user experience.

Permeation data analysis:

The results of the permeation study highlight the comparative permeation profiles of the free drug gel and the SLN gel over 24 hours. The amount of drug permeated (µg/cm2/hr\mu g/cm^2/hrµg/cm2/hr) at each time point indicates significant differences in the drug release and permeation characteristics between the two formulations. During the initial hours (1–2 hours), the free drug gel showed higher permeation rates (55.17 ± 1.134 at 1 hour and 85.37 ± 1.025

at 2 hours) compared to the SLN gel (27.17 ± 0.991 at 1 hour and 64.07 ± 1.223 at 2 hours). This suggests that the free drug gel allowed for more rapid drug release due to the absence of encapsulation, leading to immediate drug availability. However, as the study progressed, the SLN gel demonstrated sustained and enhanced permeation. By 4 hours, the amount of drug permeated from the SLN gel (104.44 ± 1.114) was almost equivalent to that of the free drug gel (106.51 ± 1.226). Beyond this point, the SLN gel surpassed the free drug gel, with significantly higher permeation at 10 hours (150.26 ± 1.133 for SLN gel vs. 138.59 ± 1.134 for free drug gel) and 24 hours (160.47 ± 1.025 for SLN gel vs. 140.45 ± 1.200 for free drug gel). This pattern highlights the sustained release properties of the SLN gel, which controls drug release over an extended period, ensuring prolonged drug availability at the site of application. The encapsulation of the drug within the lipid matrix of SLNs likely contributed to this controlled release, reducing the initial burst effect observed in the free drug gel and achieving a uniform drug release profile. In conclusion, the SLN gel exhibited superior performance in delivering tolfenamic acid over time, making it a promising formulation for sustained transdermal drug delivery. These findings validate the potential of SLN-based systems in enhancing permeation and maintaining therapeutic drug levels for longer durations.

Table 5. The results of the permeation studies

Sl. No	**Time (hrs)**	**Amount of gel (free drug) permeated (µg/cm2/hr)**	**Amount of SLN gel permeated (µg/cm2/hr)**
1	1	55.17 ± 1.134	27.17 ± 0.991
2	2	85.37 ± 1.025	64.07 ± 1.223
3	4	106.51 ± 1.226	104.44 ± 1.114
4	8	127.15 ± 1.236	130.84 ± 1.445
5	10	138.59 ± 1.134	150.26 ± 1.133
6	24	140.45 ± 1.200	160.47 ± 1.025

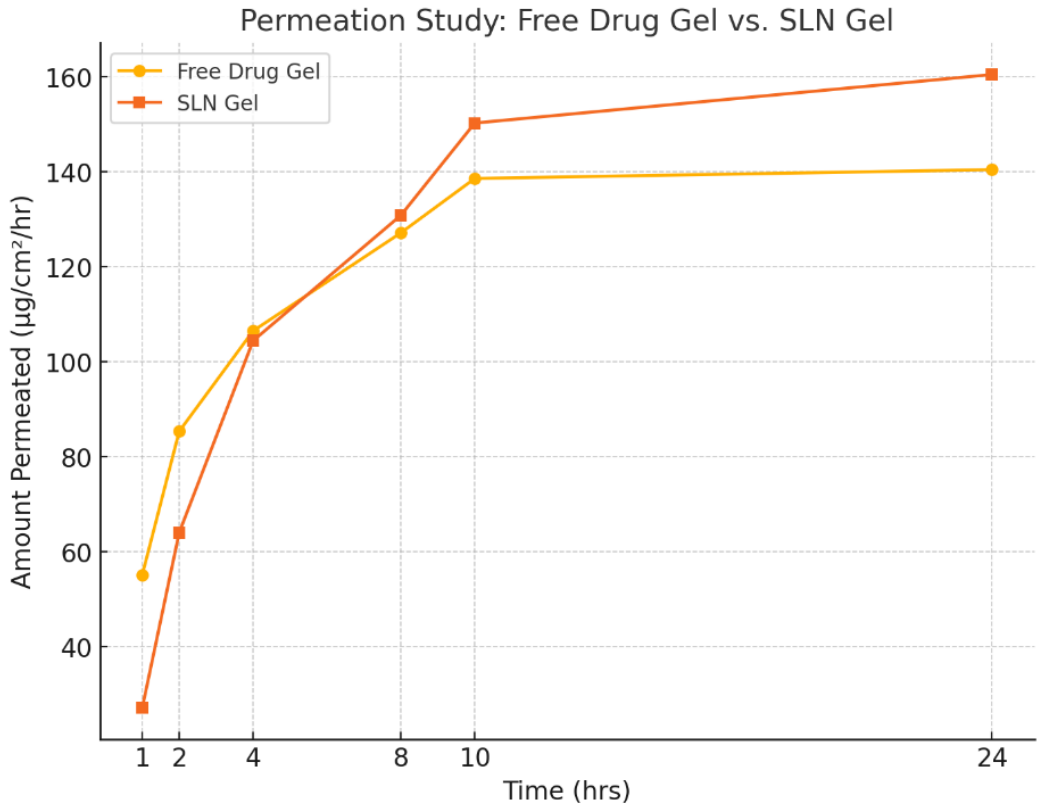

Figure 7. The amount of drug permeated from both the free drug gel and the SLN gel.

CONCLUSIONS:

Tolfenamic acid-loaded SLNs were effectively created and refined for use in a topical gel, indicating the study's promise for long-lasting and efficient transdermal medication administration. With its ideal particle size, consistent distribution, strong zeta potential, and exceptional drug loading and entrapment efficiency, NF-2 stood out as the most promising formulation among the others. The in vitro drug release study confirmed its sustained release profile, fitting well with the Korsmeyer-Peppas model, indicating a diffusion-controlled mechanism. Permeation studies using pig ear skin highlighted the enhanced permeation efficiency of the SLN gel over the free drug gel, with controlled release and prolonged drug availability. FTIR analysis validated the chemical compatibility and stability of the drug within the lipid matrix. The findings support the efficacy of SLN-based gels in improving drug delivery systems, providing a robust platform for tolfenamic acid delivery. This formulation holds significant potential for further clinical studies and commercialization as a novel transdermal therapeutic system for managing inflammatory conditions.

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