International Journal of Pharmaceutical Drug Design (IJPDD)
Website: https://ijpdd.org/
ISSN: 2584-2897
Vol. 2, Issue 3, March, 2025
Page No.: 73-83

Research

ADVANCED FORMULATION AND OPTIMIZATION OF FLURBIPROFEN-LOADED NANOEMULGEL: EXPLORING NOVEL NANO-CARRIERS AND DRUG DELIVERY ENHANCEMENTS

Souvik Giri*, Archana Pattanik, Debashish Mohanty, Nilima Shukla

Sri Jayadev College of Pharmaceutical Sciences, Naharkanta, Bhubaneswar, Odisha

Corresponding Author:
Souvik Giri

Email: *souvikrjgiri@gmail.com*

DOI: *10.62896/ijpdd.2.3.11*

Conflict of interest: *NIL*

ABSTRACT:

The objective of this study was to create and refine a topical nanoemulgel containing flurbiprofen in order to increase its permeability and water solubility without causing first- pass metabolism. Peppermint oil, Tween 80, and PEG-400 were selected as the oil phase, surfactant, and co-surfactant, respectively, following individual screening processes. To formulate the nanoemulsion, the ideal oil to Smix (surfactant and co-surfactant mixture) ratio was found by a pseudo-ternary phase research. A high-pressure microfluidization method was used to create the nanoemulsions (named NEF1 through NEF9). A 2^2 Central Composite Design (CCD) served as the basis for the optimisation procedure. The dependent variables were particle size and drug release percentage (%DR), while the independent variables were peppermint oil (X1) and the Smix ratio (X2).Particle sizes of 69.5±0.5 nm to 297.1±1.1 nm, zeta potentials of -35.19±0.02 to -18.91±0.27, PDI values of 0.147 to 0.666, and in vitro drug release percentages of 76±0.81% to 92.1±0.3% were all displayed by the optimised nanoemulsions. A 1% Carbopol 934 gel basis was combined with the ideal formulation (NEF1) to create a 5% w/w Flurbiprofen nanoemulgel. This nanoemulgel was evaluated for various properties, displaying a spreadability of 7.6±0.2 cm, a pH value of 5.8±0.63, a percentage drug release of 97.4±0.21%, and a flux of 5.29±1.20 µg/cm²•h. Comparative studies revealed that the 5% Flurbiprofen nanoemulgel achieved superior drug release, with an 80.12% improvement over the marketed 5% Flurbiprofen gel (Brugel). Additionally, the nanoemulgel remained stable at 4°C for 90 days.This research successfully developed and optimized a Flurbiprofen-loaded nanoemulgel with significantly improved drug release properties compared to the commercial product, showcasing favorable characteristics and stability for potential topical delivery applications.

Keywords: flurbiprofen, nanoemulsions, topical delivery applications

Article History
Received: 03/01/2025
Accepted: 22/02/2025
Published: 08/03/2025

INTRODUCTION: The burgeoning interest in nanosized systems for topical drug delivery in scientific and biomedical research is driven by their minimal side effects, superior bioavailability, and additional advantages.[1-2] Nanoemulsions, characterized as transparent, thermodynamically stable dispersions composed of oil and aqueous phases stabilized by surfactant and co- surfactant molecules (Smix), typically possess particle sizes ranging from 10 to 200 nm.[3-5] These nanoemulsions are favored for their prolonged stability, facile preparation, efficient permeation, reduced dosage requirements, enhanced patient compliance, and high drug solubilization capacity.[6-7] By improving the solubility and permeability of drugs, nanoemulsions present a promising vehicle for drug delivery. Flurbiprofen, a BCS Class-II drug frequently utilized in oral formulations for managing pain in conditions such as spondylitis, rheumatoid arthritis, and osteoarthritis, is subjected to first-pass metabolism and associated with adverse gastrointestinal effects like ulcers and bleeding. Consequently, topical administration is preferable.[17] The inclusion of natural oils in the oil phase of nanoemulsions offers therapeutic benefits alongside thixotropic properties, non-greasiness, spreadability, removability, emollience, bio-friendliness, extended shelf life, and a translucent appearance. This research employs a high-energy microfluidization technique to formulate the nanoemulsion gel and applies a 2^2 Central Composite Design (CCD) for experimental design. This study is pioneering in its formulation of a Flurbiprofen-loaded nanoemulsion using high-energy microfluidization and a 2^2 CCD approach.

MATERIALS AND METHODS

A. Materials

Flurbiprofen was sourced from Hygro Chemicals Pharmatek Pvt. Ltd., India. PEG400, isopropyl alcohol, peppermint oil, and propylene glycol (PG) were among the chemicals and solvents that were purchased from Fischer Scientifics in Mumbai. Tween 20 was purchased from S.D. Fine Chemicals in Mumbai, while castor oil, olive oil, soybean oil, isopropyl myristate (IPM), Tween 80, and Carbopol-934 were procured from R.P. Chemicals in Mumbai. Analytical grade solvents and chemicals were all utilised.

B. Screening for Oil Phase

Flurbiprofen's solubility in various oils was tested using a screening procedure.[20] Excess Flurbiprofen was mixed with 2 mL samples of different oils, surfactants, and co-surfactants in 5 mL stopper vials. The mixtures were vortexed and then orbitally agitated for 24 hours at 37±1°C. After agitation, samples were centrifuged at 3000 rpm, filtered over a 45 µm membrane, and diluted with the appropriate solvent. The solubility and concentration of Flurbiprofen were measured using a validated UV spectrophotometric technique at 247nm.

C. Screening of Surfactants

Surfactant screening included Tween 20, Tween 40, and Tween 80. A 15% w/w solution of each surfactant was made in distilled water, and the pre-screened oil phase was added dropwise to each solution while vigorously vortexing until cloudiness occurred. The surfactant retaining solubility without clouding was chosen, with a preference for surfactants with an HLB value greater than 10.[21]

D. Screening of Co-Surfactants

For co-surfactant screening, a combination of oil and surfactant was used, resulting in a translucent emulsion with little flask inversion. Flurbiprofen's solubility was tested in co- surfactants including methanol, isopropyl alcohol (IPA), PEG-400, propylene glycol, and glycerol. Several weight ratios of surfactant to co-surfactant (Smix) were evaluated, and the mixture that produced clarity was chosen as the stable Smix ratio for nanoemulsion development. The percentage transmittance was determined by diluting 1 mL of the produced surfactant and co-surfactant ratios with 100 mL of double-distilled water and analysing at 247 nm with water as a blank.[3]

E. Selection of Surfactant-Co-Surfactant Ratio for Nanoemulsion Formulation

A ternary phase study was conducted using various ratios of surfactant to co-surfactant (1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 2:1, 3:1) combined with different oil ratios (1:9, 1:8, 1:7, 1:6, 1:5, 1:4). A pseudo-ternary phase diagram was used to identify the optimal Smix and oil ratio. The emulsification efficiency was evaluated based on % transmittance.[12][3]

Drug-Excipients Interaction Study

i. Infrared Spectroscopy

Fourier Transform Infrared (FTIR) spectroscopy (Shimadzu FTIR-8400S) was utilized to analyze interactions between Flurbiprofen and excipients, employing the KBr disc method with a 4 cm^{-1} resolution over a frequency range of 4000–400 cm^{-1}.[22]

ii. Differential Scanning Calorimetry (DSC)

The thermal behavior of Flurbiprofen was assessed using Differential Scanning Calorimetry (DSC, DSC-60, Shimadzu, Japan). Samples were heated in aluminum pans from 25°C to 200°C at a 10°C/min rate in a nitrogen atmosphere with a 40 mL/min flow rate.[22]

Design of Experiment and Nanoemulsion Optimization

Optimization of nanoemulsions was conducted using a 2^2 Central Composite Design (CCD) with Sigmatech® software to evaluate the effects of oil (X1) and surfactant-co-surfactant ratio (Smix) (X2) on critical quality attributes (CQAs) like particle size and % drug release. These factors were tested at two levels (high [+1] and low [−1]) to assess their main and interaction effects on the formulation. Particle size and % drug release discussed in Table 1a and 1b.

Table 1a: Factors with levels for 50ml Nanoemulsion

Levels	Values	Oil (ml)	Smix (ml)
+1	High	3.5	25

-1	Low	2.5	17.5

Table1b: Selected variables for optimization of formulations

Independent variables	Dependent variables
Concentration of oil	Particle size
Concentration of Smix	% Drug release

Method of Preparation of Nanoemulsions Preparation of Coarse Emulsion

The specified oil, surfactant, and co-surfactant ratios were employed to create the nanoemulsion. The oily phase (5-10% w/w oil and 40-60% w/w S_{mix}) was gradually introduced to the aqueous phase containing water at 30°C while magnetically stirring. The resultant emulsion was then sonicated for 30 minutes with an ultrasonic probe sonicator (Mangaldeep Tech Solutions) to reduce particle size until cooling in an ice bath to ambient temperature.

Table 2: Formulation code and interaction factor in nanoemulsion

Code	Interaction		Drug Formulation (g)	Oil Phase (ml)	Smix (ml)	Aq. Phase (ml)	% of oil	% of Smix	% of Aq-phase
	A	B							
NEF1	-α	- α	1.25	2.5	17.5	30	5	35	60
NEF2	+α	- α	1.25	3.5	17.5	29	7	35	58
NEF3	-α	+α	1.25	2.5	25	22.5	5	50	45
NEF4	+α	+α	1.25	3.5	25	21.5	7	50	43
NEF5	0	0	1.25	3.0	21.25	25.75	6	42.5	51.5
NEF6	-1	0	1.25	2.0	21.25	26.75	4	42.5	53.5
NEF7	+1	0	1.25	4.0	21.25	24.75	8	42.5	49.5
NEF8	0	-1	1.25	3.0	13.75	33.25	6	27.5	66.5
NEF9	0	+1	1.25	3.0	28.75	18.25	6	57.5	36.5

Preparation of Nanoemulsion

The coarse emulsions were further processed with a microfluidizer (LM20, Microfluidizer). This high-energy approach uses shear, cavitational, and turbulent forces to break down emulsions into smaller droplets. Each formulation completed 15 cycles at 25,000 psi, with ice injected to the cooling chamber to prevent degradation due to temperature increases.

Figure 1: Microfluidizer used in the formulation of Flurbiprofen-loaded Nanoemulsion Characterization of Nanoemulsion

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

Utilising a ZetaSizer (Horiba Scientific, Japan), photon correlation spectroscopy was employed to determine the prepared nanoemulsion's zeta potential, PDI, and particle size.

Measurements were made in triplicate on a 1 mL sample at 25°C and a 90° scattering angle.[24]

Drug Content Determination

Drug quantity was analyzed by diluting 1 mL of the sample with methanol in a volumetric flask, centrifuging the mixture at 3500 rpm for 30 minutes, filtering the supernatant, and using a UV spectrophotometer.[12]

Preparation of Flurbiprofen Nanoemulgel

As the gelling agent, 1% Carbopol 934 was used to create a 5% w/w Flurbiprofen Nanoemulgel. After constant stirring, the gel base was combined with the refined nanoemulsion formulation that contained 5% flurbiprofen. To prevent spoilage, triethanolamine was added, and sodium benzoate was used as a preservative.[25-26]

Evaluation of Flurbiprofen Nanoemulgel Homogeneity

Homogeneity was assessed by pressing a small amount of Nanoemulgel between the index finger and thumb.

Spreadability

Measurement of the spread diameter of 1 g of Nanoemulgel between two horizontal plates (20 cm x 20 cm each) was used to assess spreadability. Using the following formula, the spreadability (S) was determined:

$$S = \frac{M \times L}{T}$$

where S represents spreadability in g/s, M is the mass of the gel in grams, L is the length of the gel spread, and T is the time in seconds.

pH Measurement

A Systronics Digital 335 pH meter was implemented to measure the pH after dissolving 50 mg of gel in 10 mL of distilled water. The mean and standard deviation were then computed after several measurements.

Viscosity Measurement

Viscosity was measured using a Brookfield DVE viscometer with spindle S-64 at 37°C, with the spindle rotating at 12 rpm for 10 seconds.[27]

SEM of Nanoemulsion Gel

Employing scanning electron microscopy (SEM), the size and morphology of the nanoemulsion gel loaded with flurbiprofen were investigated. Before analysis, samples were prepared in accordance with established methods.

Drug Content Determination

To quantify Flurbiprofen content in the gel, 1 g of gel was dissolved in a pH 7.4 buffer solutions, agitated for 2 hours, filtered, and analyzed using UV spectroscopy at 247 nm.

In-Vitro Drug Diffusion Study

Drug release was examined through Franz diffusion cells with cellulose acetate membranes. A pH 7.4 phosphate buffer was maintained at 37°C and swirled at 500 rpm in the receptor compartment, while 0.5 g of gel was placed in the donor compartment. At prearranged intervals, samples were collected, new buffer was added, and UV spectroscopy at 247 nm was used to analyse the results.

Drug Release Kinetics

The drug release data was analysed using various kinetic models (including Zero-order, First- order, Higuchi, and Korsmeyer-Peppas) to identify the release mechanism.

Stability Studies

Stability studies were conducted on the optimized Flurbiprofen nanoemulgel formulation to assess its physical and chemical stability under various storage conditions. The study adhered to the guidelines established by the International Conference on Harmonization (ICH). The following conditions were tested:

- Refrigeration at 4°C (±2°C)

- Room temperature at 25°C (±2°C) with 60% (±5%) relative humidity (RH)
- Elevated temperature at 40°C (±2°C) with 75% (±5%) RH

The study duration was three months. The nanoemulgel samples were stored in lacquered aluminum collapsible tubes to prevent any interaction between the container and the formulation.

Results and Discussion Solubility

Studies

The therapeutic effect of the nanoemulsion is dependent upon Flurbiprofen's solubility in the oil phase. Identifying the right oils, surfactants, and co-surfactants is important for creating a topical nanoemulgel that works. The chosen surfactants are known to enhance skin permeation, and co-surfactants further improve nanoemulsion stability and drug incorporation. Ensuring that the drug is well-dissolved in the oil phase creates a higher concentration gradient, enhancing the drug's topical efficacy by improving its permeation across the skin. Co-surfactants are particularly important for achieving stable nanoemulsions at lower surfactant concentrations.

Discussion

The selection of surfactants and co-surfactants was guided by their efficiency in micellization, indicated by the minimal number of flask inversions required and the resulting transparency, measured by % transmittance. Among the surfactants tested, Tween 80 demonstrated the highest % transmittance, indicating superior nanoemulsification efficiency. Screening of co-surfactants revealed that PEG 400 achieved the highest % transmittance, reflecting its effectiveness in enhancing nanoemulsion stability within the phase diagram. The phase diagram study showed that a combination of Tween 80 and PEG 400 in a 1:2 ratio provided optimal nanoemulsification with the selected oil phase for the Flurbiprofen-loaded nanoemulsion.

Solubility of Drug in Surfactants and Co-Surfactants

Peppermint oil were having the highest solubility for flurbiprofen among the studied oils, at 38.28±5.77 mg/mL. Flurbiprofen demonstrated good solubility in HLB values of 15 and 12.8 for Tween 80 (41.48±0.055 mg/mL) and PEG 400 (71.29±0.975 mg/mL). Based on this information, the formulation of the nanoemulsion system was carried out using peppermint oil as the oil phase and Tween 80 and PEG 400 as the co-surfactant and surfactant, respectively.

Table 3: Solubility of Flurbiprofen in Oils at 25°C (Mean ± S.D., n=3)

Oils	Solubility (mg/ml)
Peppermint oil	48.28±0.077
Soybean oil	30.33±0.55
Olive Oil	25.95±1.65
IPM	30.06±0.92
Castor Oil	34.93±0.006

Table 4: Solubility of Flurbiprofen in surfactants and co-surfactants (Mean ± S.D., n=3)

Surfactant/Co- surfactant	Solubility(mg/ml)
Tween 80	41.48±0.95
Tween 20	35.8±3.54
PEG-400	28.59±2.14
Propylene glycol	23.24±1.97
IPA	23.27±0.89
Glycerol	22.96±1.45

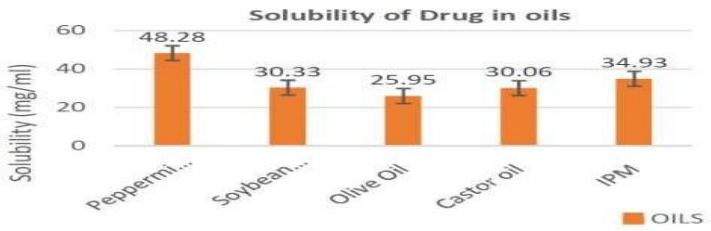


Figure 2: Solubility of Flurbiprofen in Different Oils

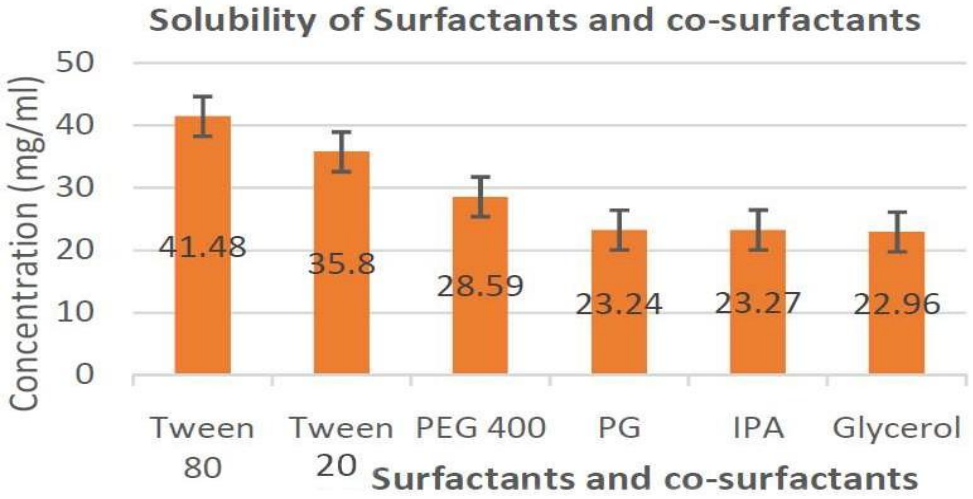


Figure 3: Solvability of drug in surfactants and co-surfactants Pseudo-

Ternary Phase Diagram

Applying the ternary phase diagram, the optimal nanoemulsion formulation the area was found. Tween 80, PEG 400, and peppermint oil were examined in many different combinations of surfactant to co-surfactant (Smix) ratios, comprising 1:1 to 1:9, 2:1, and 3:1, in conjunction with varying oil ratios (1:9, 1:8, 1:7, 1:6, 1:5, and 1:4). Durability of these combinations was determined by emulsification efficiency and transmittance percentage. For the majority of Smix combinations, stable nanoemulsions were found to be produced at ratios of 1:7 and 1:5. The biggest nanoemulsion region was obtained with a 1:2 Smix ratio, suggesting that a larger Smix concentration is necessary for stabilising nanoemulsions. For nanoemulsion stability, a formulation containing 5–10% oil and 40–50% Smix was therefore optimum. A 2^2 Central Composite Design was used to further enhance the formulation.

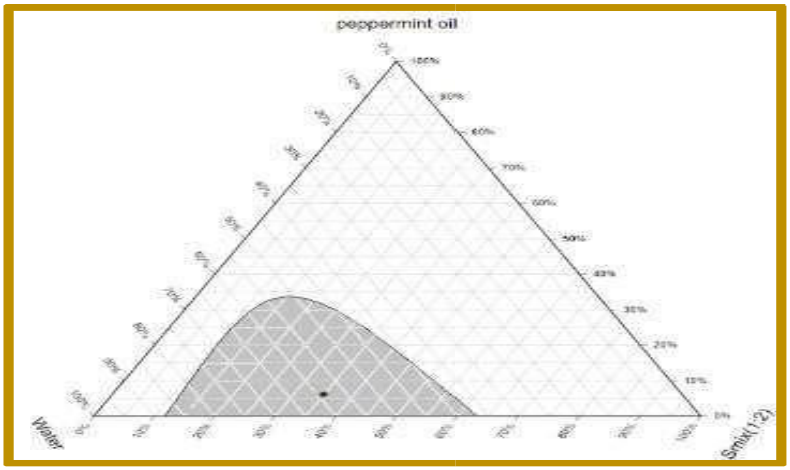


Figure 4: Pseudo ternary plot showing area of NE

Formulation and Optimization of Flurbiprofen-Loaded Nanoemulsion

Using a high-pressure homogeniser (Microfluidizer) and a probe sonicator, nanoemulsion formulations (NEF1–NEF9) with the necessary compositions were created. Nine formulations, including a midway value, were used in the optimisation process, which involved a 2^2 Central Composite Design (CCD). Particle size and percentage of medication release were dependent factors, and peppermint oil (X1) and S_{mix} ratio (X2) were independent variables. The in-vitro drug release, zeta potential, and transparency to translucency of each formulation were assessed.

CHARACTERISATION OF PREPARED FLURBIPROFEN NANOEMULSION

Particle Size

The particle sizes of the formulations ranged from 69.5±0.9 nm to 297.1±1.1 nm. All formulations had particle sizes below 300 nm, indicating their suitability for topical application. NEF1 had the smallest particle size at

69.5±0.9 nm, which is favorable for enhanced topical efficacy.

Zeta Potential

Zeta potential values indicated the stability of the formulations, with NEF1 showing the most stability at -35.19±0.02 mV. In contrast, NEF7 had the least stability at -18.91±0.27 mV.

Polydispersity Index (PDI)

Most formulations had a PDI within the acceptable range (<0.5), suggesting uniformity. However, NEF7 exceeded this limit, indicating less uniformity, likely due to its larger particle size.

% Drug Content

As per various research study, NEF1 and NEF 6 formulations shows the highest drug content with values 98.14±0.09% and 95.61±0.19%, respectively.

In-vitro Diffusion Studies

According to literature study, In-Vitro diffusion studies have been carried out over 8 hours using a dialysis membrane. Samples were performed with a UV spectrophotometer at 247nm. The highest cumulative drug release (CDR) after 8 hours was observed with formulation NEF6 at 92.02%, followed closely by NEF1 at 91.76% and NEF5 at 89.45%.

Experimental Design and Optimization of Nanoemulsions

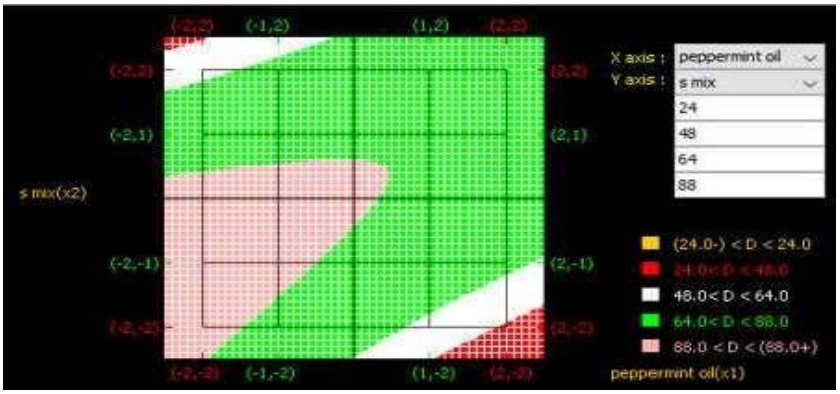
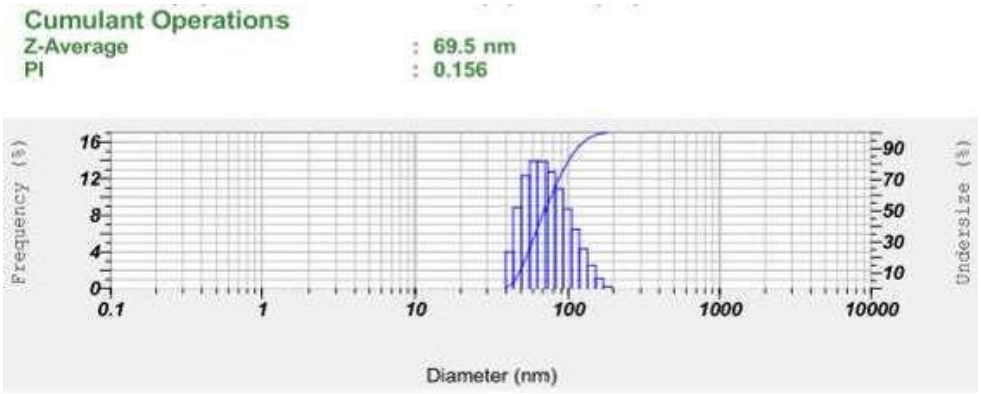


Figure 5: 2D Contour plot showing Optimized formulation

Based on particle size, drug release, coefficient of correlation value, and 2D contour plots, formulation NEF1 emerged as the optimized formulation. It shows the range of particle size of 69.5nm, a drug release of 91.76% and a coefficient of correlation value is 89.023.

Preparation of Flurbiprofen Nanoemulgel

1% Carbopol 934 was used as the gelling agent for producing a 5% Flurbiprofen nanoemulgel, with a final 5% w/w Flurbiprofen literature in the formulation.



International Journal of Pharmaceutical Drug Design (IJPDD)
Website: https://ijpdd.org/
ISSN: 2584-2897
Vol. 2, Issue 3, March, 2025
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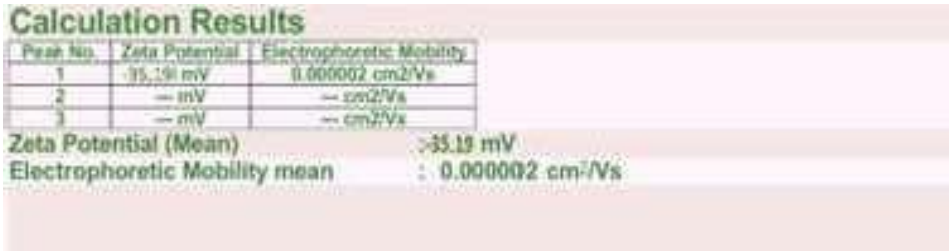
Figure 6: Particle size of optimized formulation (NEF1) CHARACTERISATION OF FLURBIPROFEN NANOEMULGEL



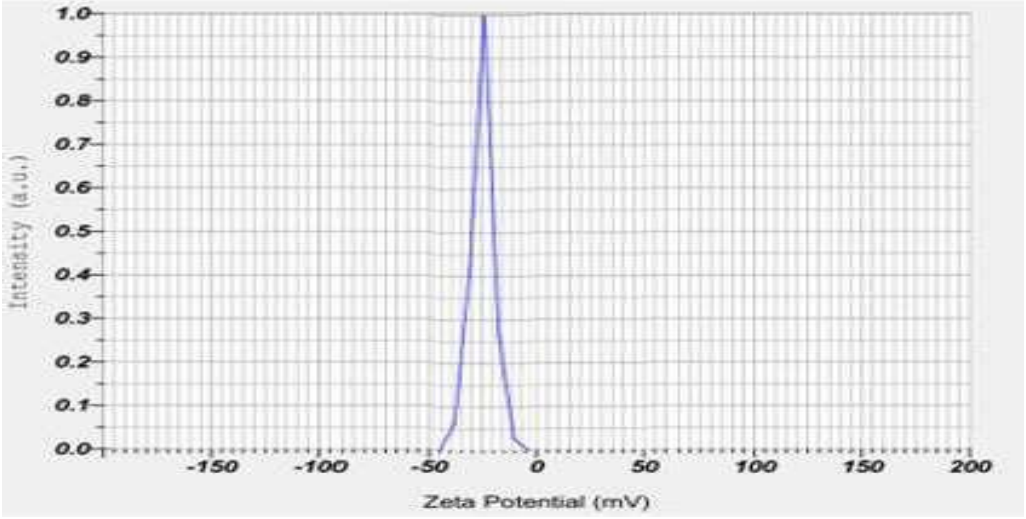


Figure 7: Zeta potential of optimized formulation (NEF1) Surface Morphology of Nanoemulgel

Figure 8: SEM image of optimize formulation

The surface morphology of the Flurbiprofen nanoemulgel was evaluated using scanning electron microscopy (SEM). The images indicated that the nanoemulsion droplets were primarily globular, characteristic of an oil-in-water nanoemulsion. The droplet size was confirmed to be within the nanometric range, varying from 51 to 120 nm.

Evaluation of Flurbiprofen Nanoemulgel

The Flurbiprofen nanoemulgel appeared transparent and smooth with a uniform texture. It demonstrated good spreadability, with a value of 76±0.22 cm, indicating its ease of application to the skin. The formulation's pH was 5.8±0.63, falling within the acceptable range for topical products. The drug content was 98.2±0.41%, reflecting excellent content uniformity. The viscosity of the nanoemulgel was measured at 16,254±0.74 cps.

Table 5: Evaluation of Flurbiprofen Nanoemulgel

Evaluation	Results
Homogenicity	Good
Spreadability(cm)	76±0.22
Viscosity(mPas)	131±0.25
pH measurements	5.8±0.63
Drug content (%)	98.2±0.41
In-vitro drug release(%)	91.4±0.21

Stability Studies

Stability studies showed that the Flurbiprofen nanoemulgel remained stable when stored at 4°C, compared to other temperatures.

Summary of Findings

A topical nanoemulgel was developed employing the non-steroidal anti-inflammatory medication (NSAID) flurbiprofen. The highest level of solubility was achieved with peppermint oil (48.28±0.077 mg/mL), Tween 80 (41.48±0.95 mg/mL) as the surfactant, and PEG 400 (28.59±2.14 mg/mL) as the co-surfactant, based on solubility studies deploying various oils, surfactants, and co-surfactants. The surfactant conjunction (Smix) was selected in a 1:2 ratio according to the transmittance value (88.5%). At a ratio of 1:7 for oil to Smix, the pseudo-ternary phase diagram indicated an increasing nanoemulsion region. The high- pressure microfluidization technology was used for producing the flurbiprofen-loaded nanoemulsion. Nine formulations have been developed through standardisation applying a 2^2 central composite design (CCD) (NEF1-NEF9). Formulation NEF1, resulting in a correlation coefficient of 89.023, a drug release rate of 91.76%, a particle size of 69.5 nm, and a composition of 35% Smix, 60% aqueous phase, was determined to be optimal. All the formulations exhibited particle sizes within 69.5±0.9 nm and 297.1±1.1 nm, although NEF1 and NEF6 had the smallest sizes, ranging 69.5±0.9 nm and 65.81±0.8 nm, respectively. Zeta potential was employed to measure stability; NEF1 (-35.19±0.02 mV) indicated the greatest stability, while NEF7 (-18.91±0.27 mV) demonstrated the lowest. Each symbol formulation had a polydispersity index (PDI) of less than 0.5, which suggests homogeneity. The drug content of formulations NEF1 and NEF6 was discovered to be high, at 98.14±0.09% and 95.61±0.19%, respectively. After eight hours, diffusion tests revealed that NEF6 had the highest cumulative drug release (CDR) (92.02%), followed by NEF1 (91.76%) and NEF5 (89.45%).A 5% flurbiprofen nanoemulgel was developed using 1% gelling agent Carbopol 934. The globular, 51–120 nm-sized nanoemulsion droplets were verified by scanning electron microscopy (SEM). The drug content of the nanoemulgel were 98.2±0.41%, its pH was 5.8±0.63, its viscosity was 16,254±0.74 cps, and its spreadability was 76±0.22 cm. According to stability studies, the nanoemulgel held its stability at 4°C.

Conclusion

The research demonstrated that peppermint oil serves as an effective and cost-efficient excipient for nanoemulsion delivery systems. Utilizing a 1:2 ratio of Tween 80 and PEG 400 as the surfactant and co-surfactant significantly improved the stability of the nanoemulsion. The pseudo-ternary phase diagram revealed an expanded nanoemulsion region at a 1:7 ratio of oil to S_{mix}. High-pressure microfluidization effectively produced the flurbiprofen-loaded nanoemulsion, with NEF1 identified as the optimized formulation. Scanning electron microscopy (SEM) confirmed that the nanoemulsion droplets were globular, consistent with oil-in-water nanoemulsions. Comprehensive testing, including viscosity, drug release, and stability assessments, validated the effectiveness of the optimized formulation. The 5% flurbiprofen nanoemulgel showed considerable promise for topical drug delivery, with in- vitro diffusion studies confirming a high cumulative drug release of 97.4±0.11%.

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