Design, Optimization, in vitro and in vivo Evaluation of Flurbiprofen Loaded Solid Lipid Nanoparticles (SLNs) Topical Gel

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ABSTRACT
Background: The aim of present study was to carry out the formulation design and evaluation of Solid Lipid Nanoparticles (SLNs) loaded topical gel of Flurbiprofen. Materials and Methods: Flurbiprofen solid lipid nanoparticles (SLNs) were formulated using Glyceryl monostearate (GMS) as lipid matrix by solvent evaporation method followed by probe sonication. A 3² full factorial design was utilized to optimize the SLNs. Drug: lipid ratio and sonication time were chosen as independent variables. Particle size, entrapment efficiency (%) and PDI were dependent variables. The optimized Flurbiprofen SLNs was formulated as topical gel and screened for pH, spreadability, drug content, viscosity, drug release study, TEM analysis and in vivo skin irritation studies. Results: The optimized SLNs-gel had particle size of 369.8 nm, entrapment efficiency of 70.66% and PDI of 0.241. The zeta potential was found to be -33.4 mV resembling the better stability. Drug release study showed sustained release pattern with maximum release of 96.78% up to 10 hr following Highuchi kinetics mechanism. TEM study revealed that formulation had particle size in the nano scale range. In vivo skin irritation study on wister rats proved that formulation didn’t had any signs of irritation. Conclusion: Based on the obtained results, the study concluded that SLNs based Flurbiprofen gel can be better and promising topical drug delivery approach.

Keywords: SLNs, DoE, Flurbiprofen, Particle size, Zeta Potential, in vitro drug release.

INTRODUCTION
Nanotechnology and the use of nanoparticles in commercial applications have grown in popularity over the last few decades. This technology has revolutionized the manufacture of bio products, drug delivery systems and diagnostics in the field of life sciences. Nanoparticles can be produced using a variety of methods. Nano-formulations refer to tiny particles with having size range of 100-1000 nm. The medication penetrates further into systemic circulation due to its reduced particle size. Solid lipid nanoparticles (SLNs) are unlike any other smaller molecules in terms of their characteristics. Today most of the formulations are in the form of nanoparticles because of their wide application on drug penetration.

SLNs not only improve the dissolution because of their increased surface area but also provide better saturation solubility of the poor watersoluble drug. As a result, they allow simple drug accumulation in the skin, resulting in a higher concentration gradient, which promotes skin penetration and in turn increases the bioavailability of drug. Flurbiprofen is a non-steroidal anti-inflammatory drug (NSAID) with antipyretic and analgesic properties. It is also used to treat gout, the majority of osteoarthritis, rheumatoid arthritis, and sunburns in addition to inflammation. It has a number of systemic adverse effects, including stomach distress,
heartburn, and abdominal discomfort, as well as other gastrointestinal problems that are more noticeable during oral treatment.\(^7\)

Flurbiprofen has a relatively short half-life and must be taken in numerous dosages three to four times each day. As a result, topical formulations are chosen to avoid gastrointestinal irritation, and also to achieve local and systemic effects, and deliver sufficient amount of drug to its targeted site. As a result, topical Flurbiprofen formulation promotes skin permeability, which is beneficial in the treatment of inflammation and pain.\(^8\)\(^9\)

Even yet, significant drawbacks emerged, such as poor solubility, a reduced dosage, and some skin allergic reactions. As a result, the use of nanotechnology of pure drug has become a viable option for improving bioavailability. The production of solid lipid nanoparticles (SLNs) is one of the more intriguing techniques.\(^10\)

The aim of current study was to develop and characterize Flurbiprofen nano gel for topical use. The Flurbiprofen loaded SLNs were prepared by solvent evaporation method using Glyceryl monostearate (GMS) as a lipid. Design Expert software was used to investigate the effect of independent variables on dependent variables. A three-factor three-level study design was opted to optimize the Flurbiprofen SLNs with response surface methodology.\(^14\)

The independent factors were selected at three different levels, low (-1), medium (0) and high (+1) resulting in a three-level factorial randomized quadratic design with 10 experimental trials. The implied model can be explained by following quadratic equation exhibiting coefficient effects, interactions and polynomial terms.\(^15\)\(^-\)\(^16\)

\[
Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_1^2 + b_{22}X_2^2 + \ldots
\]

\(\text{(Equation 1)}\)

Where, \(Y\) is measured response associated with each factor level combination, \(b_0\) is an intercept, \(b_1\) to \(b_{22}\) are regression coefficients computed from the observed experimental values of \(Y\) and \(X_1\) and \(X_2\) are the coded levels of independent variables. The experimental design and the actual values of the independent variables are given in Table 1 and the experimental runs at different drug: lipid ratios and time of sonication were represented in Table 2.

### Preparación de Flurbiprofen SLNs

Flurbiprofen-loaded SLNs were prepared by solvent evaporation followed by probe sonication method. In this method, at first Flurbiprofen and GMS were dissolved in equal ratio of methanol and chloroform.

| Table 1: 3 Level factorial design variables for SLNs of Flurbiprofen with independent and dependent variables. |
|-------------------------------------------------|---------------------------------|-----------------|
| **Factors**                                     | Levels, actual (coded)          |                  |
|                                                 | -1 (Low)                       | 0 (Medium)      | +1 (High) |
| **Independent variables**                       | A = Flurbiprofen: GMS ratio (W:W) | 1:1             | 1:2       | 1:3       |
|                                                 | B = Sonication time (min)      | 5               | 7.5       | 15        |
| **Dependent variables**                         | R1 = Particle size (nm) (R1)   |                  |           |
|                                                 | R2 = Entrapment efficiency (%) (R2) |               |           |
|                                                 | R3 = PDI (R3)                  |                  |           |

### MATERIÁLIS Y METODOS

**Materiales**

Flurbiprofen pure drug was procured from FDC limited, Mumbai, India. GMS, HPMC, Carbopol-934, PVA, Pluronic F-127 chloroform and methanol were procured from HiMedia lab, Mumbai, India. Methyl paraben, Propyl paraben and Triethanolamine were purchased from Loba chemie, Mumbai, India. All the chemicals and reagents used were of analytical grade.

**Métodos**

**FTIR spectral study for drug-polymer compatibility**

The compatibility between the drug and polymers used was evaluated by FTIR spectroscopy using ATR-Bruker FTIR spectrophotometer. The FTIR spectra of pure drug of Flurbiprofen, GMS and their physical mixture were obtained and from the IR interpretation study the compatibility was confirmed.\(^13\)
This organic solvent mixture containing drug and lipid was added drop wise to aqueous solution of Pluronic F-68 which was maintained at temperature of 65-70°C followed by magnetic stirring at 1200 rpm. The heating was continued till the organic solvent evaporated and stirring was continued for 5 hr for pre-size reduction. The SLNs were then sonicated with a probe sonicator for required time duration asper the experimental design. The optimized batch of SLNs was incorporated to formulate the nano gel of Flurbiprofen.17,18

**Evaluation of Flurbiprofen SLNs- topical gel**

**Particle size and PDI**

The particle size and polydispersity index (PDI) of all the formulated SLNs and the optimized SLNs gel was determined using the Malvern Zetasizer which employs dynamic light scattering. The measurement was done in triplicate at a fixed angle of 90° at temperature of 25°C.19

**Entrapment efficiency (%)**

Centrifugation method was used to measure the entrapment efficiency of SLNs dispersion. The SLNs dispersion was centrifuged for 40 min at 10000 rpm in a cold centrifuge, to collect the supernatant liquid. After dilution with a fresh phosphate buffer pH 7.4, the recovered liquid was filtered to determine the free drug concentration. The drug concentration was determined by UV spectrophotometer at 247nm.20

The drug entrapment efficiency was calculated by following equation:

\[
\text{Entrapment efficiency (\%) = } \left[ \frac{(C_t - C_f)}{C_t} \right] \times 100
\]

Where, \(C_t\) is the amount of total drug and \(C_f\) is the concentration of un-entrapped drug.

**Zeta potential measurement**

The zeta potential predicts the physical stability of nano-formulations. The optimized formulation was suitably diluted with ultra-pure water and the zeta potential was measured by dynamic light scattering effect at 25°C using Malvern zeta sizer.21

**Transmission Electron Microscope (TEM) analysis**

TEM analysis of optimized formulation was carried out to check the mean particle size and surface morphology. For the TEM analysis a drop of diluted sample was placed on the carbon coated copper grid surface, after staining with one drop of 2% phosphotungestic acid aqueous solution. The sample was then left for some time to dry and there after the TEM images were taken.22

**pH**

The pH of the optimized SLNs-gel was determined by using digital pH meter.23

**Spreadability**

A wooden block apparatus with a pulley at one end was used to check the spreadability. The wooden block was fitted with a glass slide. On the ground slide about 1g of gel was placed. The gel was then sandwiched between two glass slides with the same dimensions. A mass of 100g was put on top of the two slides to expel air and establish a homogenous gel coating between the slides.24

The spreadability of the formulated SLNs-gel was measured using the formula:

\[
S = M \times L / T
\]

Where, \(M = \) weight (g) applied on upper glass slide.
\(L = \) length (in cm) of the gel expanded
\(t = \) time (sec)

**Drug content**

The drug content of the optimized formulation was measured by dissolving an accurately weighed quantity in 100 ml of phosphate buffer pH 7.4. The solutions were transferred to volumetric flasks and diluted with the phosphate buffer pH 7.4. The resulting solutions were then filtered and spectrophotometrically examined at 247 nm and % drug content was determined.25

**Viscosity**

The Viscosity of the Flurbiprofen SLNs-gel was determined using Brookfield viscometer (DV-II+Pro Model) with spindle no. 96 at 25°C at different shear rates of 5, 10, 20, 50 and 100 rpm.26
Drug release study
The drug release study of the optimized Flurbiprofen SLNs topical gel was carried out using Franz diffusion cell. 100 mg of gel was placed over the dialysis membrane which was sandwiched between the donor and receptor compartment. The receptor compartment was filled with phosphate buffer pH 7.4 as dissolution medium with a magnetic bead inside. The diffusion assembly was mounted on a magnetic stirrer hot plate by maintaining temperature of 37 ± 0.5°C and magnetic stirring was operated at 50 rpm. Aliquots of 1 ml were withdrawn at every 1 hr and study was continued for 10 hr. The samples were diluted with 10 ml of buffer and absorbance of all the diluted samples was measured using UV spectrophotometer at 247 nm. The percentage cumulative drug release (% CDR) was calculated. The obtained data was further subjected to curve fitting for drug release study.27-28

Drug Release Kinetics
The data obtained from the drug release study was fitted to different release kinetic models (zero order, first order, Higuchi, and Korsmeyer-Peppas model) to understand the mechanism of drug release for the optimized formulation. The best model was selected that showed higher regression coefficient.29-30

In vivo Skin irritation study
The in vivo skin irritation study was conducted using wister rats after getting ethical approval from IAEC of NGSM Institute of Pharmaceutical Sciences, Mangaluru and Karnataka, India with IAEC certificate no. NGSMIPS/IAEC/JUNE-2021/193. Skin irritation study was carried out for optimized SLNs topical gel on Wister rats. The animals were divided into three groups (n=3). In test group, optimized Flurbiprofen gel was used, in control group plain gel was used and for standard group the animals were left untreated. Both the formulation and plain gel were applied on the hairless skin area of the animals. The animals were observed visually for any dermal reactions such as erythema and oedema for about 24, 48 and 72 hr.31-32

RESULTS AND DISCUSSION
FTIR spectral study for drug-polymers compatibility
In the IR spectrum of Flurbiprofen pure drug the prominent peaks were observed at wave length of 2917, 3387, 1739 and 1116 cm⁻¹ exhibiting the O-H (Alcohol) stretching, C-H (Aromatic) stretching, C=O (Carboxylic acid) stretching and C-F (Fluorine) stretching functional groups. The IR spectrum of pure GMS showed the characteristic peaks at 2955, 1737 and 1270 cm⁻¹ indicating the presence of O-H (Alcohol) stretching, C=O (Carboxylic acid) stretching and C-O (ether) stretching functional groups. The IR spectrum of physical mixture of Flurbiprofen with pluronic F-68 showed the peaks at 2894, 1691, 1116 and 1278 cm⁻¹ exhibiting the functional groups of O-H (Alcohol) stretching, C=O (Carboxylic acid) stretching, C-F (Fluorine) stretching and C=O (Carboxylic acid) stretching respectively. For the physical mixture of Flurbiprofen with carbopol 934 the IR peaks were obtained at 2894, 3390, 1692 and 1115 cm⁻¹ with the functional groups of O-H (Alcohol) stretching, C-H (Aliphatic) stretching, C=O (Carboxylic acid) stretching, C-F (Fluorine) stretching respectively. In the physical mixture of Flurbiprofen with GMS the prominent peaks were observed at 2955, 1737 and 1270 cm⁻¹ resembling the O-H (Alcohol), C = O (Aromatic) stretching and C-O (ether) stretching functional groups. FTIR study clearly revealed that prominent peaks obtained with drug, lipid (GMS) and their physical mixture were found to be same and there was no appearance of any additional IR peaks and no chemical interaction found. Hence both the drug and lipid used were found to be compatible with one another. The FTIR peaks of pure drug, lipid and their physical mixture were represented in Figure 1.

Optimization of Flurbiprofen SLNs
From the DoE approach, the quadratic model suggested 10 formulation batches varying the concentration of lipid and sonication time as independent factors. All
the 10 SLNs were formulated by solvent evaporation followed by probe sonication at the suggested levels. All the formulated SLNs were evaluated for the particle size, PDI and % entrapment efficiency. The obtained results of particle size, PDI and % entrapment efficiency were shown in Table 3. The particle size of all the formulated SLNs of Flurbiprofen was in the range of 347.3 to 428.6 nm, PDI was in the range of 0.173 to 0.292 and entrapment efficiency (%) was found to be in the range of 0.173 to 0.292. The study model was found to be significant with the obtained results of all the dependent variables which were verified with the ANOVA and regression analysis.

**Effect of particle size (R1) on independent factors**

From the ANOVA and regression analysis, the F and P value were found to be 12.58 and 0.0048 and the model was found to be significant. The Predicted $R^2$ of 0.5413 is in reasonable agreement with the Adjusted $R^2$ of 0.7201; i.e. the difference was less than 0.2. For particle size, the following quadratic equation was generated from the output of ANOVA results for Flurbiprofen SLNs.

**Particle size** $= +386.21 - 0.7500A - 27.75B$ (coded terms)

Where, A- Drug: lipid ratio (w:w) and B- sonication time (min)

From the plots obtained with particle size, it was observed that as the concentration of drug-lipid was increased the particle size was decreased constantly and as the sonication time was increased the particle size was found to be reduced gradually, hence the effects of both drug-lipid ratio and sonication time on particle size was found to be more significant. The contour and 3D-surface plots for particle size against the independent variables are depicted in Figure 2.

**Effect of % entrapment efficiency (R2) on independent factors**

For the factor, entrapment efficiency (%), the ANOVA study revealed the F and P value of 5.20 and 0.0414 and the model was found to be significant. The predicted $R^2$ of 0.2381 was not as close to the Adjusted $R^2$ of 0.4825 as one might normally expect; i.e. the difference was more than 0.2. For entrapment efficiency (%) the following quadratic equation was generated from the output of ANOVA results for Flurbiprofen SLNs.

**Entrapment efficiency (%)** $= +68.23 - 0.1550A + 3.52B$

Where, A- Drug: lipid ratio (w: w) and B- sonication time (min)

From the surface plots of entrapment efficiency (%), it was observed that as the concentration of drug-lipid ratio was increased the % entrapment efficiency was substantially increased and as the sonication time was increased the % entrapment efficiency was found to increase constantly, hence the effect of both drug-
lipid ratio and sonication time on entrapment efficiency (%) was also found to be significant. The contour and 3D surface plots for the factor entrapment efficiency (%) against the independent variables were depicted in Figure 3.

Effect of PDI (R3) on independent factors

For PDI, the ANOVA study suggested F value of 5.47 and p-value of 0.0372 indicating the model was found to be significant. The predicted $R^2$ of 0.1675 was not as close to the adjusted $R^2$ of 0.4981 as one might normally expect; i.e. the difference was more than 0.2. The following quadratic equation was generated from the output of ANOVA results.

$$\text{PDI} = +0.2302 - 0.0052A - 0.0333B$$

Where, A- Drug: lipid ratio (w:w) and B- sonication time (min)

With the surface plots of PDI, it was observed that as the concentration of drug-lipid ratio was increased the % entrapment efficiency was substantially increased and as the sonication time was increased the % entrapment efficiency was found to increase constantly, hence effect of both drug-lipid ratio and sonication time on entrapment efficiency (%) was found to be significant. The contour and 3D- surface plots for the factor entrapment efficiency (%) against the independent variables are depicted in Figure 4.

Numerical optimization

The desirability was found to be 1 for the selected formulation. For the selected formulation the predicted results of particle size, % entrapment efficiency and PDI by DoE software was found to be 374.8 nm, 72.8% and 0.268 and the software generated the optimized level of 0 for drug-lipid ratio and level of +1 for sonication time. At the selected levels the SLNs- topical gel of Flurbiprofen was prepared and evaluated for the parameters.

Preparation of optimized SLNs loaded Flurbiprofen topical gel

The optimized batch of Flurbiprofen SLNs was incorporated into Carbopol 934 at a concentration of 1% to get the topical nano gel. HPMC was used as viscosifier. PVA was used as stabilizer. Propyl and methyl parabens were added as preservatives. Triethanolamine in quantity sufficient amount was used as solvent to bring the pH to skin range. The final quantity of gel was made by adding distilled water as quantity sufficient. The formulation composition of optimized Flurbiprofen SLNs-gel is shown in Table 4.

Percentage error between predicted and observed results

The optimized Flurbiprofen SLNs loaded gel showed the mean particle size of 369.8 nm, % entrapment efficiency of 70.66 % and PDI of 0.241. The actual results of particle size, PDI and % entrapment efficiency for the optimized Flurbiprofen SLNs- gel were found be within ± 5% error and the study indicated that results were statistically significant at 95% of confidence interval which is highly appreciable. The selected solution and % error between predicted and observed results for the factors are shown in Table 5.
Particle size and PDI of optimized formulation

The mean particle size of optimized Flurbiprofen SLNs gel was found to be 369.8 nm and PDI was found to be 0.241. The mean particle size of the optimized formulation was found to be in the nano scale range and the PDI less than 0.3 indicates particles were mono-disperse with uniform distribution without any signs of particle segregation. The results of particle size and PDI of optimized formulation were shown in Figure 5.

Zeta potential

The zeta potential indicates the stability of nano-formulations. Zeta potential generally predicts the charge contributed to the formulation and it basically depends upon composition of dispersion and type of nano-carrier used for the formulation. The zeta potential of optimized Flurbiprofen SLNs topical gel was found to be -33.4 mV. The general thumb rule is that zeta potential more than ±25 mV is a good indication of stability for the SLNs. The negative sign in the zeta potential indicate the presence of repulsive forces and also the possible reason for negative sign corresponds to presence of fatty acids in GMS which releases them by hydrolysis in SLNs. The zeta potential of optimized Flurbiprofen SLNs topical gel is shown in Figure 6.

Transmission Electron Microscope (TEM) analysis

The surface morphology of the optimized formulation was determined with TEM analysis. The particles were found to be spherical in shape with good particle size distribution and the particles were also found to be in the nano scale range. The TEM images of the optimized Flurbiprofen SLNs topical gel were depicted in Figure 7.

Evaluation of pH, spreadability and drug content (%)

The pH of the optimized formulation was in the acceptable range of skin pH, the results of spreadability and drug content were also found to be satisfactory. The results of pH, spreadability and drug content of the optimized formulation were represented in Table 6.
Viscosity of optimized formulation

Viscosity of the optimized Flurbiprofen SLNs-topical gel was ranging from 824.66 to 8342 cps. The results of viscosity were in the acceptable range of 50-50,000 cps. The rheogram plotted demonstrated that formulation had shear thinning effects. The viscosity of the formulation was reduced as the shear rate was increased, indicating the pseudoplastic flow nature which is ideal for the semisolid formulations. The rheogram of optimized formulation was shown in Figure 8.

Drug release study

The drug release of the optimized formulation was lasted for 10 hr, and the formulation exhibited maximum of 96.78% of drug release. Initially there was burst release till 1 hr, which might be due to the easy permeation of drug through the diffusion membrane and as time proceeded, there was slow release which might be due to the swelling and erosion of polymeric lipid entrapped drug within the SLNs- gel. The study revealed that formulation had sustained release over a prolonged period. The drug release profile of the optimized formulation was shown in Figure 9.

In vivo skin irritation study

The wister rats were divided into three groups, each containing three animals, one of which served as a control and the other two were used as plain gel and test group. The animals were observed for signs of edema and erythema for about 72 hr. The results of in vivo skin irritation study revealed that that the formulation was non-irritant and non-toxic to the animal skin and found to be safe for the topical application. The images of in vivo skin irritation study for optimized formulation were shown in Figure 11.

Drug release kinetics

The release kinetics of the optimized SLNs- gel was studied using various kinetic models. Good linearity was observed with a regression coefficient ($R^2$) of 0.995 with Higuchi drug release kinetics model. The formulation followed Higuchi release kinetics mechanism indicating that release of drug might be prolonged because of polymeric swelling and by the polymeric erosion of lipid layers of nanoparticulate gel formulation. The Higuchi release kinetics mechanism of optimized formulation is shown in Figure 10.
CONCLUSION
The present study was an attempt to develop and evaluate Flurbiprofen loaded SLNs incorporated topical gel. The SLNs of Flurbiprofen were successfully prepared using GMS as nano carrier by solvent evaporation method followed by probe sonication. The prepared SLNs were optimized by Design of experiments (DoE) approach with 3² full factorial design and the best optimized batch of SLNs was incorporated as topical gel. The optimized Flurbiprofen SLNs- gel showed the results of particle size, % entrapment efficiency and PDI within the acceptable limits of predicted values. The formulation had good zeta potential for enhancing the stability. The pH, viscosity, spreadability of the topical SLNs-gel was found to be acceptable. The formulation sustained the drug release up to 10 hr and followed Higuchi release kinetics mechanism. From the in vivo animal study results, it was confirmed that the optimized formulation was non-irritant and non-toxic to animal skin. Overall the study concluded that SLNs loaded topical gel of Flurbiprofen can be effective novel drug deliver approach to enhance the bioavailability of the drug.

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CONFLICT OF INTEREST
The authors declare that there is no conflict of interest.

ABBREVIATIONS
SLNs: Solid lipid nanoparticles; GMS: Glyceryl monostearate; ANOVA: Analysis of variance; DoE: Design of experiments; PDI: Polydispersibility index; FTIR: Fourier transform infrared; TEM: Transmission electron microscope; % EE: Percentage entrapment efficiency; Cps: Centipoise; Rpm: Revolutions per minute; % CDR: Percentage cumulative drug release; hr: hours; NSAID: Non-steroidal anti-inflammatory drug; RSD: Response surface methodology.

REFERENCES
In the present investigation, Flurbiprofen loaded SLNs topical gel was prepared by solvent evaporation method. The SLNs were optimized by $3^2$ full factorial design with DoE approach, considering dependent and independent factors. The best optimized batch of SLNs of Flurbiprofen was incorporated into topical gel and it was evaluated.

- The optimized formulation showed good results of particle size, PDI, zeta potential, % entrapment efficiency, pH, viscosity and spreadability. The formulation sustained the drug release upto 10 hr with 96.78%. The in vivo animal studies proved that the formulation was non-irritant and non-toxic. The results of overall studies revealed that Flurbiprofen SLNs-gel can be better novel alternative drug delivery approach for topical application.

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**Ms. Keerthana K** has done her Post graduation in the Department of Pharmaceutics under the guidance of Dr. Sandeep DS at NGSMIPS, NITTE (Deemed to be University), Mangalore. Ms. Keerthana has completed her Master thesis on the topic “Development and Evaluation of Nanoparticles loaded with Flurbiprofen for Topical Application”

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