International Journal of PharmTech Research
CODEN (USA): IJPRIF, ISSN: 0974-4304, ISSN(Online): 2455-9563
Vol.9, No.6, pp 210-223, 2016

# Nanoemulsion Based Emulgel Formulation of Lipophilic Drug for Topical Delivery

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**Abstract : Objective:** The aim of the present work was to investigate the potential of flurbiprofen loaded nanoemulsion based emulgel for topical delivery. In order to improve the solubility, and increasing the bioavailability of drug nanoemulsion based emulgel was formulated.
**Methods:** Flurbiprofen loaded nanoemulsion were successfully prepared by using high pressure homogenizer. Selection and area of nanoemulsion based emulgel was identified by constructing pseudo ternary phase diagram in which varying concentration of oil, surfactant, co surfactant and distilled water, andthe drug concentration was same in all the formulations. The prepared nanoemulsion was subjected to the various thermodynamic stability studies. The nanoemulsion formulations were evaluated for their particle size analysis, turbidimetric evaluation, transmission electron microscope, and viscosity. The emulgel was selected by performing the spreadability test and swelling index determination. The prepared nanoemulgel were further evaluated for their pH, drug content, viscosity, *in-vitro* and *in-vivo* studies. The in-vitro skin permeation study of the optimized formulation was compared with that of marketed gel.
**Results:** A significant increase in permeability was observed in optimized nanoemulsion based emulgel (84%).The anti – inflammatory effect of the formulation F1 showed a significant increase ($p<0.05$)in percent inhibition value after 12 hours when compared with marketed gel and nanoemulsion based emulgel on carrageenan induced paw edema of rats.
**Conclusion:** These results suggested that nanoemulsion based emulgel have great potential for topical delivery of flurbiprofen.
**Keywords:** nanoemulsion, emulgel, flurbiprofen, topical delivery.

## Introduction

Recently much attention has been focused on the colloidal drug delivery systems such as micro emulsions, solid lipid nanoparticles and liposome's for topical delivery of drugs because of low side effects, high bioavailability, good patient compliance etc [1]. Nanoemulsion is a promising tool for topical delivery system and is defined as a dispersion consisting of oil, surfactant, co surfactant, and aqueous phase, which is a single optically isotropic and thermodynamically stable liquid solution with a droplet diameter usually in range of 10–200nm [2].

Nanoemulsion offers several significant advantages including low skin irritation, powerful permeation ability and high drug loading capacity for topical delivery when compared with other carriers such as micro emulsions, liposome's, or solid lipid nanoparticles. The most commonly used method for producing nanoemulsion is high pressure homogenization which can be used at both laboratory and Industrial scale.

The design of effective formulations for drugs has long been a major challenge because drug efficacy can be severely limited by instability or poor solubility in the vehicle. One of the most promising technologies is the nanoemulsion drug delivery system, which is being applied to enhance the solubility and bioavailability of lipophilic drug. The nano sized droplets leading to an enormous increase in interfacial areas associated with nanoemulsion would influence the transport properties of the drug.

Non-steroidal anti-inflammatory drugs are the most commonly used drugs to relieve pain, arthritis and inflammation [3]. Flurbiprofen, a non steroidal anti-inflammatory drug is preferred orally for the treatment of pain, inflammation, rheumatoid arthritis, osteo arthritis and sun burn. Almost 40% of potential new drugs identified by pharmaceutical companies are poorly soluble in water. The flurbiprofen shows poor aqueous solubility and oral administration causes irritation of gastro intestinal tract, renal toxicity, high first pass metabolism which results into lack of bioavailability.

In this review, one of the nano ionization techniques that seek to overcome these limitations for drug solubilization is nanoemulsion drug delivery system. Nanosized droplets leading to an enormous increase in total surface areas would influence the transport properties of the drug.

Nanoemulsion are thermodynamically stable translucent dispersions of oil and aqueous phase stabilized by an interfacial film of surfactant and co-surfactant having a droplet size of less than 300 nm [4, 5] prepared by high pressure homogenization method, followed by being dispersed into carbopol 934 based gel matrix to form nanoemulsion based emulgel . Nanoemulsion has improved permeation of many drugs over the conventional topical formulations such as emulsions [6, 7] and gels [8, 9]. This article describes the potential of nanoemulsion systems of flurbiprofen using nonirritating, pharmaceutically acceptable ingredients.

The promising method to diminish its adverse effects is to deliver the drug via skin [10]. The objective of the research work was to investigate the potential of flurbiprofen loaded nanoemulsion based emulgel for topical delivery. In order to improve the solubility and increasing the bioavailability of drug nanoemulsion based emulgel is formulated. This alternate topical route eliminates oral side effects, increases patient compliance, avoids high first pass metabolism and maintains the plasma drug level for a long period of time. Selection and area of nanoemulsion was identified by constructing pseudo ternary phase diagram by varying the concentration of oil, surfactants, co-surfactants and distilled water, whereas drug concentration in all formulations were same. The formulated nanoemulsion was evaluated for different characterization studies.

## Materials and Methods

Flurbiprofen was a gift sample from Seeko laboratories , Andhra Pradesh, triacetin, tween 80, tween 20 , carbopol 934, from Himedia laboratories Pvt ., Ltd, Mumbai, linseed oil, span 20, span 80, propylene glycol, PEG -4000, PEG- 400, iso propyl myristate, trietanolamine ( Loba Chemicals Pvt Ltd., Mumbai), olive oil from scientific chemicals, Chennai and all others were of analytical grade.

### Excipients selection

The excipients selected should be pharmaceutically acceptable, nonirritating, and non-sensitizing to the skin and fall into the generally recognized as safe (GRAS) category. Higher solubility of the drug in oil phase is an important criterion as it may help the nanoemulsion to maintain the drug in solubilized form. Another important criterion to select the surfactant is required HLB value to form the oil in water nanoemulsion should be greater than 10. Composition of mixing of low and high HLB surfactants leads to the formation of a stable nanoemulsion formulation [11, 12].

### Solubility study of flurbiprofen

The solubility of flurbiprofen in various oils, surfactants, and co-surfactants was measured, respectively. An excess amount of flurbiprofen was added into 2 ml of each of the selected oils (triacetin, linseed oil, isopropyl myristate, olive oil and combination of triacetin and linseed oil) surfactants (tween 80, tween 20, span 80, span 20), co-surfactants (propylene glycol, ethanol and poly ethylene glycol-4000) and distilled water in 5-ml stopper vials separately, and mixed by vortexing. The mixture vials were then kept at25 $\pm$ 1.0$^{o}$ C in an isothermal shaker for 72 h to reach equilibrium. The equilibrated samples were removed from

shaker and centrifuged at 3500 rpm for 10 min. The supernatant was taken and filtered through a 0.45 µm membrane filter. The concentration of flurbiprofen was determined in the various oils, surfactants, co-surfactants and water using UV- spectrophotometer at 247 nm [11]. As the ratio of co-surfactants to surfactants is the same, the turbidity of resulting nanoemulsion will help in assessing the relative efficacy of the co-surfactants to improve the nano emulsification ability of surfactants [13].

**Construction of pseudo-ternary phase diagram**

A pseudo-ternary phase diagram (Chemix Software) was constructed by titration of four component mixtures of oil, surfactant and co-surfactant with water at room temperature. After equilibrium, the mixture was observed. The sample clear or slightly bluish in appearance was determined as nanoemulsion.Based on solubility studies of drug, select the oil phase, surfactants and co-surfactants. Distilled water was used as an aqueous phase for the construction of phase diagrams. Surfactant : co-surfactant (S mix) are mixed in different weight ratios 1:0, 1:1, 1:2, 2:1, 3:1. These S mix ratios were chosen in increasing concentration of surfactant with respect to co-surfactant and increasing concentration of co-surfactant with respect to surfactant for detailed study of the phase diagrams. For each phase diagram, oil and specific S mix ratio was mixed thoroughly in different weight ratios from 1:9 to 9:1 in different glass vials. Sixteen different combinations of oil and S mix, [1:9, 1:8, 1:7, 1:6, 1:5, 2:8(1:4), 1:3.5, 1:3, 3:7(1:2.3), 1:2, 4:6(1:1.5), 5:5(1:1), 6:4(1:0.7), 7:3 (1:0.43), 8:2(1:0.25), 9:1(1:0.1),] were made so that maximum ratios were covered for the study to delineate the boundaries of phases precisely formed in the phase diagrams. Pseudo ternary phase diagrams were developed using aqueous titration method [14]. Slow titration with aqueous phase was done to each weight ratio of oil and S mix and visual observation was carried out for transparent and easily flowable o/w nanoemulsion. The mixture was visually examined for transparency. After equilibrium was reached, the mixtures were further titrated with aliquots of distilled water until they showed the turbidity. Clear, isotropic samples were deemed to be within the nanoemulsion region. All studies were repeated thrice, with similar observations being made between repeats and results of phase diagram [11, 12].

**Preparation of drug loaded nanoemulsion**

Different formulations were selected from the pseudoternary phase diagram based on the nanoemulsion region. In order to prepare flurbiprofen loaded nanoemulsion F1 – F4 as in the (Table-1), the clear oil phase was obtained by mixing menthol, camphor and methyl salicylate with combination of linseed oil and triacetin. Exactly 2.5%w/w of flurbiprofen was kept constant in all selected formulations and which dissolved in the oil phase of nanoemulsion formulations. The aqueous phase was prepared by dissolving tween 80, propylene glycol, PEG 400, egg lecithin into distilled water under magnetic stirring. Then aqueous phase was blended with oil phase using high pressure homogenizer(Micron Lab APV, Denmark) at 10000 rpm for 30 minutes and then nanosize range of flurbiprofen loaded nanoemulsions were obtained and subjected to thermodynamic stability studies

**Table 1: Formula for preparation of drug loaded nanoemulsion**

| | **S mix (2:1)** | | **S mix (3: 1)** | |
|---|---|---|---|---|
| | **F1** | **F2** | **F3** | F4 |
| **Flurbiprofen** | 2.5g | 2.5g | 2.5g | **2.5g** |
| **Triacetin + Linseed oil** | 9 ml | 14 ml | 9 ml | **14 ml** |
| **Menthol** | 2 g | 2 g | 2 g | **2 g** |
| **Camphor** | 2 g | 2 g | 2 g | **2 g** |
| **Methyl salicylate** | 2 ml | 2 ml | 2 ml | **2 ml** |
| **Tween 80** | 18 ml | 18 ml | 20 ml | **22 ml** |
| **Propylene glycol** | 9 ml | 9 ml | 10 ml | **11 ml** |
| **Distilled water** | 58 ml | 53 ml | 55 ml | **47 ml** |
| **Total** | **100 ml** | **100 ml** | **100 ml** | **100 ml** |

Egg lecithin (0.25g) and PEG 400(0.5ml) added while homogenization and acted as emulsifying agent and permeation enhancer respectively

**Characterization of nanoemulsion formulation**

**Turbidimetric evaluation**

Nanoemulsion (0.2 ml) was added to 0.1 mol/1 hydrochloric acid (150 ml) with continuous stirring (50 rpm) on a magnetic stirrer (Remi 5-MLH DX) at ambient temperature, and the increase in turbidity was measured until equilibrium was achieved using a turbidimeter (Digital nephlo-turbidity meter 132,Systronics, India) [13].

**Nanoemulsion particle size analysis**

Droplet size distribution of nanoemulsion diluted with water was determined using a photon correlation spectrometer (Zetasizer 3000 HAS, Malvern Ltd., UK) based on the laser light scattering phenomenon. Samples were diluted 200 times with purified water. Diluted samples were directly placed into the module and measurements were made in triplicate after 2-min stirring. Droplet size was calculated from the volume size distribution.

**Transmission electron microscopy**

Transmission electron microscopy (TEM), with TOPCON 002B (CECRI) operating at 100kV (Topcon, paramus, NJ) performed to characterize the morphology and structure of nanoemulsion capable of point to point resolution. To perform the transmission electron microscopy observations, a drop of the nanoemulsionwas directly deposited on the holey film grid and observed after dryingwith an experimental conditions as formulation count – 1 (flurbiprofen loaded nanoemulsion,F1), HV = 30 kV, Direct mag : 5000x , Cal: 1.577 pix/nm.

**Viscosity determination**

The viscosity of formulations (0.5 g) of each formulation F1 to F4 was taken and was determined by using Brookfield viscometer LV using spindle size 63 or 64 at 100 rpm (25°C).

***In-vitro* skin permeation studies**

*In-vitro* skin permeation study was performed on Franz diffusion cell which is a reliable method for prediction of drug transport across the skin [15] with an effective diffusional area of 0.785 cm2. 1ml of Nanoemulsion formulation (25 mg/ml flurbiprofen) were taken from each formulation (F1-F4) and placed into each donor compartment. The receiver fluid was stirred with a tiny magnetic bead at an agitation speed of approximately 100 rpm and temperature 32 ± 1° C were maintained during the experiment. Samples of 3ml were withdrawn at predetermined time interval upto 12 hr. Samples were analyzed for drug content using uv spectrophotometer at 247 nm. In the *in-vitro* release study, formulation F1 showed highest release over rat abdominal skin due to their nano-size range when compare with other 3 formulations. So formulation F1 was selected to incorporate with gelling agent for further studies [11, 12].

**Emulgel**

**Selection of gelling agent**

To design the flurbiprofen emulgel as a drug delivery system for pain, inflammation, different polymers like carbopol 934, hpmc and gelatin were used as gelling agents. They were evaluated for spreadability and swelling index determination. The spreadability and swelling index determination were performed to select the better gelling agent.

**Spreadability test**

Two glass slides of standard dimensions (2cm×5cm) were selected.100gm was placed upon the upper slide and the gel between the two slides is pressed uniformly to form thin layer. The two slides in position were fixed to stand (at angle 45º) without any disturbance and in such a way that only the lower slide was held firmly by the clamp following the upper slide to travel the distance at 0.5 cm under the direction of weight was noted. The weight was removed and the excess of gel adhering to the slide was scrapped off. The experiment was repeated and mean time taken for three such determinations was calculated.

**Swelling index determination**

1g of carbopol 934, hydroxyl propyl methyl cellulose (HPMC) and gelatin were weighed separately and added to 25 ml measuring cylinder. Distilled water was added up to the required volume. All the gelling agents

were allowed to swell for 3 hrs. The initial and final volumes occupied by the gelling agents were performed thrice for each gel base and reported [16].

**Preparation of nanoemulsion based emulgel**

1 gm of Carbopol 934 which was selected as a gelling agent in a sufficient quantity of distilled water. After complete dispersion, the carbopol 934 was kept in the dark for 24 hrs to swell completely. Triethanolamine was added into swollen carbopol 934 to adjust the pH value of gel matrix (7.4). Flurbiprofen loaded nanoemulsion formulation (F1) which showed highest invitro release were taken and incorporated with gel matrix and nanoemulsion based emulgel were prepared after stirring by remi stirrer for 15 minutes at 250 rpm.

**Evaluation of nano emulgel**

**pH determination**

Two grams of prepared gel were dissolved in the 100 ml of phosphate buffer solution and pH of the resulting solution was studied by digital pH meter with glass electrode. Add triethanolamine if required to maintain pH to 7.4, to ensure non irritating nature of the formulation [20]. The average triplicate observations were recorded.

**Drug content determination**

The amount of flurbiprofen present in the prepared gel was analyzed by using u.v.spectrophotometer. Accurately weighed quantity of prepared gel equivalent to 50 mg of flurbiprofen was extracted with 50 ml of ethanol, filtered and it was diluted to 200 ml. Further 5 ml of this filtrate was diluted to 100 ml in volumetric flask with ethanol. The absorbance of this solution was measured at 247 nm by using u.v spectrophotometer.

**Viscosity**

The viscosity of formulation nano emulgel (NEG) (0.5g) was determined by using Brookfield viscometer LV which is thermostatic at a temperature of 35 ± 1ºC. The spindle size and rpm used were 7 and 20 rpm respectively.

***In-vitro* skin permeation study across rat abdominal skin**

*In-vitro* skin permeation studies were performed on a Franz diffusion cell with an effective diffusional area of 0.785 cm2. The full thickness rat abdominal skin was excised and hair was removed with a sterilized blade. The skin was kept at room temperature and mounted between the donor and receptor compartment of the Franz diffusion cell where the stratum corneum side faced the donor compartment and the dermal side faced the receptor compartment. The whole system was sandwiched between donor and receptor compartment and secured with clamp. The receptor compartment was filled with ethanolic phosphate buffered saline pH 7.4 (20:80% v/v). The formulation F1 provided the highest release as compared with other nano emulsion formulations. The formulation and was coded as NEG (Nano emulgel). The skin permeation profile of the optimized nanoemulsion based emulgel (25mg/g) was compared with marketed available gel (50mg/g).1g of nanoemulgel(25 mg/g flurbiprofen) and 1g of marketed gel(50mg/g flurbiprofen) were taken separately and placed into each donor compartment. The receiver fluid was stirred with a tiny magnetic bead at an agitation speed of approximately 100 rpm and temperature 32 ± 1° C were maintained during the experiment. Samples of 3ml were withdrawn and replaced with fresh medium at predetermined time interval upto 12 hr. Samples were analyzed for drug content using u.v spectrophotometer at 247 nm.

***In-vivo a*nti-inflammatory effects**

Procedures and protocols were reviewed by the institutional animal ethical committee [Reg.No:688/02/C/CPCSEA] and IAEC guidelines were followed for the studies. Anti-inflammatory effects of the optimized formulations nanoemulgel and marketed gel were evaluated by carrageenan induced paw edema method developed by *Winter etal* in Wistar rats. Albino rats of wistar strains of either sex weighing between 140 – 170 grams were selected for the studies. The animals were divided into four groups in each group as control, optimized nanoemulgel, and marketed available gel comprising four animals each. The animals were kept under standard laboratory conditions at a temperature of 25 ± 1°C and relative humidity of 55 ± 5%. The animals were housed in polypropylene cages, with free access to standard laboratory diet.25mg and 50mg of

respective formulation of nanoemulgel and marketed gel were applied (except in control group) to the right paw half an hour before carrageenan was injected (0.1 ml 1%) subcutaneously. Paw edema was induced by injecting 0.1 ml of 1 % w/v dispersion of carrageenan in distilled water. Paw volume was measured by plethysmometer at different time intervals (1, 2, 4, 8 and 12 hours). The amount of paw swelling was determined time to time and expressed as percent edema. Percent inhibition of edema produced by each formulation-treated group was calculated against the respective control group. Test was carried out for a period of 12 hours and % inhibition of edema was calculated. Results of these studies were compared using Dunnett test of one-way analysis of variance (ANOVA) [16, 17].The average paw swelling in this drug test group was compared with that of control rats and percentage inhibition was determined.

**Skin irritation study**

Skin irritation study was carried out on swiss albino mice weighing 20-30 g. The animals were kept under standard lab conditions. The animals were housed in polypropylene cages with free access to a standard laboratory diet. A single dose of nano emulgel was applied once a day for seven days to the left ear of the mice with right ear as control and the site was observed for any sensitivity and reaction. The development of erythema was monitored for 7 days using the reported method of van-abbe et al [17].

**Thermodynamic stability studies**

To overcome the metastable formulation, thermodynamic stability tests were performed. Selected formulations were centrifuged at 3500 rpm for 30 min. Those formulations that did not show any phase separation were taken for heating and cooling cycle. Six cycles between refrigerator temperature (40$^{o}$C) and 450$^{o}$C with storage at each temperature of not less than 48 hours were studied. Those formulations which were stable at these temperatures were subjected to freeze thaw cycle test. Three freeze thaw cycles between -210$^{o}$C and +250$^{o}$C with storage at each temperature for not less than 48 hours were done for the formulations. The formulations survived thermodynamic stability tests were selected for further studies.

## Results and Discussion

**Screening of excipients**

**Excipients selection**

In this study, combination of triacetin and linseed oil were selected as oil phase because it shows higher solubility of drug and higher emulsification ability. Tween 80 was selected as a surfactant due to an HLB value of 15, their higher solubility with drug and higher emulsification ability. Transient negative interfacial tension and fluid interfacial film are rarely achieved by the use of single surfactant. The addition of co-surfactant is necessary. Thus, the co-surfactant selected was propylene glycol due to their solubility potential with drug and higher emulsification ability.

The values of the oil, surfactant and co-surfactant showed that solubility of the lipophilic drug flurbiprofen was found to be highest in combination of triacetin and linseed oil 10.78± 0.072 (mg/ml) followed by triacetin9.21 ± 0.147(mg/ml), isopropyl myristate3.35 ± 0.0818 (mg/ml) at 25$^{o}$c. Solubility of drug in these oils was significantly high than in linseed oil and olive oil. All the surfactants showed good solubility of the drug .Among the surfactants tested in this study, tween 80 showed 388.122± 0.084 (mg/ml) at 25$^{o}$c, a medium-length alkyl chain with HLB 15 was selected as appropriate surfactant becausenon-ionic surfactants were less toxic than ionic surfactants, and has good biological acceptance, powerful permeation enhancer, less affected by pH, ionic strength, and highest solubility of flurbiprofen was obtained. Furthermore, propylene glycol, ethanol160.95 ± 0.246 mg/ml and PEG 4000 121.46 ± 0.582 mg/ ml were taken as co surfactant in the solubility study of the drug. In this propylene glycol 172.91 ± 0.706 (mg/ml) at 25$^{o}$c were selected as co-surfactant because of their potential to solubilize the drug

From results it was inferred that the oily phase as combination of triacetin and linseed oil exhibited the highest emulsification efficiency with tween 80 (98%), requiring only 5 flask inversions for homogenous emulsion formation. Addition of a co-surfactant to the surfactant-containing formulation was reported to improve dispersibility and drug absorption from the formulation. In as reported in( Table.2), the combination of triacetin and linseed oil exhibited good emulsification with both co-surfactants, i.e. propylene glycol showed maximum transmittance (92.6%) followed by ethanol (88%).

**Table 2: Percent transmittance of surfactants and co surfactants**

| Surfactant | % transmittance Tween 80 | Co surfactants | % transmittance Triacetin + Linseed |
|---|---|---|---|
| Triacetin + Linseed Oil | 98.00 ± 0.24 | Propylene glycol | 92.61 ± 0.69 |
| Triacetin | 82.01 ±0.66 | Ethanol | 88.02 ± 0.63 |

**Values are expressed as mean** ±SD (n= 3)

**Construction of pseudo ternary phase diagram**

The pseudo-ternary phase diagrams were constructed to identify the nanoemulsion regions with maximum drug loading and to optimize the concentration of oil, surfactant and co-surfactant in the nanoemulsion formulations and to obtain transparent and stable o/w nanoemulsions. The shaded areas in the pseudo-ternary phase-diagrams shown in figures represents the existence field of stable, clear and transparent o/w nanoemulsions containing triacetin and linseed oilas oil phase with the tween 80:propylene glycol as fixed mixing ratio and the rest of the region on the phase diagram represents the turbid and conventional emulsions based on visual observation [15]. For any selected composition of surfactant and co-surfactant ratio from self-emulsifying region of ternary phase diagram (shaded) the addition of great volumes of continuous phase (aqueous phase) allowed the clear system.

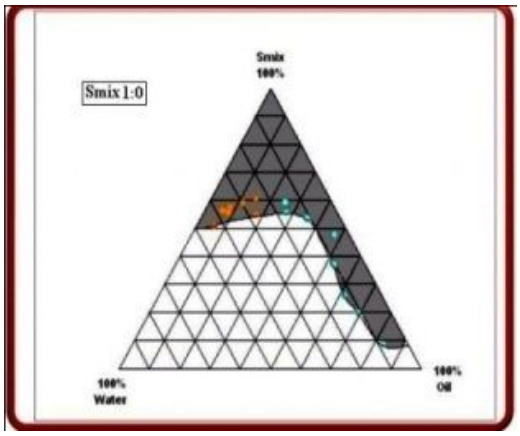


**Fig. 1:Pseudoternary phase diagram of S mix ratio 1:0**

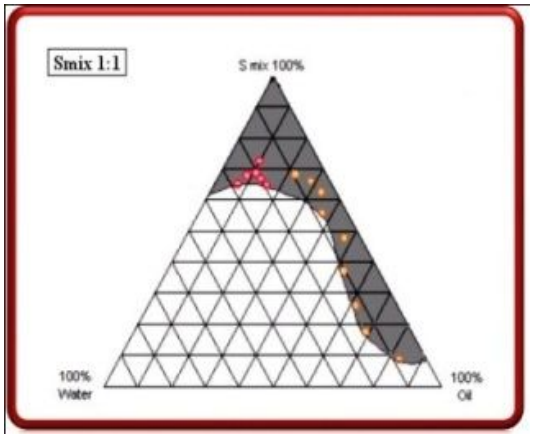


**Fig .2: Pseudoternary phase diagram of S mix ratio 1:1**

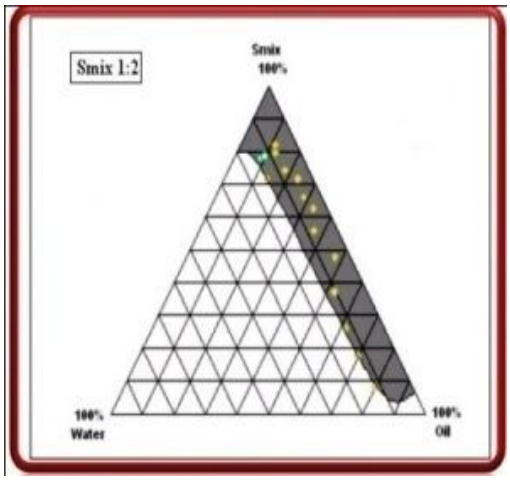


**Fig .3: Pseudoternary phase diagram of S mix ratio1:2**

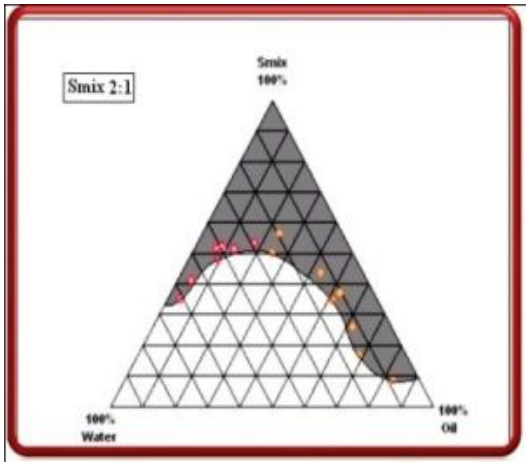


**Fig. 4: Pseudoternary phase diagram of S mix ratio 2:1**

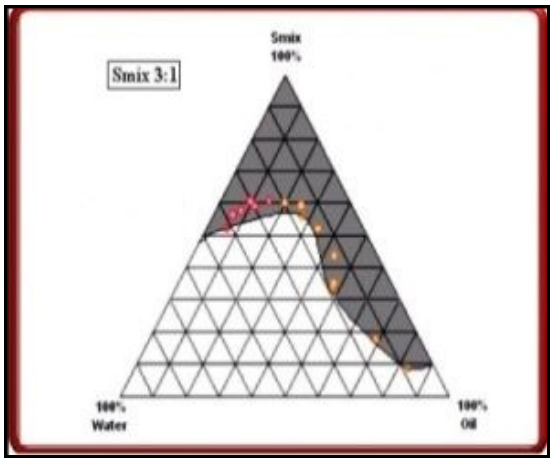


**Fig. 5: Pseudoternary phase diagram of S mix ratio 3:1**

Above (Fig1-5**)** represents the phase diagram of triacetin + linseed oil (oil phase) s mix (tween 80 and propylene glycol) -water system having different s mix ratio (1:0, 1:1, 1:2, 2:1, 3:1). The nanoemulsion region exists at s mix ratio 1:0 (i.e. without co-surfactant). However, equal mixture of surfactant and co-surfactant decreases the nanoemulsion region (Fig.2). Increasing the concentration of surfactant (2:1) resulted in even large area of nanoemulsion region (Fig.4). Further increasing surfactant concentration from 2:1 to 3:1 resulted little reduction on nanoemulsion region (Fig. 5). The influence of concentration of co-surfactant on the nanoemulsion region was also seen by constructing the phase diagram in ratio of 1:2. It was seen that the region of nanoemulsion was decreased with increase in concentration of co-surfactant **(**Fig. 3). The existence of large or small nanoemulsion region depends on the capability of a particular surfactant or surfactant mixture to solubilize the oil phase. With further increase in surfactant from 1:1 to 2:1, further drop in interfacial tension and free energy was achieved resulting in maximum region of nanoemulsion/self-emulsifying formation. 3:1 showed little reduction in nanoemulsion area when compare with 2:1. Thus, pseudo-ternary phase diagram for s mix 2:1 and 3:1 were selected for the formation of drug loaded nanoemulsion drug delivery system.

**Selection of formulation from pseudo ternary phase diagram**

From the data shown in different pseudo-ternary phase diagrams (Fig. 4, 5), it was understood that oil could be solubilized up to the extent of 30% w/w. Therefore, from phase diagram different concentrations of oil, which formed nano emulsions, were selected at a difference of 5 %,( Table 3) so that maximum formulations could be prepared covering the nanoemulsion/self-emulsification area of the phase diagram. There was no sign of change in the phase behavior and nanoemulsion area of phase diagrams, when flurbiprofen was incorporated into the formulations, which was indicated as the formation and stability of nanoemulsions consisting of nonionic components, is not affected by the pH or ionic strength[12, 13].

**Table 3: Compositions of selected nanoemulsion formulations**

| S mix Ratio | Oil | S mix | Water | Oil : S mix | Code |
|---|---|---|---|---|---|
| **2:1** | 15 | 27 | 58 | 1:1.8 | **F1** |
| **2:1** | 20 | 27 | 53 | 1:1.35 | **F2** |
| **3:1** | 15 | 30 | 55 | 1:2 | **F3** |
| **3:1** | 20 | 33 | 47 | 1:1.55 | **F4** |

**Characterization of nanoemulsion formulations**

**Turbidimetric evaluation**

The results of turbidimetric evaluation of nanoemulsion are presented in (Table 4).Formulations F1 and F3 showed low turbidity values, owing to the presence of adequate amounts of surfactant (tween 80), which primarily governs the resultant droplet size and its distribution. Formulations F2 and F4 with moderate quality of emulsion formation because of high concentration of oil and showed very high and variable turbidity and

coarser droplets. Thus the droplet size distribution is strongly dependent on concentration of surfactant / co surfactant.

**Particle size analysis**

Size characterization of nanoemulsionis essential for ensuring safe and efficient dosage and drugbioavailability [18]. The droplet size increases with the increase in concentration of oil in the formulations. The droplet size of formulation F1 which contains 15% of oil was lowest (191±1.24). The droplet size of formulation F4 was highest (219±4.33). All the formulations had droplets in the nano scale range. Droplet size analysis revealed the effect of varying amounts of tween 80 and propylene glycol in the formulated nanoemulsion. Changes in tween 80 to propylene glycolratios are most likely to alter the resultant HLB of the system and the properties of liquid crystal interfaces. This in turn governs the size of droplets formed. Thus it is the appropriate choice of surfactant and co-surfactant together with their proper concentrations, which provides an optimum self-emulsifying formulation. The mean droplet sizes of the reconstituted nano emulsions are in between 190 to 219 nm as reported in(Table.4). The data for particle size analysis for various flurbiprofen loaded nanoemulsion were measured by laser scattering spectroscopy.

**Viscosity determination**

The viscosity of the selected formulations was determined and the viscosity of formulation F1 (135.01 ± 1.41 cp) was lower than that of any other formulation (Table 4). The viscosity of formulation F4 was highest (150.71 ± 2.75 cp), but it was observed that the viscosity of the nanoemulsion formulations generally was very low and also confirmed formulation of o/w type of nanoemulsions [19].

**Table 4: Characterization study of nanoemulsion formulations**

| Formulation Code | Oil | S Mix | Aqueous phase | Droplet Size (nm) | Viscosity (cp) | Turbidimetry (ntu) |
|---|---|---|---|---|---|---|
| F1 | 15 | 27 | 58 | 191.0±1.24 | 135.01±1.12 | 23.1±1.28 |
| F2 | 20 | 27 | 53 | 205.0±1.96 | 147.58±1.14 | 41.1±3.41 |
| F3 | 15 | 30 | 55 | 199.0±1.34 | 139.55 ±1.14 | 31.2±2.87 |
| F4 | 20 | 31 | 49 | 219.0±2.33 | 150.71±1.13 | 82.1±12.8 |

**Transmission electron microscopy (TEM)**

The morphology and structure of the nanoemulsion were studied using (TEM). A combination of bright field imaging at increasing magnification and of diffraction modes was used to reveal the form and size of the nanoemulsion. To perform TEM, nanoemulsion was diluted with water (1/100). A drop of the diluted nanoemulsion was directly deposited on the holey film grid and observed after drying. Some droplet sizes were measured using TEM, as it is capable of point-to-point resolution. The droplet size was in agreement with the results obtained from particle size analysis. A positive image was seen using TEM (Fig. 6).

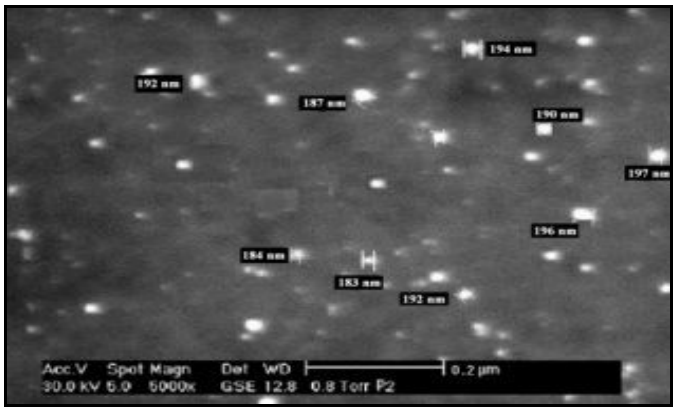


**Fig.6: Transmission electron microscopic (TEM) image of flurbiprofen nanoemulsion showing size of some of the oil droplets**

*In-vitro* skin permeation studies were performed to compare the release of the drug from 4 different nanoemulsion formulations (F1 to F4) which is having the same quantity of flurbiprofen (2.5% wt/wt). *Invitro* skin permeation was highest in formulation F1 (91.42%) and lowest in formulation F4 (62.31%). The maximum release in formulation F1 could be due to having the lowest droplet size and lowest viscosity of the nanoemulsion(Fig-7). In this *in-vitro* permeation study across rat abdominal skin, formulation F1 showed highest release and selected to incorporate with the carbopol 934 gel base for further studies.

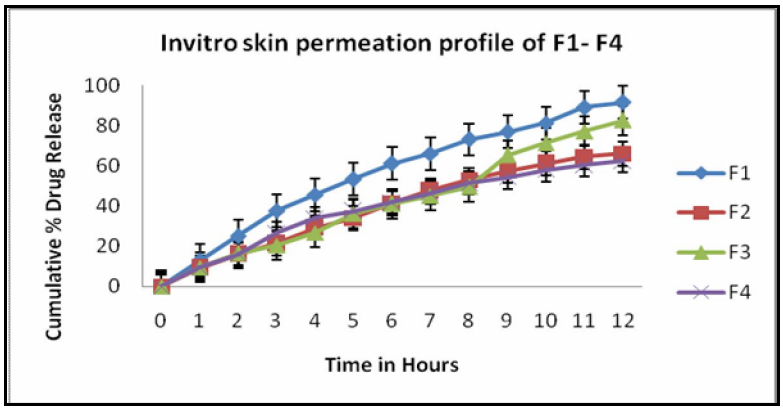


**Fig.7: Graph of *in vitro* skin permeation study for the formulations F1-F4**

**Thermodynamic stability studies**

Nanoemulsions are thermodynamically stable systems and are formed at a particular concentration of oil, surfactant and water, with no phase separation, creaming or cracking. It is the thermo stability which differentiates nanoemulsion from emulsions that have kinetic stability and will eventually phase separate. Thus, the selected formulations were subjected to different thermodynamic stability testing by using heating cooling cycle, centrifugation and freeze thaw cycle stress tests. The formulations that survived thermodynamic stability tests were selected for further study (Table 5). Thus it was concluded that the efficiency of surfactant and co-surfactant mixture was unaffected after exposing to extreme conditions.

**Table 5: Data for thermodynamic stability test of different formulations**

| Group I and Group II (S mix 2:1, S mix 3:1) | Thermodynamic stability studies | | |
|---|---|---|---|
| **Formulations** | **Heating cooling cycle** | **Centrifugation** | **Freeze thaw cycle** |
| F1 | √ | √ | √ |
| F2 | √ | √ | √ |
| F3 | √ | √ | √ |
| F4 | √ | √ | √ |

**Emulgel: selection of gelling agent**

**Spreadability test & swelling index determination**

This method was developed to assess the spreading properties of various gelling agents. The test method was developed to study the amount of spreadability of sample exhibits and be able to characterize materials based on their spreadability. Determination of swelling index was based on the addition of distilled water and allowed to swell for 3 hours. The initial and final volumes occupied by the gelling agents were performed thrice and mean taken was reported in the table. Carbopol 934 showed maximum swelling property and selected to incorporate with nanoemulsion(Table.6).

**Table 6: Spreadability and swelling index determination**

| S. No | Gelling Agents | Swelling Index (ml) | Spreadability Test (cm) |
|---|---|---|---|
| 1 | Carbopol 934 | 8.5 ±0.23 | 7.08 ±0.25 |
| 2 | HPMC | 5.0 ±0.25 | 5.66 ± 0.21 |
| 3 | Gelatin | 4.7 ± 0.31 | 4.95 ± 0.30 |

**Values are expressed as mean** ±SD (n= 3)

**Evaluation of nano emulgel (neg)**

The average pH of optimized Nano Emulgel was found to be 7.4 permitting the use of the formulation on the skin. The amount of drug present and percentage purity of the Nano Emulgel were found to be 2.46g and 98.44% w/w respectively. The results showed that the drug was minimum while formulating nanoemulgel.

The viscosity of nanoemulgel (0.5g) were determined by using Brookfield viscometer LV using spindle size 7 at 20 rpm (35°± 1ºC) and the viscosity was reported as 6650 cp. The thickened system is expected to offer good biophysical and sensorial benefits for the topical delivery [21] influence of order of addition of carbopol 934 on the formation of nanoemulgel was also investigated [21,22] .

Carbopol 934 was swollen in aqueous phase and its pH was adjusted with triethanolamine and then it was incorporated in oily phase containing surfactant, co surfactant and drug resulting in homogeneous nanoemulgel formation. The gelling behavior of carbopol may be due to non- covalent intermolecular associations deriving from forces such as columbic vanderwaals and hydrogen bond interaction. These physical interactions could lead to formulation of three dimensional gel networks and dispersed oil droplets were reasonably hosted within meshes of these networks [23].

**Comparative *in-vitro* skin permeation study of the optimized nanoemulgel and marketed product**

The *in-vitro* diffusion profile of the optimized emulgel (2.5%) and marketed available gel (5.0%) were compared .The optimized nanoemugel showed a maximum cumulative release of 84.86 % over a period of 12 hr, while marketed gel showed a maximum cumulative release of 77.48 %. It may be due to the high drug loading capacity and powerful permeation ability. The maximum release in the optimized formulations may be due to having the lowest droplet size and lowest viscosity of all the formulations. The comparative skin permeation study of optimized nanoemulgel and marketed gel was showed on the (**Fig.8**).

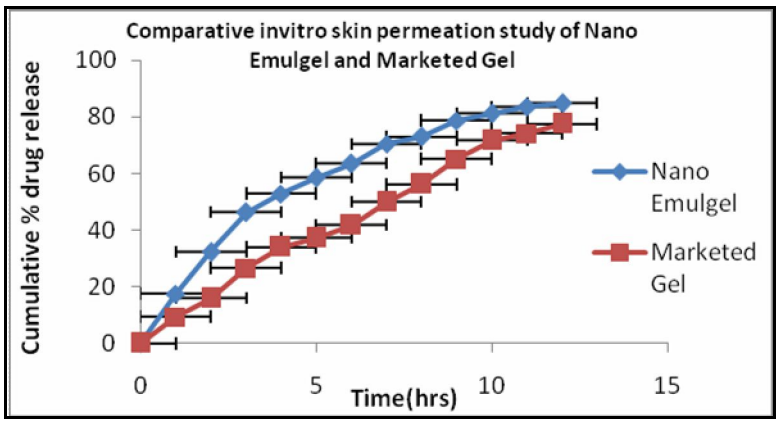


**Fig.8: Comparative i*n vitro* skin permeation study of optimized nanoemulgel formulation and marketed gel**

***Invivo* anti-inflammatory effects**

The selected nanoemulgel and marketed gel were used for the study of in-vivo anti-inflammatory effects. The anti-inflammatory and sustaining action of the optimized formulation was evaluated by the carrageenan induced paw edema method developed by Winter et al [24] in rats. The percentage inhibition value after 12 hours of administration was found to be high for nanoemulgel (85.2%) as compared with (68.0%) for

marketed gel(Fig - 9).The difference between nanoemulgel and marketed gel percentage inhibition was significant (< 0.5) (Table 7). The enhanced anti-inflammatory effects of optimized nanoemul gel formulation could be due to the enhanced permeation of flurbiprofen through the skin.

**Table 7:Anti-Inflammatory effects of NEG and MG in carrageenan induced rat paw edema**

| Group | Formulation | N | Time (hrs) | Mean % Edema ± SD | % Inhibition |
|---|---|---|---|---|---|
| I | Control | 3 | 1<br>2<br>4<br>8<br>12 | 4.46±0.10**<br>4.56±0.10**<br>2.95±0.17<br>2.49±0.27<br>1.525±0.07 | -<br>-<br>-<br>-<br>- |
| II | Carrageenan | 3 | 1<br>2<br>4<br>8<br>12 | 6.51±0.07**<br>7.39±0.14**<br>7.98±0.17**<br>4.49±0.23**<br>2.53±0.72** | -<br>-<br>-<br>-<br>- |
| III | Optimized Nano emulgel | 3 | 1<br>2<br>4<br>8<br>12 | 5.22±0.22**<br>7.15±0.22**<br>7.22±0.90**<br>4.02±0.11**<br>2.01±0.08** | 19.87<br>40.0<br>57.1<br>75.0<br>85.2 |
| IV | Marketed Gel | 3 | 1<br>2<br>4<br>8<br>12 | 3.95±0.07*<br>6.37±0.11**<br>6.89±0.71*<br>4.22±0.19**<br>1.92±0.12* | 17.66<br>35.55<br>51.1<br>61.7<br>68.0 |

N= Number of rats in each group; SD = Standard deviation
Values are expressed mean ± SEM
(*) P<0.01 when compared to normal control
(**) P<0.01, P<0.05 when compared to Carrageenan Control
[Dunnet test of one-way analysis of variance (ANOVA)]

Based on higher drug permeation, nano size and low viscosity, formulation $F_1$ was selected to incorporate with gelling agent and optimized a nanoemulgel. The selected nanoemulgel and marketed gel were used for the study of invivo anti-inflammatory effects. The anti-inflammatory and sustaining action of the optimized formulation was evaluated by the carrageenan induced paw edema method developed by winter et al [24].in rats. The percentage inhibition value after 12 hours of administration was found to be high for nanoemulgel (85.2%) as compared with (68.0%) for marketed gel (Table 7). The difference between nanoemulgel and marketed gel percentage inhibition was significant (< 0.5). The enhanced anti-inflammatory effects of optimized nanoemulgel formulation could be due to the enhanced permeation of flurbiprofen through the skin.

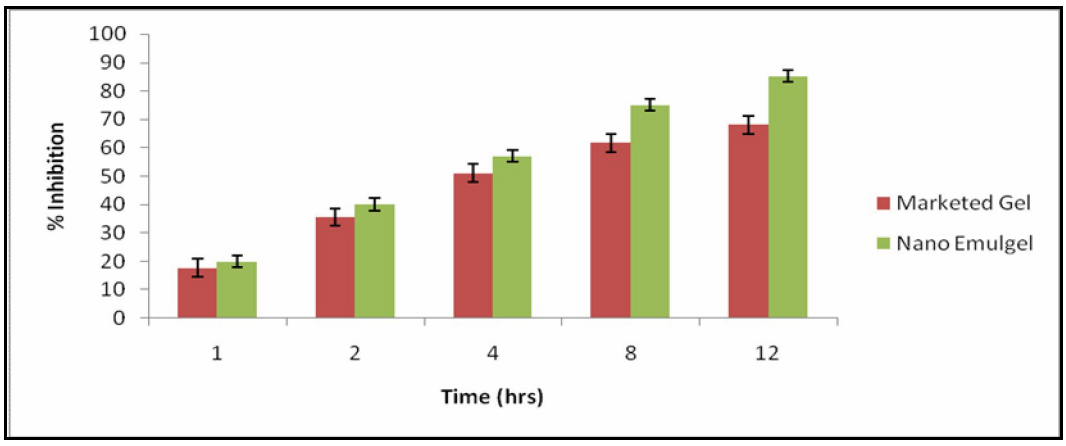


**Fig.9: Comparison of anti-inflammatory effects of optimized nanoemulgel and marketed gel**

### Skin irritation study

Skin irritation study was performed to confirm the safety of the optimized nano emulsion based emulgel van-abbe et al [24] mentioned that a value between 0 and 9. It indicates that the applied formulation is generally not an irritant to human skin. The mean skin irritation score for optimized nanoemulgel formulation and marketed gel were found to be 1.8 ± 0.53 and 2.6 ± 1.03 respectively (Table.8).Optimized nanoemulgel was showed less irritation profile when compared with Marketed product. It may be due to the fewer dose of flurbiprofen (2.5%) in nanoemulgel. From this study it was concluded that the optimized nanoemulgel formulation was safe to be used for topical drug delivery.

**Table 8: Data for skin irritation study**

| S.No | Group | Score after (Days) | | | | | Mean score | Standard deviation |
|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 5 | 7 | | |
| 1 | **Nano Emulgel** | 2 | 2 | 2 | 2 | 1 | 1.8 | 0.53 |
| 2 | **Marketed gel.** | 3 | 2 | 3 | 3 | 2 | 2.6 | 1.03 |

## Conclusion

A nanoemulsion based emulgel with flurbiprofen was formulated and concentrations were optimized after the evaluation of in vitro studies The in-vivo studies of the emulgel was compared with marketed gel, which revealed a significant increase in skin permeation profile and anti-inflammatory effects. From in vitro and in vivo data it can be concluded that optimized Flurbiprofen loaded Nanoemulsion based emulgel have great potential for topical delivery.

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