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FORMULATION AND EVALUATION OF ZALTOPROFEN NANOEMULSION GEL

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Abstract:

Aim: The aim of the present study was to formulate and evaluate nanoemulsion gel of Zaltoprofen to sustain drug release, retard dosing frequency and minimise side effects associated with oral administration.

Method: Solubility studies were conducted to select the oil, surfactant and co-surfactant. Phase diagrams were constructed by aqueous phase titration method. The prepared nanoemulsions were subjected to thermodynamic stability tests, *in-vitro* diffusion studies, viscosity, percent transmittance, globule size, zeta potential and polydispersity index. Nanoemulsion gel was evaluated for pH, physical appearance, viscosity, drug content, drug-excipient compatibility studies and *ex-vivo* skin permeation study.

Result: The results showed that all the formulations had a good stability. Based on the *in-vitro* drug release studies the formulations were optimized. The optimized formulation F7 containing Capryol®90 (23.91%), Cremophor®EL (10.87%), Carbitol® (32.61%) and water (32.60%) showed 83.22% drug release in 12 h. The droplet size of the optimized formulation (F7) was found to be 82.0 nm and zeta potential was found to be -36.6 mV. *Invitro* permeation rate of nanoemulsion and conventional gel of Zaltoprofen were determined. Permeability parameters like steady-state flux (Jss), permeability coefficient (Kp), and enhancement ratio (Er) were significantly increased in nanoemulsion F7 and nanoemulsion gel F7G as compared to conventional gel.

Conclusion:

The results indicate that nanoemulsion gel of Zaltoprofen can be used successfully for transdermal delivery and improved patient compliance.

Keywords: Nanoemulsion gel, sustained release, Topical drug delivery, Zaltoprofen.

1. Introduction:

Nanoemulsions are thermodynamically stable transparent dispersions of oil and water stabilized by an interfacial film of surfactant and co-surfactant molecules having a droplet size of less than 100 nm. The nanosized droplets leading to enormous interfacial areas associated with nanoemulsions would influence the transport properties of the drug, an important factor in sustained drug delivery. The attraction of formulating nanoemulsion systems lies in their ability to incorporate hydrophobic drugs into the oil phase thereby enhancing their solubility [1]. Nanoemulsions are made from surfactants approved for human consumption and common food substances that are 'Generally Recognized as Safe' (GRAS) by the FDA [2].

Nanoemulsions are more thermodynamically stable as compared to traditional formulations such as creams and ointments, and also have a malleable nanostructure ideal for skin permeation [3]. The advantages associated with transdermal use of nanoemulsion are as enhanced drug solubility, good thermodynamic stability and enhancing effect on transdermal ability. The nanoemulsions increase the concentration gradient and thermodynamic activity towards skin along with permeation enhancement activity of its components and make the system ideal for transdermal delivery. However, the low viscosity of nanoemulsion is a constraint in its proper application. Biocompatible gels having weak interaction with surfactants have already been explored to modify the rheological behaviour of nanoemulsion [4]. Nanoemulsion as vehicle may enhance transdermal penetration by various mechanisms. Molecules are solubilised in nanoemulsion and induce a change in the thermodynamic activity of the drug they contain, modifying their partition coefficient and thus favour penetration into stratum corneum. Furthermore, surfactant included in these formulations reduces the functional barrier of stratum corneum [5].

Zaltoprofen [2-(10, 11–dihydro–10–oxodibenzo [b, f] thiepin–2-yl) propionic acid], a propionic acid derivative, is a non-steroidal anti-inflammatory drug (NSAID) and has strong inhibitory effects on acute and chronic inflammation [6]. Zaltoprofen is used in treatment of rheumatoid arthritis and osteoarthritis as well as to relieve inflammation and pain due to surgery and any kind of injury [7]. In addition, this drug has more powerful inhibitory effects on bradykinin nociception than other NSAIDs [6]. Although it is well tolerated orally compared to other NSAIDs, it has to be administered with a dose of 80 mg three to four doses per day and is associated with ulcerogenicity, bellyache and indigestion. This makes it unsuitable for patients with gastric ulcer and other gastric disorders[8]. Therefore, it is important to develop an alternative dosage form which sustains drug release, provides local effect and makes it easier to administer. The transdermal route meets all the above advantages.

An eventual need has emerged to develop a transdermal dosage form of Zaltoprofen to provide relatively consistent drug levels for prolonged periods. The major problem associated with transdermal drug delivery is barrier properties of the stratum corneum, which is considered one of the most impermeable epithelia of the human body to exogenous substances. These permeation problems can be minimized by use of chemical permeation enhancers [9]. But, the use of these chemical enhancers may be harmful especially in chronic applications, since many of them are usually irritants. It is therefore desirable to develop a novel transdermal vehicle system that does not necessitate the use of chemical enhancers to facilitate drug permeation through the skin. One of the most promising techniques in this regard is the nanoemulsion. Furthermore, the components of nanoemulsion system are expected to act themselves as permeation enhancers thereby, circumventing the use of irritable chemical penetration enhancers [10]. The present study aims at formulating novel nanoemulsion gel of Zaltoprofen for sustained release, better applicability and better permeation potential through the skin.

2. Materials and Methods

2.1 Materials

Zaltoprofen was a kind gift from IPCA labs Ltd, Mumbai, Maharashtra, India. Labrafil M 2125 CS (Linoleoyl macrogolglycerides), Plurol Oleique® (Polyglycerololeate), and Capryol®90 (Polypropylene glycol monocaprylate) were received as a gift sample from Gattefosse, Mumbai, Maharashtra, India. Cremophor® EL (Polyoxyl 35 hydrogenated castor oil) and Cremophor® RH 40 (Polyoxyl 40 hydrogenated castor oil) were gifted by Croda Chemicals (India) Private Ltd., Mumbai, Maharashtra. Capmul® MCM (Glycerol mono-dicaprylate) and Capmul® GMO (Glycerol mono/di-oleate) were received as gift samples from Abitec, India. Other chemicals like Oleic acid, Iso-propyl myristate, Ethanol, Propylene glycol, Span® 20 (sorbitan -monolaurate), Span® 80 (sorbitan-monooleate), Tween® 20 (polyoxyethylene sorbitan monolaurate), Tween®80 (polyoxyethylene sorbitan monooleate), Polyethylene glycol 400 (PEG 400), polyethylene glycol 600 (PEG 600), Glycerol, Olive oil, Linseed oil, Castor oil and Methanol were purchased from Merck India. Carbopol®934 and Carbopol®940 were obtained as gift samples from Loba Chemie, Mumbai, Maharashtra, India. Transcutol® P (Carbitol ®) (Monoethyl ether of diethylene glycol) was purchased from Avra Laboratories Pvt. Ltd., Hyderabad, India. All other reagents used were of analytical grade.

2.2 Method

2.2.1 Selection of Nanoemulsion components [11]

Oil (Solubility studies)

The solubility of Zaltoprofen in oils, surfactants, and co-surfactants was measured using the shake flask method. An excess amount of Zaltoprofen and individual excipients (2 mL) were taken in 5 mL vials and sealed. The vials were placed on a table top Orbital Shaker (Eltek®India) was used to facilitate the solubilisation at 30°C for 72 h. Each vial was then centrifuged at 15,000 rpm for 10 minutes using a research centrifuge (REMI, Mumbai, India) followed by the removal of undissolved Zaltoprofen by filtering with a membrane filter (0.45 µm). Samples were suitably diluted with methanol and drug concentration was obtained via a validated UV method at 332 nm using methanol as a blank (R^2 = 0.9994, linearity = 10–70 µg/mL) using a double-beam UV visible spectrophotometer (Lab India UV 3000$^+$). The experiment was repeated thrice. Results were represented as mean values (mg/mL ± SD).

Surfactant (Emulsification study)

Different surfactants (Cremophor® EL, Span® 20, Span® 80, Tween® 20, and Tween®80) were screened for the emulsification ability of the selected oil phase. Surfactant selection was done on the basis of percentage transmittance and ease of emulsification. Briefly, 300 mg of the surfactants was added to 300 mg of the selected oily phase. The mixtures were gently heated at 50°C for the homogenization of the components. Each mixture, 50 mg, was then diluted with distilled water to 50 mL in a stoppered conical flask. Ease of emulsification was judged by the number of flask inversions required to yield a homogenous emulsion. Emulsions were allowed to stand for 2 h and their percent transmittance was evaluated at 560 nm by a double-beam UV spectrophotometer using distilled water as a blank. Emulsions were furthermore observed visually for any turbidity or phase separation.

Co-surfactant (Emulsification study)

Three co-surfactants were screened for nanoemulsion formulation, which included Carbitol®, PEG 400 and Plurol Oleique®. The screening of the co-surfactant was conducted on the basis of percent transmittance and ease of emulsification. Mixtures of 100 mg of the co-surfactant, 200 mg of the selected surfactant and 300 mg of the selected oil were prepared and evaluated for percent transmittance at 560 nm.

Drug - Excipient compatibility studies

Fourier transform infrared analysis (SHIMADZU) was conducted to study the drug excipient interactions. Samples were scanned in the range from 400-4000 cm^{-1}. The drug excipients compatibility study was determined using Potassium bromide (KBr) pellet method and scanned in the range of 4000 cm^{-1} to 400 cm^{-1}. The IR spectrum of the pure drug was compared with IR spectrum of optimised formulation to check the interaction.

2.2.2 Construction of the ternary phase diagrams

On the basis of solubility and emulsification study Capryol 90®, Cremophor®EL and Carbitol® were selected as oil, surfactant and co-surfactant, respectively. To determine the concentration of components for the existing range of the nanoemulsion, a pseudo-ternary phase diagram was constructed using an aqueous titration method at ambient temperature (25°C). The surfactant and co-surfactant were mixed in different volume ratios (1:1, 1:2, 1:3, 1:4, 4:1, 3:1, and 2:1). Oil and surfactant/co-surfactant (S_{mix}) were mixed thoroughly in different volume ratios (1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1) and titrated with water by drop wise addition under gentle agitation. 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 nanoemulsions. The proper ratio of one excipient to another in the nanoemulsion formulation was analyzed and the pseudo-ternary plot was constructed using TRIPLOT V14 software (version 4.1.0.2). All studies were repeated three times, with similar observations being made between repeats [12].

2.2.3 Selection of formulations from phase diagrams

From each phase diagram constructed, different formulations were selected from nanoemulsion region so that drug could be incorporated into the oil phase on the following basis. The selection criteria, for the formulation in nanoemulsion region were the complete solubility of single dose of drug in given oil concentration, effect of drug on the phase behaviour and nanoemulsion area of the phase diagram and to use low concentration of the surfactant and co-surfactant.

Selected formulations were subjected to thermodynamic stability [13].

2.2.3.1 Thermodynamic stability studies

1. Heating cooling cycle: Six cycles between refrigerator temperature (4°C) and (45°C) with storage at each temperature of not less than 48 h was studied. Those formulations, which were stable at these temperatures, were subjected to centrifugation test.
2. Centrifugation: Stable formulations were centrifuged at 3500 rpm for 30 min. Those formulations that did not show any phase separation were taken for the freeze thaw stress test.
3. Freeze thaw cycle: Three freeze thaw cycles between (-21°C) and (+25°C) with storage at each temperature for not less than 48 h was done for the formulations.

The formulations that passed the thermodynamic stability were selected for further studies [13].

2.2.4 Preparation of Zaltoprofen Nanoemulsion

Zaltoprofen (80 mg) was added in accurately weighed amount of oil into a beaker. The surfactant and co-surfactant were added to the oily mix using a positive displacement pipette and stirred with a magnetic bar. The formulations were further sonicated (Sonica ultrasonic, 2000 MH, Spinotech Pvt Ltd, India) for 15 minutes and stored at room temperature until their use in subsequent studies.

2.2.5 Evaluation parameters of Zaltoprofen Nanoemulsion

In vitro drug release studies

In-vitro drug release of Zaltoprofen from the nanoemulsion formulation was determined by using locally fabricated Franz diffusion cell. Nanoemulsion equivalent to single dose (80 mg of Zaltoprofen) was placed in donor compartment of diffusion cell. Receptor compartment was filled with pH 6.8 Phosphate buffer solution with continuous stirring at 350 rpm. The receptor and donor compartments were separated by Hi-media dialysis membrane 150 (molecular cut off 12000-14000 Dalton, pore size 0.4 nm). Samples were withdrawn at specific intervals (0, 1, 2, 3, 4, 5, 6, 7 and8 h) and an equal volume of medium was replaced to maintain sink condition. The samples were analyzed by the UV-Visible spectrophotometer at 338 nm to determine the concentration. The experiment was repeated thrice. Results were represented as mean values (% Cumulative Drug Release) (%CDR) ± SD). Formulation having highest %CDR was considered as optimised formulation and used for subsequent studies. Studies were repeated in triplicate manner and results are described in mean ± SD.

Globule size, polydispersity index and zeta potential determination

50 mg of the optimized nanoemulsion formulation was diluted with water to 100 mL in a flask, and gently mixed by hand. The globules size distribution, polydispersity index and zeta potential of the resultant nanoemulsion was determined by laser diffraction analysis using a particle size analyzer (Horiba Scientific nanopartica, UK), that analyzes the fluctuations in light scattering due to Brownian motion of the particles. Light scattering was monitored at 25°C at a 90° angle. Studies were repeated thrice and results are described in mean ± SD.

Percent Transmittance

The percent transmittance of the nanoemulsion was measured using UV-Visible double beam spectrophotometer keeping distilled water as blank at 560 nm.

Viscosity

Viscosity of the samples was measured as such without dilution using Brookfield viscometer LVDV-II+P fitted with an S-34 spindle at 25°C. Studies were repeated three times and results are described in mean ± SD.

2.2.6 Preparation of Zaltoprofen Nanoemulsion Gel and Zaltoprofen gel

1 % w/w Carbopol 934 was dispersed in distilled water and added to previously optimised nanoemulsion (F7). The contents were stirred for few minutes and glycerol (10%) was added. The gel was then neutralized with 1% triethanolamine in water. Zaltoprofen loaded gel (ZG) was prepared by dispersing the 1 % w/w Carbopol 934 in a mixture of distilled water and glycerol (10%) with 80 mg Zaltoprofen being kept under magnetic stirring until a homogeneous dispersion formed. The mixture was made neutral by adding triethanolamine (1%). Prepared Zaltoprofen nanoemulsion gel (F7G) and Zaltoprofen loaded gel (ZG) were stored at room temperature until their use in subsequent studies.

2.2.7 Evaluation parameters of Zaltoprofen Nanoemulsion gel

pH

The pH values of 1% aqueous solutions of the prepared nanoemulsion gel was measured by a pH meter (Systronics, μ pH System 361) at 25°C ± 1°C. Studies were repeated thrice and results are described in mean ± SD.

Viscosity

Viscosity of Zaltoprofen nanoemulsion gel was determined using Brookfield Viscometer, LVDV-II+ Pro Spindle S-64. Studies were repeated three times and results are described in mean.

Drug content

Quantity of Zaltoprofen in nanoemulsion gel was determined by UV-Spectrophotometer. 1.0 g of formulation was accurately weighed, dissolved in 50 mL of methanol. It was filtered and diluted if required. Absorbance was determined using UV spectrophotometer. Studies were repeated thrice and results are described in mean ± SD.

Ex-vivo Skin permeation study

Male albino rats weighing 180g-200g were sacrificed by cervical dislocation of spinal cord. Abdominal skin hair was removed by using a depilatory. The skin was carefully removed and washed after removing subcutaneous fat and other visceral tissue. Freshly excised skin was mounted on Franz diffusion cells with an effective diffusion area of 2.0 cm^2 and diameter of 16 mm and receptor volume of 12.5 mL, to assess in vitro drug permeation. Donor and receptor

compartments were separated by freshly excised rat skin. The receptor compartment was kept at 37°C. The receptor fluid was selected as pH 6.8 phosphate buffer and the hydrodynamics in the receptor compartment was maintained by stirring continuously with magnetic stirrer at 500 rpm. Each formulation equivalent to single dose was placed in the donor compartment. Permeation experiments were carried out for 24 h after application. Samples were taken from the receiver compartment at scheduled time intervals (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12 and 24 h) and immediately replaced with the same volume of fresh receptor fluid. The amount of Zaltoprofen in the samples was determined by UV-visible spectrophotometer at 338 nm using freshly prepared pH 6.8 phosphate buffer as blank. Studies were repeated thrice and results are described in mean ± SD.

***Permeation Data Analysis*[14]**

The permeation profiles were constructed by plotting the cumulative amount of Zaltoprofen permeated per unit rat skin area (μg/cm^2) versus time. Linear regression analysis was used to calculate the steady state flux (J_{ss}, μg/cm^2/h) of Zaltoprofen by using the slope of the plot. The following equation was used to determine the permeability co-efficient (K_p) of the drug through the stratum corneum:

$$Kp = \frac{Jss}{C}$$

Where, C is the initial concentration of the drug in the donor compartment. The penetration enhancing effect was calculated in terms of enhancement ratio (Er) by using the following equation:

$$Er = \frac{Jss\ of\ Nanoemulsion\ gel\ formulation}{Jss\ of\ control\ fomulation}$$

3. Results and Discussion

3.1 Selection of Nanoemulsion components

Oil (Solubility studies)

The most important criterion for screening of excipients is the ability of oil, surfactants and cosurfactants to solubilise the poorly soluble drug. The solubility of Zaltoprofen in different oils, surfactants and cosurfactants was determined. As described in Table 1, the solubility of Zaltoprofen was found to be highest in Capryol®90 as compared to other oils. Hence, Capryol®90 was selected as the oil phase. Drug was found to be more soluble in Cremophor®EL, Span®20, Span®80, Tween®20 andTween®80 among surfactants and in Carbitol®, Plurol Oleique® and PEG 400 among cosurfactants. Among the various oils that were screened, Capryol®90 could solubilise the target amount of

Zaltoprofen (80 mg) at a relatively small amount of 1 mL. The selection of the surfactant or co-surfactant in the further study was governed by the emulsification efficiency rather than the ability to solubilise Zaltoprofen.

Table-1: Solubility study of Zaltoprofen in various excipients at 30°C.

Oil	Solubility[a] in mg/ml ± SD	Surfactant	Solubility[a] in mg/ml ± SD	Co-surfactant	Solubility[a] in mg/ml ± SD
Capmul MCM	76.92 ± 1.48	Cremophor EL®35	86.26 ± 0.57	Ethanol	111.19 ± 0.35
Capryol 90	80.42 ± 0.20	Cremophor RH®40	41.39 ± 0.06	Glycerol	1.34 ± 0.13
Castor oil	40.70 ± 0.25	Span®20	19.30 ± 0.35	Propylene Glycol	32.84 ± 0.50
Iso Propyl Myristate	9.87 ± 0.13	Span®80	39.52 ± 0.04	Plurol Oleique®	22.70 ± 0.27
Linseed oil	12.22 ± 0.08	Tween®20	113.13 ± 1.33	PEG 400	157.10 ± 0.44
Labrafac	14.68 ± 0.14	Tween®80	69.34 ± 0.35	PEG 600	91.50 ± 0.49
Oleic acid	18.40 ± 0.50			Transcutol®P	338.68 ± 0.40
Olive oil	6.65 ± 0.11			Labrafil®M 2125 CS	31.60 ± 0.37

[[a] Data are expressed as mg/mL ± SD (*n* = 3)]

Preliminary Screening of Surfactants

The excipients selected were needed to be pharmaceutically acceptable, nonirritating and nonsensitizing to the skin and should fall into the GRAS (generally regarded as safe) category. Safety is a major factor in choosing a surfactant, as a large amount of surfactants may cause skin irritation [15]. Nonionic surfactants are considered to be less toxic than ionic surfactants and therefore Cremophor®EL, Span®20, Span®80, Tween®20 andTween®80 were selected, out of which some are reported to have bioactive effects, such as lymphotropic characters by Tween®20, Tween®80, and Span®80, and the inhibitory effect on P-gp and CYP enzymes by Cremophor®EL [12].

Results indicated that Capryol®90 exhibited the highest emulsification efficiency with Cremophor®EL [% transmittance: 99.5%, 5 flask inversions] for the homogenous emulsion formation. On the other hand, Capryol®90 showed poor emulsification properties with other surfactants employed, requiring a higher number of flask inversions [Table 2]. Therefore, Capryol®90 with Cremophor®EL as surfactant was optimised.

Table-2: Emulsification efficiency of various surfactants.

Surfactant	Number of Inversions[a]	% Transmittance[a]
Cremophor EL 35	5	99.50
Tween 20	8	92.69
Tween 80	6	95.89
Span 20	30	68.45
Span 80	35	62.93

[[a] Data are expressed as mean (*n* = 3)]

Preliminary screening of co-surfactants

Addition of a co-surfactant to the surfactant-containing formulation was reported to improve transparency and drug permeation from the formulation. Additionally, co-surfactants decreases the bending stress of interface and allows the interfacial film sufficient flexibility to take up different curvatures required to form nanoemulsion over a wide range of composition [16]. In the current investigation, three co-surfactants, namely, Carbitol®, PEG 400 and Plurol Oleique® were compared.

As described in Table 3, Capryol®90 exhibited good emulsification with all co-surfactants, with Carbitol® showing the maximum transmittance (99.99%). Herein, the solubility of the drug in different co-surfactants may judge the final selection. Results of the solubility study demonstrated in Table 1 indicate a higher solubility in Carbitol®. It is worthy to note that all dispersions exhibited an instantaneous emulsion formation with only one flask inversions [Table 3]. This could contend the importance of co-surfactant addition to the surfactant-containing dispersions.

Table-3: Emulsification efficiency of various Co-surfactants.

Co-surfactant	Number of Inversions[a]	% Transparency[a]
PEG 400	1	99.90
Plurol Oleique	6	80.41
Transcutol P	1	99.99

[[a] Data are expressed as mean (*n* = 3)]

Drug - Excipient compatibility studies

To test for possible intramolecular interaction between Zaltoprofen and excipients, FTIR studies was used. FTIR spectra of drug and optimized formulations were recorded by FTIR spectrophotometer. The IR spectrum of the pure

Zaltoprofen and optimized formulation F7 nanoemulsion gel were recorded by FTIR spectrometer, as depicted in Fig. 1 and Fig. 2 respectively. Pure Zaltoprofen presents a characteristic broad band in the region 3800 cm^{-1} to 2800 cm^{-1}, represents O-H stretching in carboxylic acid functional group, while 1280 cm^{-1} suggest C-O stretching. It also exhibits characteristic band in the C=O stretching region of carboxyl functional group, strong band at 1672 cm^{-1} were obtained.

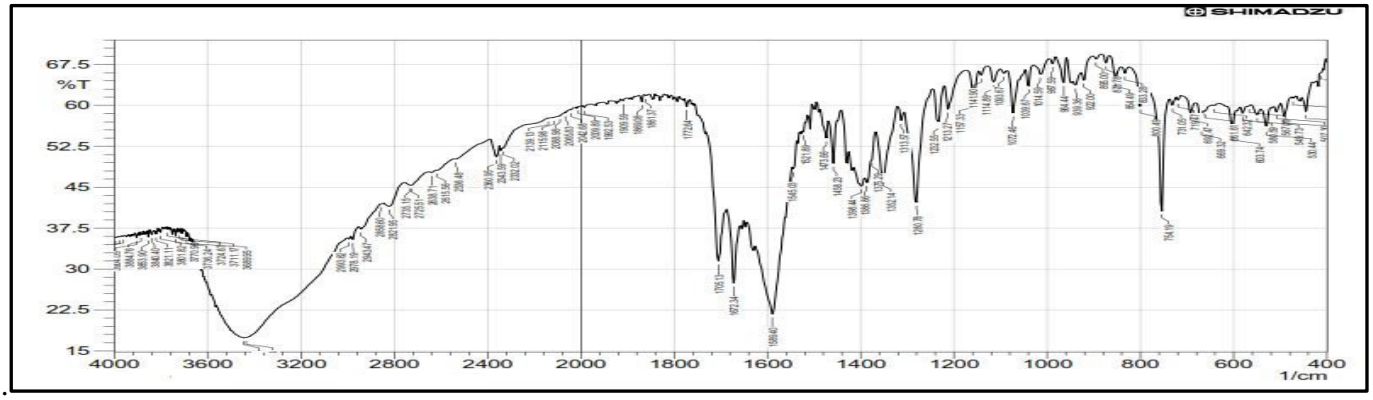

Figure-1: FTIR spectra of pure Zaltoprofen drug.

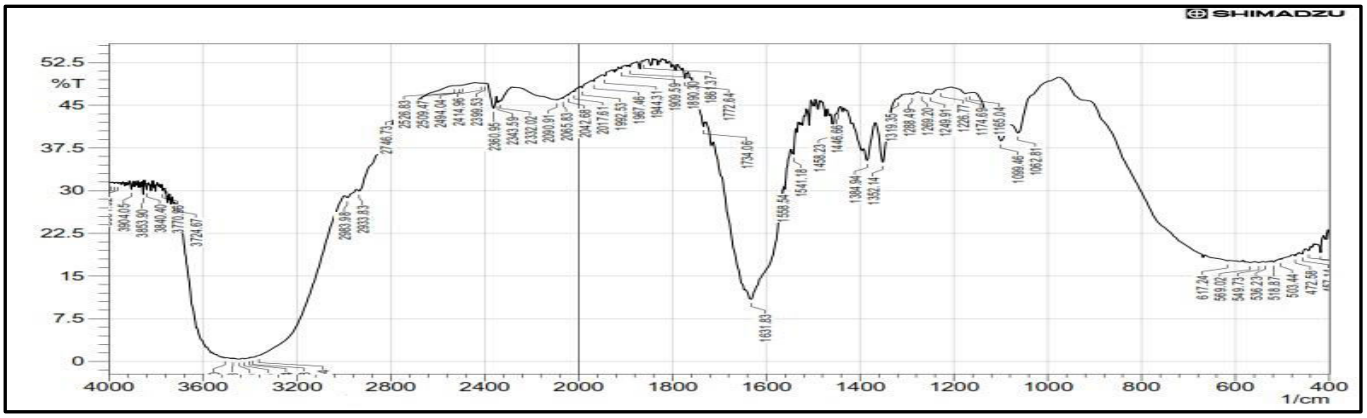

Figure-2: FTIR spectra of pure Zaltoprofen drug.

The characteristic peaks of the optimized formulation followed the same trajectory as that of the drug alone with minor differences. Thus there were no drug-excipient interactions.

3.2 Construction of Pseudoternary phase diagram

The aim of the construction of pseudoternary phase diagram was to find out the existence range of nanoemulsion. Care was taken to ensure that observations are not made on metastable system. Pseudoternary phase diagrams were constructed separately for each S_{mix} ratio for getting o/w nanoemulsion regions. The area of nanoemulsion isotropic region changed slightly as the ratio of surfactant in S_{mix} was increased. In the phase diagrams, the existence of large or small nanoemulsion region depends on the capability of the particular S_{mix} to solubilise the oil phase. The extent of solubilisation results in a greater area with the formation of more clear and homogenous solution. The ratio of

surfactant to cosurfactants was very effective for a stable and an efficient nanoemulsion formation. It is important to determine the optimum concentration of surfactant in a formulation because a high amount of surfactants can cause skin irritation.

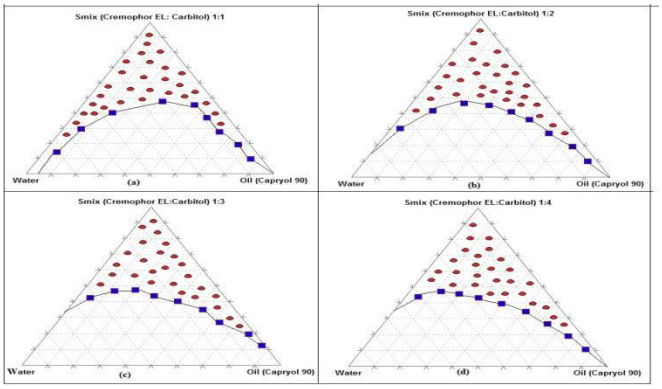

Figure-3: Pseudoternary phase diagrams indicating o/w nanoemulsion region at different S_{mix} ratios.

The phase diagrams were constructed at surfactant/co-surfactant ratios of 4:1, 3:1, 2:1, 1:1, 1:2, 1:3 and 1:4 (w/w). The gel-like region and highly viscous nanoemulsion was found to become large with the increasing concentration of Cremophor®EL, while nanoemulsion region expanded with the amount of Carbitol® increasing. Those which did not show a change in the meniscus after tilting to an angle of 90° were classified as metastable nanoemulsions and were not considered for further studies.

Nanoemulsions prepared with S_{mix} ratios 2:1, 3:1 and 4:1 were observed to be more viscous. Hence, they were not selected for further studies. When S_{mix} was incorporated in the ratio (1:1) [Fig. 3(a)], 59% nanoemulsion region was found. With increase in the concentration of co-surfactant to (1:2) [Fig. 3(b)] a larger area of nanoemulsion (61%) was obtained. Increasing co-surfactant concentration further from (1:2) to (1:3) [Fig. 3(c)] even larger area of nanoemulsion (65%) was formed. Further increasing co-surfactant concentration, (1:4) [Fig. 3(d)], resulted in the reduction of the nanoemulsion area to 62%.

Table-4: Pseudoternary phase diagrams.

S_{mix} Ratio	Figure No.	Percent area of nanoemulsification (%)	Inference
1:1	2(a)	41	Large area of emulsification was observed with all S_{mix} ratios.
1:2	2(b)	45	
1:3	2(c)	48	
1:4	2(d)	46	

3.3 Selection of formulation from phase diagram

Large amount of surfactant causes skin irritation and toxicity-related problem; therefore it is preferable to use the minimum amount of surfactant and co-surfactant in the formulation. The surfactant concentration should be selected so that it gives the maximum flux, which is an important criterion but its level should not be toxic to cause any irritation to the skin. This is usually not obtained with formulations that contain the highest amount of surfactant because high surfactant concentration decreases the thermodynamic activity of the drug in the vehicle, and the affinity of the drug to the vehicle becomes greater.

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 thermostability which differentiates nanoemulsion from conventional emulsion that has only kinetic stability and which causes phase separation eventually. Thus, the selected formulations were subjected to different thermodynamic stability by using heating cooling cycle, centrifugation and freeze thaw cycle stress tests. Formulations which survived thermodynamic stability tests were taken for further studies. Formulation F12 did not pass the thermodynamic stress tests and thus was dropped for further study (Table 5). The results showed that all the formulations except F12 had a good physical stability.

Table-5: Composition of formulation in %w/w and Thermodynamic stability of Formulations.

S_{mix} ratio	F-code	Percentage w/w of different components in formulation				Observation based on Thermodynamic stability studies			Inference
		Oil	S_{mix}		Aqueous	H/C	Centri.	F/T	
			Sur.	Co-sur.					
1:1	F1	43.78	22.02	22.02	12.01	√	√	√	Passed
	F2	22.37	22.27	22.27	33.05	√	√	√	Passed
	F3	11.58	17.38	17.38	53.66	√	√	√	Passed
1:2	F4	44.05	14.53	14.53	11.01	√	√	√	Passed
	F5	23.88	15.60	31.44	29.08	√	√	√	Passed
	F6	15.50	15.65	30.99	37.85	√	√	√	Passed
1:3	F7	23.91	10.87	32.61	32.60	√	√	√	Passed

	F8	17.21	13.97	34.92	33.90	√	√	√	Passed
	F9	14.26	12.08	36.23	37.43	√	√	√	Passed
	F10	41.84	8.36	33.47	15.9	√	√	√	Passed
1:4	F11	22.95	9.09	37.05	30.91	√	√	√	Passed
	F12	16.42	9.85	38.65	34.31	√	×	×	Failed

Heating cooling cycle (H/C), centrifugation (Centri.), freeze thaw cycle (F/T), Formulation code (F-code), Surfactant (sur.), Co-surfactant (Co-sur.)

3.4 Evaluation parameters of Zaltoprofen Nanoemulsion

In-vitro drug release studies

In-vitro drug release studies were performed to compare the release of drug from eleven different nanoemulsion formulations (F1 to F11), all having the 80 mg of Zaltoprofen. The release rate of F7 (83.22% ± 3.21%, mean ± SD, n =3) was found to be the best as compared to other formulations. The release profile of formulations as shown in Fig. 4 and Fig. 5, follows order as: (F7>F8>F1>F4>F10>F9>F11>F2>F3>F5>F6). F7 was further screened for globule size analysis, zeta potential, viscosity and percent transmittance.

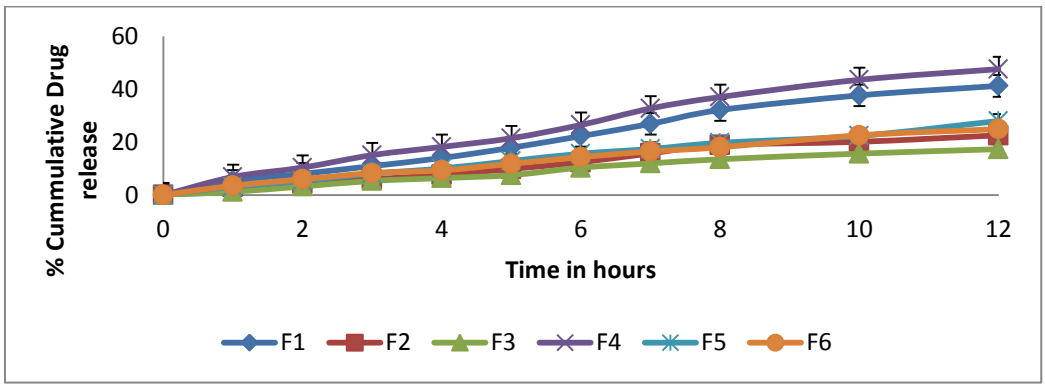

Figure-4: *Invitro* diffusion studies of Zaltoprofen formulations (F1 to F6).

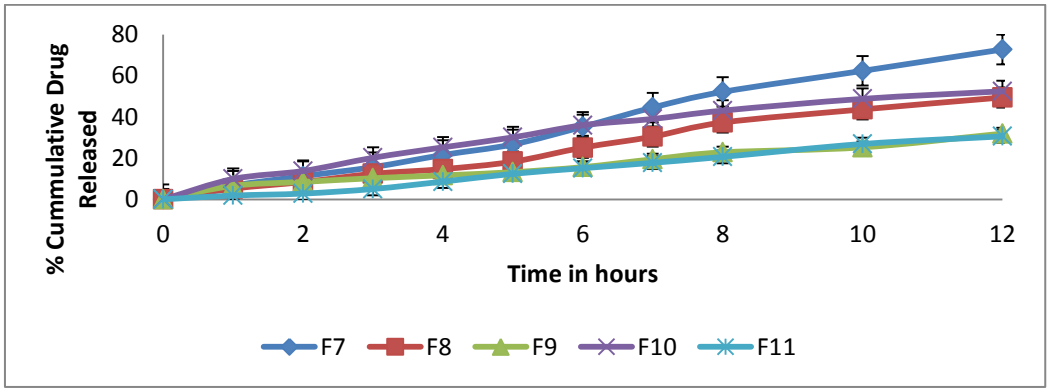

Figure-5: *Invitro* diffusion studies of Zaltoprofen formulations (F7 to F11).

Globule size, poly dispersity index and zeta potential determination

The globule size analysis, poly dispersity index and zeta potential of the optimized formulation F7 was done using particle size analyzer (Horiba).The mean globule size was found to be (82.0 ± 4.23 nm, mean ± SD, n = 3). The particle size distribution of optimized formulation F7 is shown in Fig. 6. The polydispersity index (0.333± 0.017, mean ± SD, n= 3) showed that F7 nanoemulsions had narrow size distribution.

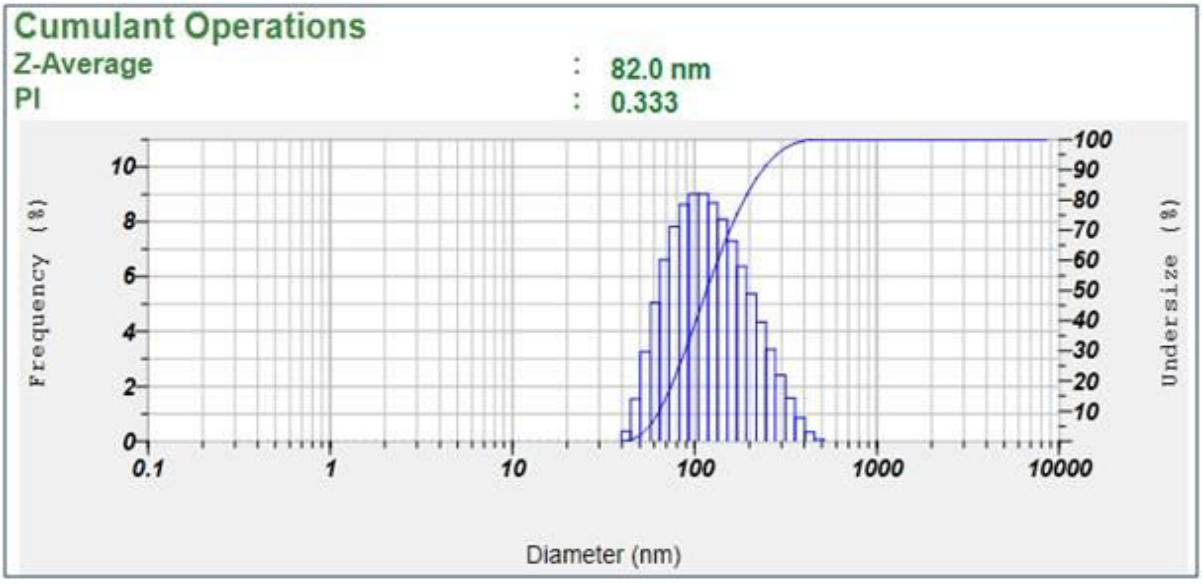

Figure-6: Globule size distribution of Zaltoprofen F7 nanoemulsion.

Zeta potential of optimized formulation was found to be (-36.6 ± 1.16 mV, mean ± SD, n = 3) mV which indicates that the particles of nanoemulsions are negatively charged and thus provides electrostatic stabilization. The zeta potential of the optimized formulation F7 is shown in Fig. 7.

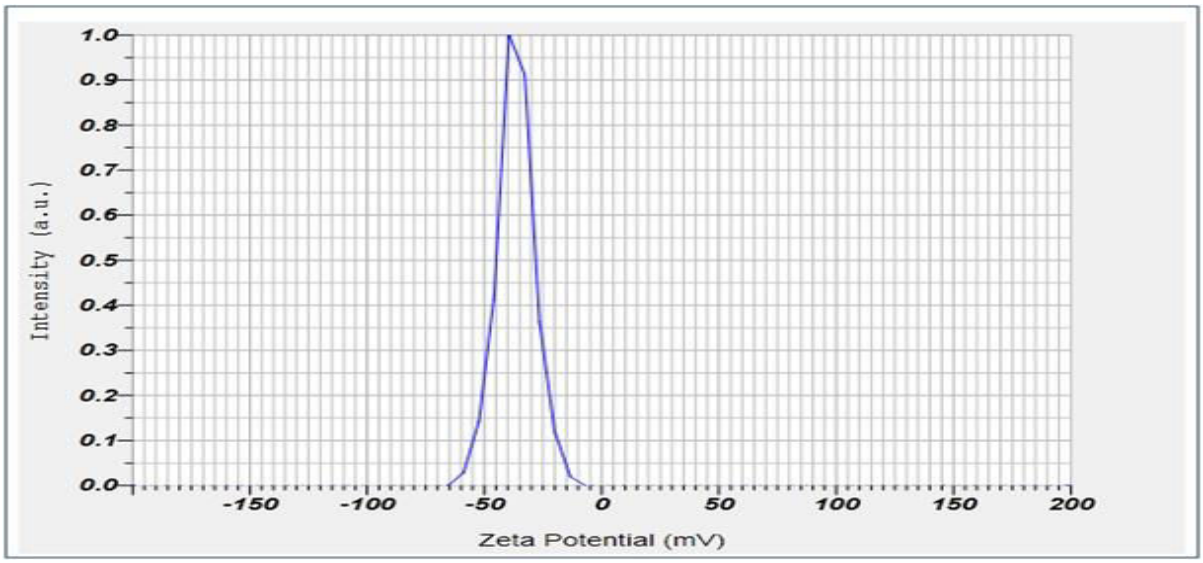

Figure-7: Zeta potential of Zaltoprofen F7 nanoemulsion.

Viscosity

Viscosity of the nanoemulsion F7 was found to be (3.089 cP ± 0.68 cP, mean ± SD, n = 3), as expected for o/w emulsion. The low viscosity of nanoemulsion may be low concentration of Cremophor®EL 35 (having high intrinsic viscosity) as compared to Carbitol® (a short chain alcohol having low intrinsic viscosity). It was not suitable for topical use, which justified the incorporation of nanoemulsion into gel matrix, resulting into nanoemulsion gel having high value of viscosity.

Percent Transmittance

The percent transmittance of the F7 formulations was measured at 560nm keeping distilled water as a blank. The percent transmittance of the optimized formulation F7 was found to be (97.6% ± 0.16%, mean ± SD, n = 3). The results of percentage transmittance revealed that F7 formulation was nearly transparent.

3.5 Evaluation parameters of Zaltoprofen Nanoemulsion gel

pH

The pH of 1% aqueous solutions of the prepared nanoemulsion gel was found to be (6.84 ± 0.18, mean ± SD, n = 3) at 25°C ± 1°C which indicates that the pH of formulation was similar to that of skin pH and thus it was non-irritant.

Viscosity

Viscosity of optimised Zaltoprofen nanoemulsion gel was determined using Brookfield Viscometer, LVDV-II+ Pro, using spindle no. 64.

Table 6 and Fig. 8 indicate that the viscosity of the gel decreases with increasing shear rate as it was pseudoplastic. This pseudoplasticity results from colloidal network structure that aligns itself in the direction of shear, thereby decreasing the viscosity as the shear rate increases. The pseudoplastic flow performance justifies that the developed system will require some force to expel.

Table-6: Viscosity of F7G Zaltoprofen Nanoemulsion gel.

SR. No.	Angular Velocity (RPM)	% Torque*	Viscosity* (cP)
1	0.3	29.4	117003
2	0.5	38.1	91660
3	0.6	37.7	75584

4	1	45.2	54228
5	1.5	55.5	44391
6	2	58.8	35212
7	2.5	65.4	31433
8	3	71.3	28474
9	4	77.2	23215
10	5	84.1	20180
11	6	90.8	18156

[[a] Data are expressed as mean (*n* = 3)]

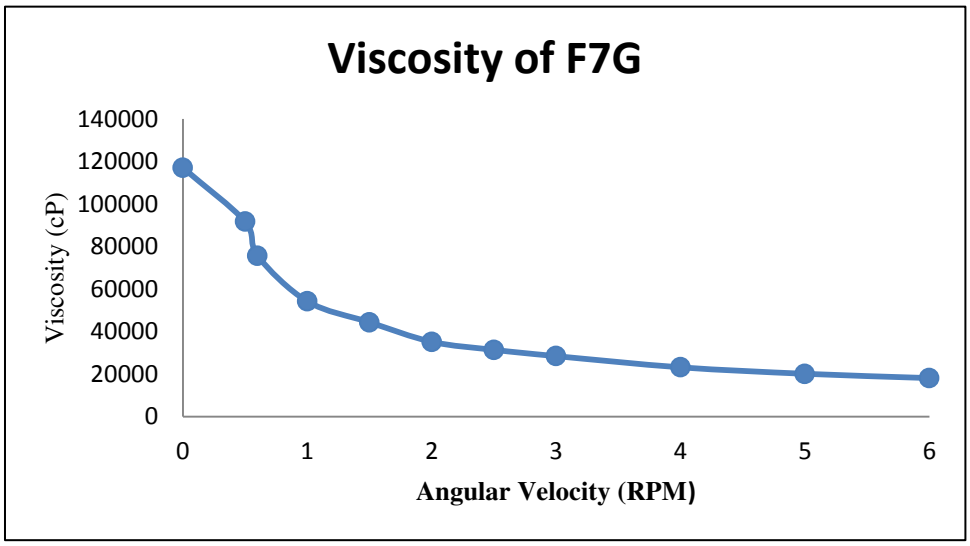

Figure-8: Viscosity of F7G Zaltoprofen Nanoemulsion gel.

Drug content

The drug content of the optimized formulation was found to be (99.30 ± 0.42) % (mean ± SD, n = 3).

Ex-vivo Skin permeation study

Ex-vivo skin permeation studies were performed to compare the release rate of the drug from the various nanoemulsion formulations (Zaltoprofen gel ZG, F7 and F7G) all having the same quantity of Zaltoprofen (80 mg). The release rate of F7 (72.72%) was found to be more as compared to F7G (66.53%) and ZG (38.88%) as shown in Figure 9. Here it was observed that the release of F7 is slightly more than F7G because the gel formulation provides a higher diffusional resistance for drug release. The comparison between F7, F7G and ZG showed that even though the release rate of F7G was less than F7, but it was significantly more than that of ZG, due to presence of drug nanocarriers.

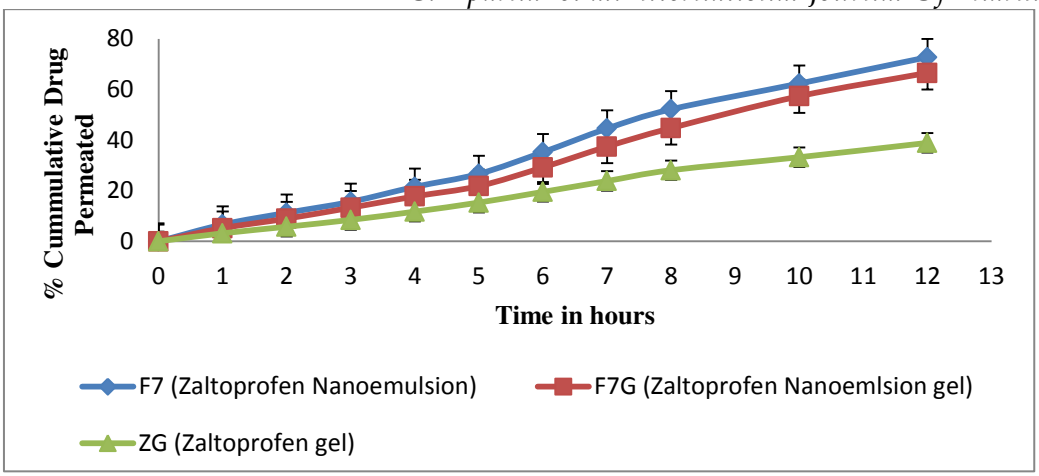

Figure-9: ***In-vitro*** **% Zaltoprofen permeated through excised rat skin.**

Permeation Data Analysis

Permeability parameters like a steady-state flux (Jss), permeability coefficient (Kp), and enhancement ratio (Er), were significantly increased in nanoemulsions F7 and the F7 gel (F7G) formulation as compared with Zaltoprofen gel (ZG). The flux value was found to be 695.8±10.3 µg/cm^2/h of normal gel (ZG) in comparison to F7 1283.7±7.55 µg/cm^2/h and F7G 1132.5±8.3 µg/cm^2/h. Permeability coefficient (Kp) and Enhancement ratio (Er) of ZG, F7 and F7G are described in Table 7.

This result indicates higher permeability of drug through the skin because of the presence of nanocarriers in the formulations. The higher value of flux in F7 compared to F7G indicates formulation provides prolonged drug release behaviour as compare to nanoemulsion. Moreover, this can be thought that F7 and F7G excipients contain permeation enhancers like Cremophor®EL and Carbitol®, which was also responsible for the increased permeation ability in comparison to the normal gel.

4. Conclusion

In this study, Zaltoprofen loaded nanoemulsion gel was formulated successfully to sustain the drug release. The optimized formulation showed low particle size, low viscosity and high percentage transmittance. The *ex-vivo* studies revealed that nanoemulsion gel has better permeation property as compared to conventional gel. From the above studies it can be concluded that nanoemulsion gel is a promising surrogate carrier for transdermal delivery of Zaltoprofen.

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