

Development and Ex Vivo Permeation of a Ketoprofen-Loaded Nanogel for Accelerated Transdermal Delivery

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Abstract

The aim of the present study was to investigate the nanoemulgel as transdermal delivery system for poorly water-soluble drug, ketoprofen, in order to overcome the troubles associated with its oral delivery. Different nanoemulsion components (oil, surfactant, and cosurfactant) were selected on the basis of solubility and emulsification ability. Pseudoternary phase diagrams were constructed using titration method to figure out the concentration range of components. Carbomer 940 was added as gel matrix to convert nanoemulsion into nanoemulgel. Drug loaded Nanoemulsions and nanoemulgel were characterized for particle size, TEM, viscosity, conductivity, spread ability, rheological behavior, and permeation studies using Wistar rat skin and stability studies. Transdermal permeation of ketoprofen from nanoemulgel was determined by using Franz diffusion cell. Nanoemulgel containing 6% oleic acid as oil, 35% Tween 80, and Transcutol P as surfactant cosurfactant mixture, 56.5% water, 2.5% drug, and 0.6% carbomer was concluded as optimized formulation (NG6). The *ex vivo* permeation profile of optimized formulation was compared with nanoemulsion and marketed formulation (*Fastum*). Nanoemulgel showed significantly higher ($P < 0.05$) cumulative amount of drug permeated and flux along with lower lag time and skin retention than marketed formulation. Thus, the study substantiated that nanoemulgel formulation can be used as a feasible alternative to conventional formulations of ketoprofen with advanced permeation characteristics for transdermal application.

Key words: Nanoemulgel, Ketoprofen, Transdermal drug delivery, Ex vivo permeation, and Transcutol P

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1. Introduction

Nanoemulsion is a promising tool for transdermal drug delivery and is defined as a dispersion consisting of oil, surfactant, cosurfactant, and aqueous phase, which is a single optically isotropic and thermodynamically stable liquid solution with a droplet diameter usually in range of 10–200 nm [1]. The ascendancies associated with transdermal use of nanoemulsion are as enhanced drug solubility, good thermodynamic stability, and enhancing effect on transdermal ability [2]. The aptness of nanoemulsion to increase the concentration gradient and thermodynamic activity towards skin along with permeation enhancement activity of its components makes the system expedient for transdermal delivery. But the low viscosity of nanoemulsion constrains its application in transdermal delivery due to cumbersome use. Biocompatible gels having weak interaction with surfactants have already been explored to modify the rheological behavior of nanoemulsion [3]. Variant gel matrices such as carbomer 940, xanthan gum, and carrageenan have been exploited to increase the viscosity of nanoemulsion for transdermal delivery [4]. Thus, the incorporation of nanoemulsion into gel matrix can result in nanoemulgel which may be more relevant for transdermal application as compared to nanoemulsion.

Ketoprofen, a nonsteroidal anti-inflammatory drug (NSAID), has been extensively utilized for treatment of rheumatism [5]. Although ketoprofen is highly permeable through the stomach, its poor water solubility (log partition coefficient is 3.11) limits its entry into systemic circulation before gastric emptying (30 min to 2 hr) occurs. During gastric emptying, ketoprofen enters the small intestine, where it cannot permeate through the membrane despite being solubilized [6]. Moreover, it is also associated with oral side-effects including gastrointestinal irritation when administered by oral route. The adverse effects may worsen to renal and cardiovascular problems ultimately leading to mortality when used chronically especially in case of geriatric population [5]. The promising method to diminish its adverse effects is to deliver the drug via skin [7]. Therefore, an eventual need has emerged to develop a transdermal dosage form of ketoprofen to minimize the oral side-effects and to provide relatively consistent drug levels for prolonged periods. The major problem associated with transdermal drug delivery is barrier properties of 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 [8]. But the use of these chemical enhancers may be harmful especially in chronic

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applications, since many of them are usually irritants [8, 9].

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 for enhancement of transdermal permeation of drugs is the nanoemulsion. In prior studies, nanoemulsion as carrier system has been exploited for transdermal delivery of various drugs (celecoxib, ibuprofen, etc.) [9, 10]. In one of the studies nanoemulsion was also used for transdermal delivery of ketoprofen and it was found to have better permeation but it was not incorporated in gel matrix system.

The present study aims to formulate novel nanoemulgel of ketoprofen for better applicability and better permeation potential through the skin. Furthermore, the components of nanoemulsion system are expected to act themselves as permeation enhancers, thereby circumventing the use of irritable chemical penetration enhancers.

2. Materials and Methods

Materials. Ketoprofen was purchased from Allwell Pharmaceutical Company (Chandigarh, India). Diethylene glycol monoethyl ether (Transcutol P) and caprylcaproyl macrogol glycerides (labrasol) were kind gift from Awamedica (Erbil). Oleic acid, ethanol, propylene glycol, and Tween 80 were purchased from S.D. Fine Chemicals (Awamedica, Erbil). All other reagents used were of analytical grade. Wistar rat skin was obtained from UIPS, Panjab University, Chandigarh. The experimental protocols were approved by Institutional Animal Ethics Committee (IAEC) as per guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of Erbil.

Methods. The solubility of ketoprofen in various oils (sesame oil, oleic acid, isopropyl myristate, and ethyl oleate), surfactants (labrasol and Tween 80), and cosurfactants (propylene glycol, Transcutol P) was determined by dissolving an excess amount of ketoprofen in 500 mg of each of the selected oils, surfactants, and cosurfactants in stoppered vials. The mixtures were continuously stirred using vortex mixer for 10 min and kept at $37 \pm 1.0^\circ\text{C}$ in an isothermal shaker for 72 h to attain equilibrium. The equilibrated samples were centrifuged (3000 rpm for 15 min) and supernatant was filtered through $0.45 \mu\text{m}$ membrane filter and diluted with mobile phase. Drug content was quantified by using UV-VIS spectrophotometer (Shimadzu-1700, Japan) at 260 nm.

Screening of Components for Nanoemulsion. On the basis of solubility studies, the oil was selected

that possesses the best solubilization capacity for ketoprofen. Screening of surfactant and cosurfactant was done on the basis of percent transmittance. Emulsification ability of surfactants (Tween 80, labrasol, and labrafac) was assessed by adding each (300 mg) to selected oil (300 mg). The mixture was gently heated at $40\text{--}45^\circ\text{C}$ for 30 sec to achieve homogenization. Out of this mixture, 50 mg was weighed and diluted up to 50 mL with double distilled water to yield fine emulsion. The resulting mixture was observed visually for the relative turbidity. The emulsions were allowed to stand for 2 h and transmittance was assessed by UV-VIS spectrophotometer (Shimadzu-1700, Japan) at 638 nm, using double distilled water as blank. Various cosurfactants (Transcutol P, propylene glycol, and ethanol) were screened for formulation of Nanoemulsions. Mixtures of cosurfactant (100 mg), selected surfactant (200 mg), and selected oil (300 mg) were prepared and evaluated in the same manner as described in the procedure of surfactant screening.

Formulation of Nanoemulsion. Ketoprofen was added to the mixtures of oil, surfactant cosurfactant mixture with varying ratios pooled from pseudoternary phase diagrams as described in Table 1, and then an appropriate amount of water was added to the mixture in a dropwise manner. The nanoemulsion containing ketoprofen was obtained by stir-ring the mixtures at ambient temperature. All nanoemulsions were stored at ambient temperature for further studies.

Component	Solubility	
Component	Solubility	
Sesame oil	4.06 ± 0.68	Tween
80	85.56 ± 0.55	
Oleic acid	28.32 ± 0.58	
Labrasol	62.65 ± 0.33	
Isopropyl myristate	11.09 ± 1.08	
Propylene glycol	48.04 ± 0.67	
Ethyl oleate		13.24 ± 0.86
Transcutol P	56.66 ± 0.58	

* Solubility = (mgmL^{-1})

Optimization of Nanoemulsion Morphology and Structure of Nanoemulsion.

The morphology and microstructure of drug loaded nanoemulsions were determined with the aid of transmission electron microscopy (TEM) (Hitachi H7500, Japan). Nanoemulsion formulations were diluted with water (1 : 10). A drop of diluted

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nanoemulsion was then directly deposited on the holey film grid, stained by 1% aqueous solution of phosphotungstic acid, and observed after drying.

Formulation of Nanoemulgel. All the formulations (N1–N9) were found in nanosize range and therefore incorporated in gel matrix resulting in nanoemulgel. Carbomer 940 was selected as gel matrix base. The oily phase was obtained by mixing oleic acid, Tween 80, Transcutol P, and drug. Carbomer 940 was swollen in little water for 24 h and a high viscous solution was obtained, and then the oily phase was slowly added to the viscous solution of carbomer 940 under magnetic stirring. The pH values were subsequently regulated to 6–9 by using triethanolamine, and nanoemulgel was obtained. The concentration of carbomer 940 in nanoemulgel was 0.6% (w/w).

Characterization of Nanoemulgel

Drug Content Determination. The amount of drug contained in the prepared nanoemulgel was determined by dissolving 100 mg of prepared nanoemulgel in 10 mL of distilled water. This mixture was analyzed by UV spectrophotometer at 260 nm against distilled water as blank. The drug content was quantified by (2):

$$\text{Concentration} = \frac{\text{Absorbance}}{\text{factor} \times 10} \times \text{Dilution}$$

$$E_{1\text{ cm}}^{1\%}$$

pH Determination: Since the formulation was a topical formulation to be applied to the skin, therefore pH measurement was essential to ensure nonirritating nature of the formulation. The pH of the formulation was determined at ambient temperature with digital pH meter (Rolex, India).

Spreadability: The spreadability of prepared nanoemul-gels was determined 48 h after preparation by measuring the spreading diameter of nanoemulgel between the two glass plates after 1 min. A weight of 500 mg of nanoemulgel was placed within a circle of diameter 1 cm premarked on glass plate over which a second glass plate was placed. The increase in diameter as a consequence of weights added leading to spreading of gel was noted. The spreadability can be calculated by using the formula

$$S = M \times L / T \quad (2)$$

Where S is spreadability, m is weight placed on upper slide, l is length of upper slide, and t is the time taken.

Viscosity Measurements and Rheological Behavior:

The viscosity of the prepared formulations was determined at different angular velocities at $32.0 \pm 0.1^\circ\text{C}$ using spindle number 4 (Brookfield DV-II+ Pro viscometer). The nanoemulgel formulation was evaluated for its rheo-logical behavior using cone and plate configuration (40 mm cone with 2.5° deg cone angle). Rheology studies were conducted in shear rate range of $54.63\text{--}497.7\text{ s}^{-1}$ at 25°C . The consistency index and flow index were calculated from the power law equation:

$$\tau = Kr^n \quad (3)$$

Where τ is shear stress, r is shear rate, K is consistency index, and n is flow index. Taking log on both sides,

$$\log \tau = \log K + n \log r \quad (4)$$

Thus, from the plot of log of shear stress versus log of shear rate, slope of the plot was taken as flow index and antilog of Y-intercept gave the consistency index.

Ex Vivo Drug Permeation Studies: The *ex vivo* per-meation studies were carried out using Franz diffusion cell, which is a reliable method for prediction of drug transport across the skin [12]. These studies were conducted employing excised skin of Wistar rats.

The hair on dorsal side of sacrificed animal was removed with surgical blade number 24 in direction of tail to head. The shaven part of the animal skin was separated; excess fat and connective tissue were removed using scalpel. The excised skin was washed with normal saline, examined for integrity, and subsequently used. The skin was mounted on diffusion cell assembly with an effective diffusion area of 9.99 cm^2 , where stratum corneum side was facing the donor compartment and dermal side was facing the receiver compartment. The receptor compartment consisted of 30 mL phosphate buffer of pH 7.4 as receptor fluid agitated at 100 rpm and maintained at $37 \pm 0.5^\circ\text{C}$ throughout the experiments. The prepared formulation was applied onto the membrane in donor compartment. An aliquot of 2 mL sample was withdrawn at suitable time intervals and replaced immediately with an equal volume of fresh diffusion medium.

Comparison of Permeation Studies of Marketed Formulation, Optimized Nanoemulgel, Nanoemulsion, and Plain Drug Gel and Drug

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Solution. The *ex vivo* permeation study of optimized nanoemulgel formulation (NG6) was compared with the marketed formulation (Fastum, Menarini Raunaq Pharma Ltd., Iraq) for permeation and retention characteristics. The cumulative amount of drug permeated through skin per unit area was plotted as a function of time. The permeation rate of drug at steady state (J_{ss} mg/cm/h) through skin was calculated from the slope of linear portion of plotted curve. The lag time (T_{lag}) was determined by extrapolating the linear portion of cumulative amount permeated versus time curve to the abscissa. Enhancement ratio (E_{pen}) was calculated by dividing J_{ss} of respective formulation by J_{ss} of control formulation [13].

The amount of ketoprofen retained in skin was determined at the end of experiment. Skin was removed where effective permeation of skin was cut, washed three times with saline solution, and washed off. The sample of skin was homogenized in 1 mL methanol. Resulting solution was centrifuged at 3000 rpm for 10 min and analyzed for retention. Local accumulation efficiency (LAE) was obtained as ratio of drug accumulated in skin to that delivered through skin [14, 15].

Release Kinetics: To study the release kinetics, data obtained from *ex vivo* permeation studies were fitted in various kinetic models: zero order as cumulative percent of drug released versus time, first order as log cumulative percentage of drug remaining versus time, and Higuchi's model as cumulative percent drug released versus square root of time. To determine the mechanism of drug release, the data were fitted into Korsmeyer and Peppas equation as log cumulative percentage of drug released versus log time, and the exponent n was calculated from slope of the straight line. For slab matrix, if exponent is 0.5, then diffusion mechanism is Fickian; if $0.5 < n < 1.0$, mechanism is non-Fickian.

Thermodynamic Stability Studies: Nanoemulsions are thermodynamically stable system and are formed at particular concentration of oil, mixture of surfactant and cosurfactant, and water, with no phase separation, creaming, and cracking. Thermodynamic stability of prepared nanoemulgel formulation was assessed by stability under centrifugation and freeze/thaw cycles [9]. The stability under centrifugation reflects the strength of interfacial film. The nanoemulgel formulation was centrifuged at 3500 rpm for 30 min. In case with freeze/thaw cycles, test tubes filled with the nanoemulgel were hermetically sealed and vertically stored for 16 h in a freezer at -21°C and then for 8 h at room temperature (25°C). The nanoemulgel was observed for any change. This cycle was repeated three times.

Sustainability Studies: Temperature stress studies were conducted by storing the formulation at different temperature conditions. Each formulation was stored in sealed glass containers in refrigerator (4°C), at ambient temperature (25°C) and at accelerated temperature (40°C) for 90 days. After 1, 7, 14, 21, 30, 45, 60, and 90 days, the formulations were evaluated for any physical change (like clarity, phase separation, precipitation of drug, and color change), drug content, and pH [9].

Statistical Analysis: All experimental measurements were performed in triplicate. Result values were expressed as mean value $\pm$ standard deviation (SD). Statistical analysis of difference in steady state flux and *ex vivo* permeation among predetermined intervals between formulations was performed by using unpaired *t*-test. The level of significance was taken at *P* value < 0.05 .

3. Result

Screening of Components: To develop a nanoemulsion system of ketoprofen for transdermal delivery, it should possess good solubility in the components of system, as only solubilized drug can permeate through the skin. The solubility of ketoprofen in various oils, surfactants, and cosurfactants was investigated (Table 1). Ketoprofen has the highest solubility in oleic acid (28.32 ± 0.62 mg/mL) followed by ethyl oleate, isopropyl myristate, and sesame oil. Among surfactants and cosurfactants, Tween 80 (85.56 ± 0.55 mg/mL) and Transcutol P (56.66 ± 0.58 mg/mL), respectively, showed the highest solubilities.

Therefore, oleic acid was screened as oil phase based on solubility studies. The criterion of selection of surfactant and cosurfactant for formulation of nanoemulsion is their percent transmittance [16]. Out of various surfactants and cosurfactants screened, Tween 80 revealed $96.34 \pm 0.24\%$ transmittance whereas the other surfactants labrasol and labrafac showed $89.41 \pm 0.66\%$ and $72.66 \pm 0.69\%$, respectively. Similarly, in case with cosurfactants, Transcutol P resulted in higher percent transmittance (98.21 ± 0.63) than propylene glycol and ethanol which showed 94.98 ± 0.57 and 88.58 ± 0.27 (Table 2). Therefore, Tween 80 and Transcutol P were selected as surfactant and cosurfactant, respectively, for

Table 2. Percent transmittance of surfactants and cosurfactants.

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Surfactant	Percent transmittance (%)	Cosurfactant	Percent transmittance (%)
Tween 80	96.34 ± 0.24	Propylene glycol	94.98 ± 0.57
Labrasol	89.41 ± 0.66	Transcutol P	98.21 ± 0.63
Labrafac	72.66 ± 0.69	Ethanol	88.58 ± 0.27

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

Phase Behaviour and Optimization of Nanoemulsion

The aim of the construction of pseudoternary phase diagrams was to find out the existence range of nanoemulsions. The translucent nanoemulsion region is presented in phase diagrams. The rest of the region on the phase diagram represents the turbid and conventional emulsions based on visual observation [11].

Pseudoternary phase diagrams were constructed separately for each surfactant to cosurfactant ratio,

so that O/W nanoemulsion regions could be identified and nanoemulsion formulations could be optimized.

In Figure 1(a), surfactant to cosurfactant ratio 1 : 0 was used; it showed significant nanoemulsion area. O/W nanoemulsion region was found towards higher concentration of surfactant, showing that Tween 80 could be used alone without cosurfactant, but a higher concentration of surfactant can cause skin irritation as reported in earlier reports [18]. So it was decided to incorporate cosurfactant with surfactant in ratio 1 : 1 (Figure 1(b)); a large O/W nanoemulsion area was again observed. The reason for it may be greater penetration of the oil phase in the hydrophobic region of the surfactant monomers. Another reason could be increase in entropy of the system.

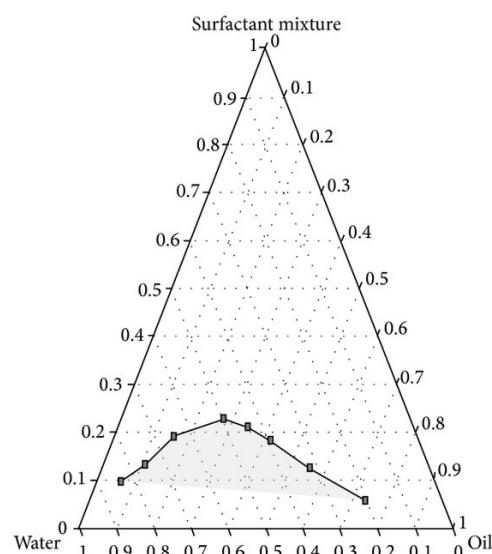


Figure 1(a): Pseudoternary phase diagram of formulation composed of oil (oleic acid), mixture of surfactant (Tween 80) and cosurfactant (Transcutol P) dispersed with water at 37°C. For surfactant: cosurfactant ratio of (a) 1 : 0, (b) 1 : 1, and (c) 2 : 1.

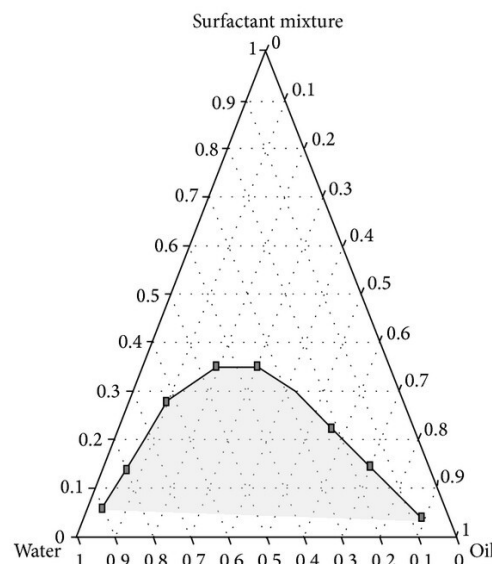


Figure 1(b): Pseudoternary phase diagram of formulation composed of oil (oleic acid), mixture of surfactant (Tween 80) and cosurfactant (Transcutol P) dispersed with water at 37°C. For surfactant: cosurfactant ratio of (a) 1 : 0, (b) 1 : 1, and (c) 2 : 1.

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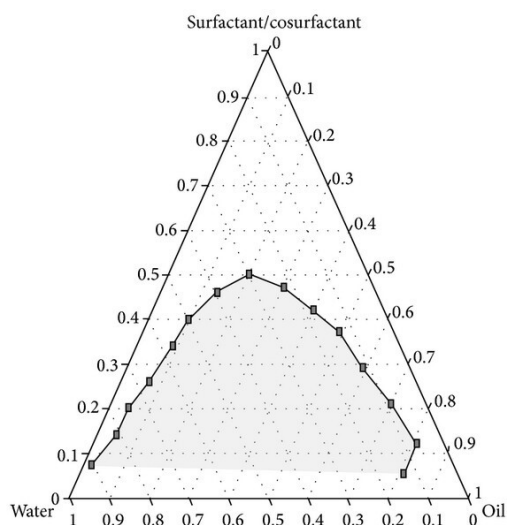


Figure 1(c): Pseudoternary phase diagram of formulation composed of oil (oleic acid), mixture of surfactant (Tween 80) and cosurfactant (Transcutol P) dispersed with water at 37°C. For surfactant: cosurfactant ratio of (a) 1 : 0, (b) 1 : 1, and (c) 2 : 1.

As we further increased surfactant concentration to 2 : 1 (Figure 1(c)), the nanoemulsion region was decreased as compared to surfactant to cosurfactant mixture ratio of 1 : 1.

Thus, 1 : 1 ratio of surfactant to cosurfactant was selected as optimized ratio from which different concentrations of components for formulation of nanoemulsion were pooled randomly (Table 3). The concentration of surfactants should be optimum enough to emulsify the system which should not act as irritant.

Table 3. Composition of ketoprofen nanoemulsions used for optimization.

Formulation code	Components (% w/w)			
	Ketoprofen	Oil	*Tween 80 : Transcutol P in 1 : 1	Water
N1	2.5	3.0	75.0	19.5
N2	2.5	3.0	55.0	39.5
N3	2.5	3.0	35.0	59.5
N4	2.5	6.0	75.0	16.5
N5	2.5	6.0	55.0	36.5

Formulation code	Components (% w/w)			
	Ketoprofen	Oil	*Tween 80 : Transcutol P in 1 : 1	Water
N6	2.5	6.0	35.0	56.5
N7	2.5	9.0	75.0	13.5
N8	2.5	9.0	55.0	33.5
N9	2.5	9.0	35.0	53.5

*Tween 80 : Transcutol P in 1 : 1—mixture of surfactant and cosurfactant which was taken in ratio 1 : 1.

For transdermal application, ketoprofen was formulated into nanoemulgel system containing carbomer 940 (0.6% w/w) as gel matrix. The thickened system is expected to offer good biophysical and sensorial benefits for transdermal delivery [10]. Here, in this study, influence of order of addition of carbomer 940 on the formation of nanoemulgel was also investigated [10, 11]. In first case, carbomer 940 was added directly to preformed nanoemulsion, and it took much more time to be swollen in nanoemulsion than in water. Even some tiny agglomerates of carbomer 940 were observed as it was not properly swollen.

In the second case, carbomer 940 was swollen in aqueous phase and its pH was adjusted with triethanolamine and then it was incorporated in oily phase containing surfactant, cosurfactant, and drug resulting in homogenous nanoemulgel formulation. Based on these studies, it can be concluded that order of addition of carbomer 940 influenced homogenization of gel matrix. The second case is found to have resulted in homogenous nanoemulgel. The gelling behavior of carbomer 940 is maybe due to noncovalent intermolecular associations deriving from forces such as coulombic, van der Waals, and hydrogen bond interaction. These physical interactions could lead to formation of three-dimensional gel network and dispersed oil droplets were reasonably hosted within meshes of these networks [19].

Characterization

Morphology and Structure

The transmission electron microscope revealed a positive image in which nanoemulsion appeared dark with bright surroundings. The average droplet size of sample was less than 100 nm. These results confirmed that the droplets were in nanosize range

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(less than 200 nm) and thus emulsion formulated was nanoemulsion. TEM of nanoemulsion is given in Figure 2.

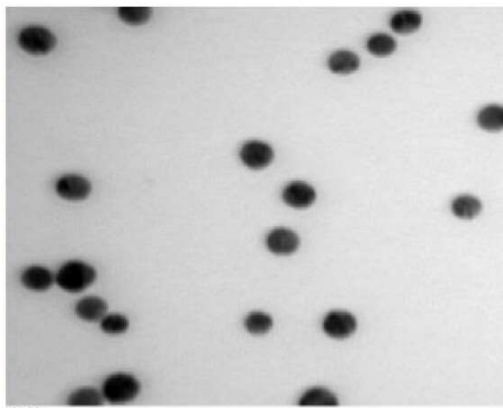


Figure 2: TEM image of ketoprofen nanoemulsion (magnification $\times 200,000$).

Micromeritics

Size characterization of nanoemulsion is essential for ensuring safe and efficient dosage [20]. As the oil content was kept minimum, that is, 3% w/w, the droplet size was found to be the lowest which was 46.30 ± 0.12 nm. When oil content was increased to 9% w/w the droplet size increased substantially, that is, up to 100 nm.

All formulations were found in nanosize range which was also depicted by the low values of polydispersity index. Polydispersity index is ratio of standard deviation to the mean droplet size and signifies the uniformity of droplet size within the formulation [20, 21]. The polydispersity values of formulation were found to be low (0.138–0.167), indicating narrow distribution of droplet size within formulation.

Viscosity and Conductivity Determination

The viscosity of nanoemulsion was found to be low (10.2 ± 0.34 to 37.2 ± 0.45 mPaS) and was not suitable for topical use which justified the incorporation of nanoemulsion into gel matrix, resulting into nanoemulgel having high value of viscosities.

The conductivity of nanoemulsion was 49.7 ± 0.02 to 160.2 ± 0.12 μ S/cm which was high (Table 4) and also confirmed formulation of O/W type of nanoemulsions as reported earlier [18].

Table 4. Characterization of nanoemulsion.

Formulation	Droplet size (nm)	PDI	Viscosity (mPa·S)	Conductivity (μ S $\cdot$ cm $^{-1}$)
N1	51.04 $\pm$ 1.12	0.174 $\pm$ 0.002	10.2 $\pm$ 0.34	58.35 $\pm$ 0.34

Formulation	Droplet size (nm)	PDI	Viscosity (mPa·S)	Conductivity (μ S $\cdot$ cm $^{-1}$)
N2	48.03 $\pm$ 0.38	0.185 $\pm$ 0.005	12.8 $\pm$ 0.43	110.2 $\pm$ 0.65
N3	46.30 $\pm$ 0.12	0.166 $\pm$ 0.005	10.5 $\pm$ 0.34	160.2 $\pm$ 0.27
N4	64.60 $\pm$ 1.13	0.167 $\pm$ 0.006	18.7 $\pm$ 0.61	75.20 $\pm$ 0.58
N5	59.08 $\pm$ 1.45	0.170 $\pm$ 0.021	22.4 $\pm$ 0.32	103.3 $\pm$ 0.45
N6	55.40 $\pm$ 0.58	0.165 $\pm$ 0.006	28.6 $\pm$ 0.27	148.6 $\pm$ 0.33
N7	76.70 $\pm$ 1.02	0.192 $\pm$ 0.003	33.0 $\pm$ 0.55	49.70 $\pm$ 0.47
N8	71.20 $\pm$ 0.77	0.171 $\pm$ 0.007	33.5 $\pm$ 0.45	100.2 $\pm$ 0.45
N9	70.00 $\pm$ 0.35	0.184 $\pm$ 0.006	37.2 $\pm$ 0.45	139.2 $\pm$ 0.43

*Values are expressed as mean $\pm$ SD ($n = 3$).

Formulation of Nanoemulgel

Though all the formulations (N1–N9) were found in nanosize range, their low viscosities obstructed their applicability and therefore found inappropriate for dermal use. In order to impart applicability to nanoemulsions, their viscosities were used to

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increase by incorporating nanoemulsions into gel matrix of carbomer 940 resulting in nanoemulgels which were found to be consistent, uniform, and highly viscous to be applied dermally.

Drug Content and pH Determination of Nanoemulgel

The drug content of nanoemulgel formulation (Table 5) was in the range of $97.96 \pm 0.38\%$ to $100.05 \pm 0.43\%$. The results showed that the drug was uniformly distributed throughout the formulation and drug loss was minimum while formulating nanoemulgel.

Table 5. Characterization of nanoemulgel.

Formulation code	Drug content (%)	pH	Spreadability (gcmS ⁻¹)	Viscosity (m·PaS)
NG1	97.9 ± 0.3 8	6.5 ± 0.34	6.0 ± 0.21	1205 ± 6.2 ± 0.44
NG2	97.8 ± 0.5 8	6.7 ± 0.43	5.5 ± 0.32	1324 ± 3.6 ± 0.68
NG3	97.6 ± 0.5 2	6.6 ± 0.67	6.0 ± 0.54	1376 ± 6.3 ± 0.62
NG4	99.4 ± 0.3 4	6.7 ± 0.33	6.0 ± 0.22	1444 ± 3.0 ± 0.83
NG5	99.8 ± 0.4 6	6.5 ± 0.76	5.8 ± 0.42	1653 ± 3.4 ± 0.96
NG6	100.5 ± 0.6 5	6.8 ± 0.84	6.0 ± 0.11	1467 ± 2.7 ± 0.32
NG7	98.3 ± 0.4 3	6.6 ± 0.33	6.0 ± 0.32	1770 ± 3.5 ± 0.63

Formulation code	Drug content (%)	pH	Spreadability (gcmS ⁻¹)	Viscosity (m·PaS)
NG8	98.4 ± 0.3 3	6.5 ± 0.45	5.5 ± 0.18	1457 ± 7.3 ± 0.43
NG9	98.7 ± 0.6 5	6.5 ± 0.44	5.8 ± 0.21	1548 ± 0.5 ± 0.43

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

The pH values of different nanoemulgel were found to be in a range of 6.5–6.8 (nearly neutral), permitting the use of the formulation on the skin.

Spreadability

The spreadability of nanoemulgel formulation was determined because the application of formulation to inflamed skin is more comfortable and it spreads easily, exhibiting maximum slip and drag [8]. The spreadability of all formulations was found to be in range of 5.5 ± 0.18 to 6.0 ± 0.11 gcmS⁻¹. The large diameter signifies better spreadability.

Rheological Behaviour and Viscosity Measurement

The performance of topical formulation is monitored by its rheological behavior, which governs its flowability, spreadability, and release of drug. The release of drug from the formulation is governed by its components and consistency of the formulation. Consistency index is the measure of consistency and is equivalent to apparent viscosity at a shear rate of 1 sec⁻¹. The consistency index of the formulation NG6 was found to be 86.4 PaS and $n = 0.32$. The flow index is the measure of the deviation of system from Newtonian behavior ($n = 1$). A value of $n < 1$ indicates pseudoplastic flow or shear thinning and $n > 1$ indicates dilatant or shear thickening flow. Flow index confers an idea of flowability of formulation from the container. Generally the thicker the base, the lower the flow index. The nanoemulgel showed flow index of 0.32, indicating pseudoplastic flow behavior. 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.

It can be observed (Table 5) that increase in surfactant concentration leads to increase in viscosity of the nanoemulgel. Tween 80, used as surfactant here, was more soluble in the external

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aqueous phase. That is, because the concentration of water soluble surfactant in the system increased, the self-association of these amphiphilic molecules increased and formed different sizes and shapes of micellar aggregates [22]. As the concentration in external phase increased, the network will form between the surfactants molecules, micelles, and oil droplets. The denser the network, the closer the distance between the dispersed phases and the higher the viscosity.

Skin Permeation Studies

The *ex vivo* skin permeation studies were carried out to confirm and to compare the permeation potential of the nanoemulgel formulations (NG1–NG9). Nanoemulgel, in particular, is known to enhance permeation rates in deep skin layers and decrease lag time as compared to conventional formulations.

The cumulative amount of drug permeated, flux, enhancement ratio, lag time, skin retention, and LAE were calculated for each formulation of nanoemulgel. The formulation NG6 having oil content 6% w/w, surfactant cosurfactant mixture 35% w/w, and aqueous phase 56.5% w/w showed the highest CADP ($1.82 \pm 0.04 \text{ mg/cm}^2$) as compared to other formulations. Also a comparatively higher flux (0.074 ± 0.04) was observed for this formulation. In addition, lower lag time (0.56 h) and less skin retention (0.68 mg/cm^2) of NG6 than the other formulations tested made it considerable for being selected as the optimized formulation (Table 6, Figure 3).

Table 6. Permeation parameters for various nanoemulgel formulations.

Formulation code	CADP (mg/cm ²)	Flux	Lag time (h)	Drug retained (mg)
NG1	1.10 ± 0.04	0.05 7 ± 0.06	2.45 ± 0.21	1.40 ± 0.07
NG2	1.15 ± 0.06	0.05 8 ± 0.35	1.02 ± 0.16	1.35 ± 0.43
NG3	1.62 ± 0.02	0.07 2 ± 0.52	0.66 ± 0.26	0.88 ± 0.24
NG4	1.41 ± 0.07	0.06 4 ± 0.06	3.05 ± 0.23	1.09 ± 0.29
NG5	1.58 ± 0.12	0.06 6 ± 0.14	1.93 ± 0.18	0.92 ± 0.11

Formulation code	CADP (mg/cm ²)	Flux	Lag time (h)	Drug retained (mg)
NG6	1.82 ± 0.23	0.07 4 ± 0.25	0.56 ± 0.64	0.68 ± 0.66
NG7	1.19 ± 0.30	0.05 7 ± 0.32	2.39 ± 0.18	1.31 ± 0.33
NG8	1.32 ± 0.28	0.05 9 ± 0.17	2.28 ± 0.11	1.18 ± 0.26
NG9	1.50 ± 0.06	0.06 3 ± 0.29	0.73 ± 0.28	1.00 ± 0.18
NS1	1.08 ± 0.09 *	0.04 1 ± 0.32 *	3.68 ± 0.38 *	1.42 ± 0.46 *
NS2	1.11 ± 0.43 *	0.04 4 ± 0.18 *	3.43 ± 0.42 *	1.39 ± 0.19 *
NS3	1.15 ± 0.42 *	0.04 5 ± 0.26 *	3.21 ± 0.42 *	1.35 ± 0.25 *

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

CADP: cumulative amount of drug permeated.

* $P < 0.05$; Student's unpaired *t*-test was used to compare NS batch (formulation prepared without cosurfactant) with NG6 (optimized formulation) for CADP, flux, and lag time.

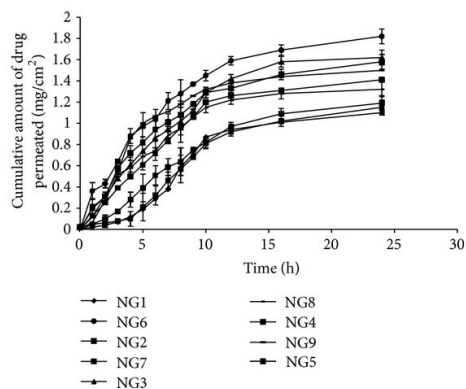


Figure 3: Permeation profile of nanoemulgel formulations.

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In the present study, oleic acid (oil phase) was employed as an integral component which is widely known for increasing permeation. The results revealed that, as the oleic acid in the formulation was increased from 3% w/w to 6% w/w, the flux (rate of permeation) was increased. This may be due to the fact that oleic acid permeates within lipid bilayers of stratum corneum by disrupting their order and management. Two mechanistic scenarios by which oleic acid increases permeation are lipid fluidization and lipid phase separation [23].

It was also observed that when the oil concentration was further increased to 9% w/w, the flux gets decreased. This may well be correlated with aggregation of oil due to its higher concentration [23]. Thus, formulation containing 6% w/w oil (NG4–NG6) attributed maximum permeation and less lag time in comparison to other nanoemulgel formulations.

Results also indicated that in nanoemulgel formulations (NG4–NG6) as the surfactant cosurfactant mixture concentration was decreased from 75% to 35%, the skin permeation rate was increased twofold. The reason attributed to the situation could be increase in thermodynamic activity of drug in nanoemulgel at lower content of surfactant [18]. It is well said that the thermodynamic activity of drug in the formulation is a sufficient driving force for the release and permeation of drug into the skin [24, 25].

The formulations (NG4–NG6) were also compared on the basis of concentration of aqueous phase, as water is the hydrophilic domain of nanoemulgel. When water content was increased, cumulative amount of drug permeated increased substantially. Also the flux calculated for formulation NG6 containing 56.5% of water was found to be significantly higher ($P < 0.05$, t -test) than formulation NG4 containing 16.5% water. The plausible rationale might be the entrance of aqueous fluid of nanoemulgel in polar pathway which increases interlamellar volume of lipid bilayer of stratum corneum, resulting in disruption of its interfacial structure and thereby enhancing permeation [8] (Figure 4).

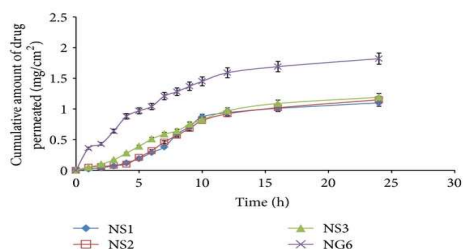


Figure 4: Comparison of permeation profile of NS (formulations without use of cosurfactant) with optimized formulation NG6.

It was well reported in previous works that nanoemulgel could perform as drug reservoir where drug is released from inner phase to outer phase and

then further into the skin [11]. The enhanced transdermal drug delivery might have resulted due to different mechanisms which include the permeation enhancement potential of different components of nanoemulgel.

Effect of Transcutol P as Permeation Enhancer. In order to investigate the effect of Transcutol P on the permeation of drug through skin, three batches (NS1–NS3) were prepared without the use of cosurfactant in the same composition as of NG4, NG5, and NG6. The NS1, NS2, and NS3 formulations showed significantly ($P < 0.05$) less permeation when compared with NG6 (optimized formulation containing cosurfactant). The lag time and drug retained are significantly higher in case of NS batch compared to NG6 formulation which explained that NS formulations showed less permeation as more amount of drug was retained in skin and took more time to permeate through the skin. The results confirmed that Transcutol P (cosurfactant) itself acted as permeation enhancer. The activity of Transcutol P was thought to result from solvation of α -keratin within the stratum corneum and occupation of proteinaceous hydrogen binding, thus promoting permeation [17].

Comparison of Permeation Studies of Marketed Formulation, Optimized Nanoemulgel, Nanoemulsion, and Plain Drug Gel and Drug Solution. The nanoemulgel formulation had higher flux (0.154 ± 0.56 mg/cm/h) than conventional marketed formulation (0.132 ± 0.34 mg/cm/h), plain drug gel (0.104 ± 0.34 mg/cm/h), and drug solution (0.089 ± 0.65 mg/cm/h) depicted in Table 7, Figure 5.

Table 7. Comparative permeation profile of different formulations.

Formulation code	^a CAD P (mg/cm ²)	^a Flux (mg/cm ² /h)	^a Lag time (h)	E_{pen}	^a Dru g retained (mg)	LAE
Dru g solution	0.75 ± 0.05	0.7 ± 0.21	3.4 ± 0.1	1	1.5 ± 0.1	2.3
Plain drug gel	1.2 ± 0.05	0.5 ± 0.12	3.0 ± 0.1	1.4 ± 0.1	1.8 ± 0.1	1.2

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Formulation code	^a CAD P (mg/c m ²)	^a Flux (mg/c m ² /h)	^a Lag time (h)	<i>E</i> _{pen}	^a Dru g retained (mg)	LAE
Marketed			2		0	
			5		6	
	1.	0.	1	1.	1	1
	2	06	.	6	.	.
	5	1	0	2	2	0
	±	±	1		5	0
	0.	0.	±		±	
	2	22	0		0	
	1		.		.	
			0		2	
N6			9		6	
	1.	0.	1	2.	0	0
	9	07	.	1	.	.
	7	8	8	0*	5	2
	±	±	9	*	3	6
	0.	0.	±		±	
	1	27	0		0	
	8*	**	.		.	
			2		2	
			5		2	
NG6	1.	0.	0	2.	0	0
	8	0.	.	0	.	.
	2	74	5	0*	6	3
	±	±	6		8	7
	0.	0.	±		±	*
	2	43	0		0	
	3*	*	.		.	
			1		2	
			2		9	
			*		*	

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

CADP: cumulative amount of drug permeated; *E*_{pen}: enhancement ratio; LAE: local accumulation efficiency = ketoprofen retained into skin/ ketoprofen permeated through skin.

**P* < 0.05; Student's *t*-test was used to compare NG6 (optimized formulation) with drug solution, plain drug gel, and marketed formulation.

***P* > 0.05; Student's *t*-test was also used to compare N6 (plain nanoemulsion) with NG6 and no significant difference was found.

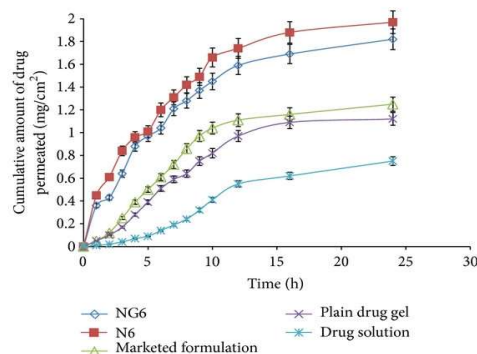


Figure 4: Permeation profile of nanoemulgel (NG6) in comparison to marketed formulation, nanoemulsion (N6), plain drug gel, and drug solution.

Furthermore, low droplet size also accounts for high permeation potential of nanoemulgel. The number of droplets that can interact on fixed area of stratum corneum will increase as droplet size decreases. Also small droplets might embed into the stratum corneum without a transfer via hydrophilic phase of nanoemulsions and drug molecules were easily permeated into the skin [9].

The lower local accumulation efficiency (LAE) of nanoemulgel (Table 7) which was found to be 0.37 elucidated the lower retention of drug in the skin and confirmed that maximum amount of drug has been permeated through skin. The LAE for drug solution was found to be the highest, that is, 2.33, showing that drug has permeated through skin to a negligible extent and has been retained in skin only.

In the present study, nanoemulgel was also compared with plain nanoemulsion. It was observed that the flux of nanoemulgel was lower than nanoemulsion, which may be due to higher viscosity of the formulation. When the flux of plain nanoemulsion (N6) was compared using unpaired student *t*-test, no significant (*P* > 0.05) difference was observed (Table 7). Though the nanoemulgel had lower flux, it can be favored over the nanoemulsion, due to prolonged effect and increased viscosity from viewpoint of its applicability on skin.

Investigation of Release Kinetics

The release kinetics was studied by plotting *ex vivo* permeation data in various kinetic models. Zero order rate describes the system where release rate is independent of its concentration (*q* versus *t*) and the first order describes the system where the release is concentration dependent (log *q* versus *t*). Higuchi's model describes the release of drugs from an insoluble matrix as a square root of time (*q* versus square root of *t*) based on Fickian diffusion [13]. The highest determination coefficient (*r*²) is preferred for selecting order of release. The best fit with the highest *r*² value was found to be shown by zero order permeation for all nanoemulgel formulations (Table 8).

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Table 8. Permeation kinetics of nanoemulgel formulations.

Formulation code	Zero order (r^2)	First order (r^2)	Higuchi (r^2)	Mechanism of release
NG1	0.984	0.785	0.878	0.345
NG2	0.979	0.868	0.899	0.376
NG3	0.959	0.780	0.795	0.267
NG4	0.988	0.925	0.911	0.368
NG5	0.993	0.888	0.800	0.422
NG6	0.996	0.980	0.985	0.327
NG7	0.959	0.855	0.922	0.353
NG8	0.967	0.915	0.912	0.412
NG9	0.961	0.899	0.944	0.333

To analyse the release mechanism of drug from nanoemulgel, the data was fit to Korsmeyer Peppas model, and n value was obtained close to 0.5 suggesting that ketoprofen was released from nanoemulgel through aqueous channels of gel matrix by Fickian diffusion release model.

Stability Studies

Nanoemulgel formulations when centrifuged showed no phase separation or drug precipitation, indicating that prepared nanoemulgel was physically stable. They showed no signs of breaking or cracking even when subjected to freeze/thaw cycles. The results of stability studies are shown in Table 9, depicting nanoemulgels remained clear even after a period of three months at temperatures $25 \pm 2^\circ\text{C}$, $40 \pm 0.1^\circ\text{C}$, and $4 \pm 0.2^\circ\text{C}$. All the formulations were found to be consistent with respect to their pH values, drug content, phase separation, and transparency during the stability study.

Table 9. Stability study parameters for nanoemulgel formulations.

Time (h)	Temp	Drug content ^a (%)	Transparency	pH ^a	Phase separation
1 day	4 ^o C	100.50 $\pm$ 0.02	+	6.6 $\pm$ 0.02	–
	25 ^o C	100.3 $\pm$ 0.04	+	6.6 $\pm$ 0.01	–
	40 ^o C	100.24 $\pm$ 0.05	+	6.6 $\pm$ 0.02	–
1 month	4 ^o C	99.85 $\pm$ 0.02	+	6.6 $\pm$ 0.04	–
	25 ^o C	99.74 $\pm$ 0.12	+	6.6 $\pm$ 0.02	–
	40 ^o C	99.64 $\pm$ 0.21	+	6.6 $\pm$ 0.01	–

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Time (h)	Temp	Drug content ^a (%)	Transparency	pH ^a	Phase separation
2 months	4°C	99.7	+	6.6	–
		2 ± 0.04		6.4	
				6.1	
	25°C	99.5	+	6.5	–
		9 ± 0.07		4.0	
				6.1	
	40°C	99.6	+	6.6	–
		1 ± 0.21		6.0	
				2.2	
3 months	4°C	99.4	+	6.6	–
		2 ± 0.14		6.3	
				6.1	
	25°C	99.5	+	6.6	–
		1 ± 0.15		1.0	
				1.1	
	40°C	99.1	+	6.6	–
		6 ± 0.21		6.3	
				0.1	

^aValues are expressed as mean ± SD (*n* = 3); +: presence; -: absence.

Carbomer 940 in nanoemulgel resulted in high viscosity and oil droplets might be distributed in gel

network, which might contribute to enhancement of stability of droplets in nanoemulsion [19]. Additionally, small droplet size of about 70 nm could reduce the creaming movement of droplets and high viscosity of nanoemulgel could also constrain the Brownian movement.

4. Discussion:

Nanoemulgel was proposed as carrier for transdermal delivery of ketoprofen due to its high solubilizing ability and permeation enhancing properties [1]. Apart from this its topical route has the potential to bypass the problems associated with chronic oral delivery of ketoprofen. The novel nanoemulgel system is drug loaded multicomponent system containing oil, surfactant cosurfactant mixture, aqueous phase, and gel base. The nanoemulsions were prepared by figuring out the concentration range of components [7]. All nine nanoemulsions (N1–N9) formed were optimized for morphological structure, droplet size, viscosity, and conductivity. The TEM image revealed spherical structure, and all formulations were in nanosize range (10–100 nm) with low PDI value indicating uniformity of droplet size in formulation. The high conductivity values confirmed the O/W structure of nanoemulsions. The viscosity values were found to be low which was not suitable for application onto skin.

Thus all nanoemulsions were incorporated in gel base, carbomer 940 (0.6%), resulting in nanoemulgel. The formulations were subjected to drug content determination which showed that drug loss during formulation occurred within the limits. The pH was near to pH of the skin which revealed nonirritating nature of the formulation. The spreadability of the all formulations exhibited slip and drag phenomenon with higher diameters. The viscosity was increased and rheogram displayed pseudoplastic behaviour which ensures that the developed system will not flow by itself and when filled into a container, namely, collapsible tube, it will require some yield value for ejection [26]. All nanoemulgels were subjected to permeation studies, and NG6 formulation showed significantly higher permeation which can be well correlated with the concentration of components. As the oil content was increased from 3% to 6% and content of surfactant cosurfactant mixture was decreased from 75% to 35% the permeation is enhanced. This synergistic effect was maybe due to the oleic acid permeation within lipid bilayers of stratum corneum by disrupting their order and management and increase in thermodynamic activity of the drug in skin at lower content of surfactant [18]. The effect of cosurfactant (Transcutol P) as permeation enhancer was also established by comparing the NG6 (optimized formulation) with NS1–NS3 (formulations prepared without cosurfactant) which showed NS formulations had significantly lower cumulative amount of drug permeated with lower

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flux. Also significantly higher lag time and skin retention than NG6 proved the permeation enhancing effect of cosurfactant.

Furthermore, the optimized formulation (NG6) was compared for various permeation parameters with plain nanoemulsion (N6), marketed formulation (*Fastum*), plain drug gel, and drug solution. Results clearly indicated that NG6 had higher cumulative amount of drug permeated, flux, and enhancement ratio and lower skin retention and LAE than marketed formulation, plain drug gel, and drug solution. But N6 showed higher flux than NG6 but not with significant difference ($P > 0.05$, t -test). Though the nanoemulgel has lower flux, it can be considered as superlative option over the nanoemulsion, because the prolonged effect of nanoemulgel can be expected. Moreover, pseudoplastic flow behaviour imparted by gel makes nanoemulgel superior in terms of ease of applicability [26].

The formulated nanoemulgel system was found to possess good permeation potential without incorporation of any chemical enhancers which are habitually irritants [9]. Hence, the novelty of this system lies here, as the components (oil, surfactant, and especially cosurfactant) of nanoemulgel themselves acted as permeation enhancers.

The stability studies were carried out at room temperature and refrigerator temperature, indicating that the formulation is stable and no change in drug content and pH was observed. Thus the nanoemulgel formulation could be beneficial in improving bioavailability and permeation of ketoprofen for transdermal fungal infections.

5. Conclusion:

The novel nanoemulgel of ketoprofen with suitable viscosity was successfully formulated for transdermal application. Nanoemulgel was formulated by addition of carbomer 940 into nanoemulsion which resulted in increase in viscosity and had no significant influence on penetration of ketoprofen. The permeation rate of nanoemulgel was 2.0 times higher than that of drug solution. The contact of the nanoemulgel with skin and effect of Transcutol P with fine permeation enhancing potential acted as key role for permeation of drug through skin. The optimized formulation was compared with conventional marketed formulation and showed significant higher permeation profile which justifies the nanoemulgel system to be a promising surrogate carrier for transdermal delivery of ketoprofen.

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