Original Research Article

# Enhanced transdermal permeability of piroxicam through novel nanoemulgel formulation

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## Abstract

**Background:** Piroxicam is a non-steroidal anti-inflammatory drug belongs to BCS class II drugs having poor solubility and is associated with a number of undesirable side-effects on the stomach and kidneys in addition to gastric mucosal damage. **Aim:** The present work was to develop and characterize nanoemulgel formulation as transdermal delivery system for poorly water soluble drug, in order to overcome the troubles associated with its oral delivery and to circumvent the need of chemical penetration enhancers, which are responsible for causing skin irritation in transdermal drug delivery. **Materials and Methods:** Different nanoemulsion components (oil, surfactant and co-surfactant) were selected on the basis of solubility and emulsification ability. Pseudoternary phase diagrams were constructed using aqueous titration method to figure out the concentration range of components. Carbopol 934 was added as gel matrix to convert nanoemulsion into nanoemulgel. Drug loaded nanoemulsions and nanoemulgels were characterized for particle size, transmission electron microscopy, viscosity, conductivity, spreadability, rheological behavior, permeation studies using Wistar rat skin and stability studies. Transdermal permeation of piroxicam from nanoemulgels was determined by using Franz Diffusion cell. **Results:** The optimized nanoemulgel (BG6) contained 10% oleic acid as oil, 35% tween 80 and ethanol as surfactant co-surfactant mixture, 55% water, 0.5% drug and 0.5% w/w carbopol. The *ex vivo* permeation profile of optimized formulation was compared with nanoemulsion and marketed formulation (Feldene®). Nanoemulgel showed higher ($P < 0.05$) cumulative amount of drug permeated and flux and significantly less drug retained along with less lag time than marketed formulation. **Conclusion:** The results indicate that nanoemulgel formulation can be used as a feasible alternative to conventional formulations of piroxicam with advanced permeation characteristics for transdermal application.

**Key words:** Carbopol 934, nanoemulsion, permeation, piroxicam, ternary diagram

## INTRODUCTION

Nanoemulsions have attracted great attention in delivery of therapeutically active agents since approximately 40% of new chemical entities are hydrophobic in nature and the delivery of these poorly water soluble drugs is a challenge for delivery of drugs. The term "Nanoemulsion" refers to a thermodynamically stable isotropically clear dispersion of two immiscible liquids, such as oil and water stabilized by an interfacial film of surfactant molecules. The emulsions and nanoemulsions differ mainly in size and shape of the particles dispersed in the continuous phase. The particle size in nanoemulsions is (10-200 nm) and those of conventional emulsions are (1-20 μm). The ascendancies associated with transdermal use of nanoemulsion are as enhanced drug solubility, good thermodynamic stability, enhancing effect on transdermal ability.[1] The aptness of nanoemulsion to increase the concentration gradient and thermodynamic activity toward skin along with permeation enhancement activity of its components makes the system expedient for transdermal delivery. However, 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.[2] Variant gel matrices such as carbomer 980, carbomer 940, carbomer 934, pluronics, xanthan gum and carrageenan have been exploited to increase the viscosity of nanooemulsion for transdermal delivery.[3] Thus, the incorporation of nanoemulsion into gel matrix can result in nanoemulgel which may be more relevant for transdermal application when compared to nanoemulsion.

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Piroxicam, a non-steroidal anti-inflammatory drug with analgesic and antipyretic effects, has been extensively utilized for treatment of musculoskeletal and joint disorders such as rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, in soft-tissue disorders, in acute gout and in post-operative pain. It is well absorbed following oral administration, however, its use has been associated with a number of undesirable side-effects on the stomach and kidneys in addition to gastric mucosal damage.[4] The adverse effects may worsen to severe renal and cardiovascular problems ultimately leading to mortality when used chronically especially in case of the geriatric population. The promising method to diminish its adverse effects is to deliver the drug through the skin.[5]

Therefore, an eventual need has emerged to develop a transdermal dosage form of piroxicam 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 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.[6] But, the use of these chemical enhancers may be harmful especially in chronic applications, since many of them are usually irritants.[6,7] 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 (ketoprofen, celecoxib, ibuprofen etc.).[7,8] The present study aims at formulating novel nanoemulgel of piroxicam 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.

## MATERIALS AND METHODS

### Materials

Piroxicam was procured from R.K.G Pharmaceutical Company, (Faridabad, India). Diethylene glycol monoethyl ether (Transcutol P®), Caprylocaproyl macrogol glycerides (Labrasol®) were a kind gift from Gattefosse (India). Oleic acid, ethanol, propylene glycol and tween 80 were purchased from S.D. Fine Chemicals (Mumbai, India). All other regents were used of analytical grade. Wistar rat skin (used rats) was obtained from NIPER, Mohali.

### Methods

#### *Solubility studies*

The solubility of piroxicam in various oils (sesame oil, oleic acid and isopropyl myristate (IPM) and soya bean oil), surfactants (labrasol, span 80 and tween 80) and co-surfactants (propylene glycol, ethanol and transcutol P) was determined by dissolving an excess amount of piroxicam in 500 mg of each of selected oils, surfactants and cosurfactants in stoppered vials. The mixtures were continuously stirred using a vortex mixer for 10 min and kept at 37 ± 1.0°C in an isothermal shaker for 72 h to attain equilibrium. The equilibrated samples were centrifuged (3000 rpm for 15 min) and the supernatant was filtered through 0.45 µm membrane filter and diluted with mobile phase. Drug content was quantified using ultraviolet (UV)-visible spectrophotometer (Shimadzu-1700, Japan) at 240 nm.

### Screening of components for nanoemulsion

On the basis of solubility studies, the oil was selected that possesses best solubilization capacity for piroxicam. Screening of surfactant and cosurfactant was done on the basis of percent transmittance. Emulsification ability of surfactants (tween 80, labrasol, span 80) was assessed by adding each; (300 mg) was to selected oil (300 mg). The mixture was gently heated at 40-45°C for 30 s 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-visible spectrophotometer (Shimadzu-1700, Japan) at 638 nm, using double distilled water as blank.

Various cosurfactants (propylene glycol, ethanol and transcutol P) were screened for formulation of nanoemulsions. Mixtures of co-surfactant (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.

#### *Construction of phase diagrams*

Pseudoternary phase diagrams were constructed using aqueous titration technique.[9] Oleic acid was used as oil phase. Surfactant cosurfactant mixture was composed of tween 80 as surfactant and ethanol as co-surfactant. Four weight ratios (1:0, 1:1, 1:2 and 1:3) of tween 80 to ethanol were optimized to determine the optimum ratio which can result in maximum nanoemulsion existence area. These Smix ratios were selected to reflect increasing concentrations of cosurfactant with respect to surfactant for detailed study of the phase diagrams in the nanoemulsion formation.

For each phase diagram, oil (oleic acid) and specific Smix ratio were mixed thoroughly in different volume ratios from 1:9 to 9:1 in different glass vials. A total of 18 different combinations of oil and Smix (1:9, 1:8, 1:7, 1:6, 1:5, 1:4, 1:3, 1:2, 1:1, 2:9, 2:8, 2:7, 2:6, 2:5, 2:4, 2:3, 2:2 and 2:1) were made for the study to delineate the boundaries of phases precisely formed in the phase diagrams. Slow titration with the aqueous phase was performed for each combination of oil and Smix separately. The amount of aqueous phase added was varied to produce a water concentration in the range of 5-95% of the total volume at around 5% intervals. The calculation for the addition of aqueous phase was done by calculating the percentage of each component of the nanoemulsion present at each 5% addition. After each

5% addition visual observation was made and the following categories were assigned:

1. Transparent and easily flowable: Oil/water nanoemulsions
2. Transparent gel: Nanoemulsion gel
3. Milky or cloudy: Emulsion
4. Milky gel: Emulgel.

For each Smix ratio, a separate phase diagram was constructed. As it can be observed in Figure 1, pseudo three-component phase diagram with 1 axis representing the aqueous phase, the second representing the oil phase and the third representing a mixture of surfactant and cosurfactant at a fixed volume ratio were constructed. Only nanoemulsion points are plotted (shaded area), so that there is no overcrowding of the phases in the diagram, as for formulation development only the nanoemulsion area is of interest.

## Formulation of nanoemulsion

Piroxicam was added to mixtures of oil, surfactant co-surfactant mixture with varying ratios pooled from pseudoternary phase diagrams and then an appropriate amount of water was added to the mixture in a dropwise manner. The nanoemulsion containing piroxicam was obtained by stirring the mixtures at ambient temperature. All nanoemulsions were stored at ambient temperature for further studies.

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

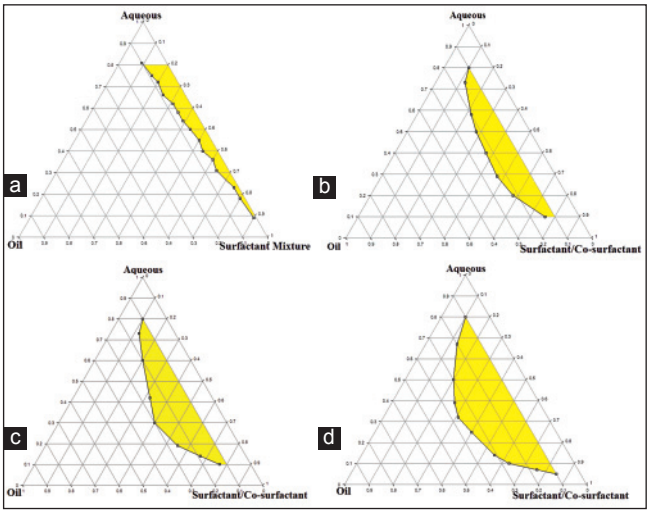


**Figure 1:** Pseudoternary phase diagram of formulation composed of oil (oleic acid), mixture of surfactant (tween 80) and cosurfactant (ethanol) dispersed with water at 37°C. For surfactant: Co-surfactant ratio of (a) 1:0 (b) 1:1 (c) 1:2 and (d) 1:3

### *Micrometrics of nanoemulsion*

Analysis of nanoemulsion's globule size and polydispersity index (PDI) measurement was carried out by dynamic light scattering with Zetasizer HSA 3000 (Malvern Instruments Ltd., UK). All samples were subjected to sonication prior to globule size and PDI determination.

### *Viscosity and conductivity of nanoemulsion*

The viscosity of true nanoemulsions was determined without any dilution using Brookfield Viscometer. The sample (1 g) was taken in a beaker and allowed to equilibrate for 5 min before measuring the dial reading using a spindle 40 and the measurement was started by operating the viscometer at 0.6 rpm, the speed was gradually increased and the measurement was recorded when the torque reached 10%. The speed was gradually increased at a constant rate for all tested samples until the torque reached 90%, with 30 s between each successive speed at 0.5, 1, 2.5 and 5 rpm respectively. At each speed, the corresponding dial reading on the viscometer was noted.[10] The electrical conductivity of nanoemulsion was determined using conductivity meter (EC Testr 11+, USA) at 25°C. The experiment was conducted in triplicate.

## Formulation of nanoemulgel

All the formulations (B1-B9) were found in nano size range and therefore incorporated in the gel matrix resulting in nanoemulgel. Carbomer 934 was selected as gel matrix base. The oily phase was obtained by mixing oleic acid, tween 80, ethanol and drug.

Carbomer 934 was swelled in a 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 934 under magnetic stirring. The pH values were subsequently regulated to 6-9 by using triethanolamine and nanoemulgel was obtained. The concentration of carbomer 934 in nanoemulgel was 0.5% (w/w).[11]

## Characterization of nanoemulgel

### *Drug content determination*

The amount of drug contained in the prepared nanoemulgel was determined by diluting required amount of prepared formulation using phosphate buffered saline (PBS) 7.4. This mixture was analyzed by UV spectrophotometer at 240 nm against PBS 7.4 as blank.[11]

### *pH determination*

Since the formulation was a topical formulation to be applied to the skin, therefore pH measurement was essential to ensure non irritating 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 nanoemulgels was determined 48 h after preparation by measuring the spreading diameter of nanoemulgel between the two glass plates after 1 min. A weight

of 350 mg of nanoemulgel was placed within a circle of diameter 1 cm pre-marked on the 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

$$S = \frac{m.l}{t} \quad (2)$$

Where, S = Spreadability, m = Weight placed on upper slide, l = Length of upper slide and t = The time taken.

### *Viscosity measurements and rheological behavior*

A Brookfield LVT DV-II Programmable Viscometer of Engineering Laboratories, Inc., (Middleboro, MA, USA) was connected to a thermostatic water bath adjusted to 25°C. Viscosity was measured on each base by using spindle 40. A defined amount (1 g) of each gel base was placed inside the plate and carefully closed. The measurement was started by operating the viscometer at 0.6 rpm, the speed was gradually increased and the measurement was recorded when the torque reached 10%. The speed was gradually increased at a constant rate for all tested samples until the torque reached 90%, with 30 s between each successive speed. The rheological parameters, including viscosity, shear rate, shear stress and yield value, were directly obtained from the monitor. The speed was then reduced gradually, using the same order as the increasing speeds, until reaching the starting rpm. A complete rheogram was obtained by plotting the shear rate as a function of the shear stress.[10]

For pseudoplastic flow, the exponential formula has been used most frequently.

$$\eta' = (FN)/G \quad (3)$$

The exponent *N* (Farrow's constant) rises as the flow becomes increasingly non-Newtonian. The term $\eta'$ represents viscosity coefficient. By the arrangement of the above equation,

$$\log G = N \log F - \log \eta' \quad (4)$$

An equation for a straight line is obtained. Many pseudoplastic systems fit this equation when log G is plotted as a function of log F.

## Ex vivo drug permeation studies

The *ex vivo* permeation 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 the dorsal side of the sacrificed animal was removed with a surgical blade no. 24 in the 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 receptor compartment of the diffusion cell was filled with 20 mL phosphate buffer pH 7.4. The whole assembly was fixed on a magnetic stirrer and the solution in the receptor compartment was constantly and continuously stirred using magnetic beads at 100 rpm and the temperature was maintained at 37 ± 0.50°C throughout the experiments.

The skin was mounted on diffusion cell assembly with an effective diffusion area (orifice area) of 4.91 $cm^2$. The prepared formulation (1 g) was applied onto the membrane in donor compartment. An aliquot of 2 mL sample was withdrawn at a suitable time intervals and replaced immediately with an equal volume of fresh diffusion medium. Samples were analyzed spectrophotometrically. The drug permeated per $cm^2$ of membrane was calculated and plotted against time and the flux was calculated as drug permeated per $cm^2$/h.

### *Comparison of permeation studies of marketed formulation, optimized nanoemulgel, nanoemulsion, plain drug gel and drug solution*

The *ex vivo* permeation study of optimized nanoemulgel formulation (BG6) was compared with the marketed formulation (Feldene®, Pfizer Ltd., Mumbai, India) for permeation and retention characteristics. The cumulative amount of drug permeated through the 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 the linear portion of plotted curve. The lag time ($T_{lag}$) was determined by extrapolating the linear portion of the cumulative amount permeated versus time curve to the abscissa. Enhancement ratio ($E_{pen}$) was calculated by dividing $J_{ss}$ of respective formulation with $J_{ss}$ of control formulation.[13]

The amount of piroxicam retained in the skin was determined at the end of the experiment. Skin was removed where effective permeation of skin was cut, washed 3 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 the ratio of drug accumulated in the skin to that delivered through the 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 the slope of the straight line. For slab matrix, if the exponent is 0.5, then diffusion mechanism is fickian; if $0.5 < n < 1.0$, mechanism is non-fickian.

## Stability studies

### *Thermodynamic stability studies*

Nanoemulsions are thermodynamically stable system and are formed at a particular concentration of oil, mixture of surfactant

and co-surfactant 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.[7]

The stability under centrifugation reflects the strength of interfacial film. The nanoemulgel formulation was centrifuged at 3500 rpm for 30 min. In the 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 3 times.

### *Stability studies at different temperature conditions*

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 (such as clarity, phase separation, precipitation of drug, color change), drug content and pH.[7]

### Statistical analysis

All experimental measurements were performed in triplicates. Result values were expressed as the mean value ± standard deviation. 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 $P < 0.05$.

## RESULTS

### Screening of components

To develop a nanoemulsion system of piroxicam for transdermal delivery, it should possess good solubility in the components of the system, as only solubilized drug can permeate through the skin. The solubility of piroxicam in various oils, surfactants and co-surfactants was investigated [Table 1]. Piroxicam had highest solubility in oleic acid (5.20 ± 0.58 mg/mL) followed by IPM. Oleic acid (Octadec-9-enoic acid) is widely used as oil phase in emulsion system and is responsible for permeation enhancement and can solubilize variety of drugs.[16] Oleic acid is an omega-nine fatty acid that is found naturally in many vegetable sources and animal products. It can impart stability to the formulations. It is rich in antioxidant that helps in fighting the effects of free radicals in the body; it strengthens the cell membrane integrity and helps in replacing cells and tissues damage. Therefore, oleic acid was screened as oil phase based on solubility studies.

**Table 1: Solubility of piroxicam in oils, surfactants and co-surfactants**

| Component | Solubility (mg/mL) | Component | Solubility (mg/mL) |
|---|---|---|---|
| Sesame oil | 0.92±0.68 | Tween 80 | 18±0.55 |
| Oleic acid | 5.20±0.58 | Labrasol | 3.7±0.33 |
| IPM | 2.51±1.09 | Propylene glycol | 7.1±0.58 |
| Soyabean oil | 0.90±0.86 | Span 80 | 4.2±0.58 |
| Transcutol P | 5.9±0.55 | Ethanol | 8.1±0.02 |

IPM: Isopropyl myristate

Among surfactants and co-surfactants, tween 80 (18 ± 0.55 mg/mL) and ethanol (8.1 ± 0.02 mg/mL) respectively showed highest solubilities. The criterion of selection of surfactant and co-surfactant for formulation of nanoemulsion is their percent transmittance.[17] Out of various surfactants and co-surfactants screened, tween 80 revealed 94.34 ± 0.21% transmittance whereas other surfactants span 80 and labrasol showed 60.66 ± 0.69% and 84.41 ± 0.66% respectively. Similarly, in case with co-surfactants, ethanol resulted in higher percent transmittance (88.58 ± 0.27) than propylene glycol (78.98 ± 0.57) and transcutol P (69.21 ± 0.63). Therefore, tween 80 and ethanol were selected as surfactant and co-surfactant, respectively, for the phase study.

Moreover, tween 80 is a non-ionic surfactant which is non-toxic when compared with ionic surfactants and has appropriate blend of low and high hydrophilic lipophilic balance (HLB), (HLB = 15), which can result in stable nanoemulsion. Ethanol, selected as co-surfactant has already been reported an efficient permeation enhancer.[18]

### Phase behavior 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.[9] Phase diagrams are useful tools to determine the number and types of phases, the wt% of each phase and the composition of each phase at a given temperature and composition of the system.[19] Pseudoternary phase diagrams were constructed separately for each surfactant to the cosurfactant ratio, so that o/w nanoemulsion regions could be identified and nanoemulsion formulations could be optimized.

As can be seen in Figure 1a, surfactant to the cosurfactant ratio 1:0 (w/w) was used, it showed decreased nanoemulsion area. It might be due to aggregation of the surfactant, leading to decrease in nanoemulsion existence area. As shown in Figure 1b, surfactant to the co-surfactant ratio 1:1 (w/w) was used, it showed significant nanoemulsion area. Nanoemulsion o/w region was found as we incorporated cosurfactant with the surfactant. However, still surfactant is used in the same concentration as that of cosurfactant. As reported in earlier reports higher concentration of surfactant can cause skin irritation.[20] Hence, it was decided to increase the co-surfactant concentration in the ratio 1:2 (w/w) [Figure 1c], a large o/w nanoemulsion area was observed. The reason attributed to the condition may be greater penetration of the oil phase in the hydrophobic region of the surfactant monomers by decreasing oil phase size due to the use of cosurfactant. Another reason could be an increase in entropy of the system.

As we further increased cosurfactant concentration to 1:3 (w/w) [Figure 1d], the nanoemulsion region was further enhanced when compared with a surfactant to the co-surfactant mixture ratio of 1:2 (w/w) which leads to much better results. Increasing the amount of cosurfactant i.e., ethanol in nanoemulsion may also have favorable effects on the skin permeation of piroxicam since ethanol has been used as a permeation enhancer for many drugs.[18] Thus, 1:3 (w/w) ratio of surfactant to co-surfactant was selected as optimized ratio, which can be used further in the formulation of nanoemulsion from which different concentrations of components (oil, surfactant co-surfactant mixture and water) for formulation of nanoemulsion were pooled randomly [Table 2]. Concentration of piroxicam used for preparation of nanoemulsion was 0.5% w/w. The concentration of surfactants should be optimum enough to emulsify the system only and not being the irritants.

## 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 the sample was less than 200 nm [Figure 2]. These results confirmed that the droplets were in nano size range (less than 200 nm) and thus emulsion formulated was nanoemulsion [Table 2].

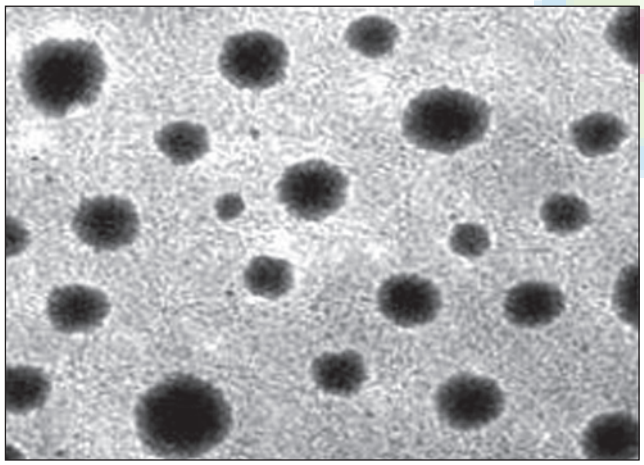

**Figure 2:** Transmission electron microscopy image of piroxicam nanoemulsion

### *Micrometrics*

Size characterization of nanoemulsion is essential in ensuring safe and efficient dosage.[21] As the oil content was kept minimum, i.e., 5% w/w, the droplet size found to be lowest, which was 125.1 nm. When oil content was increased to 15% w/w the droplet size increased substantially. All formulations were found in nano size range which was also depicted by the low values of PDI. PDI signifies the uniformity of droplet size within the formulation.[22] PDI below 0.3 indicates good uniformity in the droplet size distribution after dilution with water.[23,24] the polydispersity values of formulations were found in low range (0.164-0.192), indicating narrow distribution of droplet size within formulation [Figure 3 and Table 2].

The type of surfactant did not considerably affect the droplet size, while the co-surfactant (ethanol) containing nanoemulsions produced largest droplets as well as highest viscosity.[25] An increase in the ratio of the oil phase (oleic acid) also resulted in a proportional increase in particle size. It is well-known that the addition of surfactants to these systems causes the interfacial film to stabilize and condense, while the addition of co-surfactant causes the film to expand; thus, the relative proportion of surfactant to co-surfactant has varied effects on the globule size. Furthermore, it has been reported that the smaller particle size of the emulsion globules may lead to more rapid absorption and improve the bioavailability.[26]

Data of nanoemulsion formulations reveals that there is not much difference in the zeta potential of the formulations [Figure 4 ]. A dividing line between stable and unstable aqueous dispersions is generally taken at either ± 30 mV. Particles with zeta potentials more negative than −30 mV are normally considered as

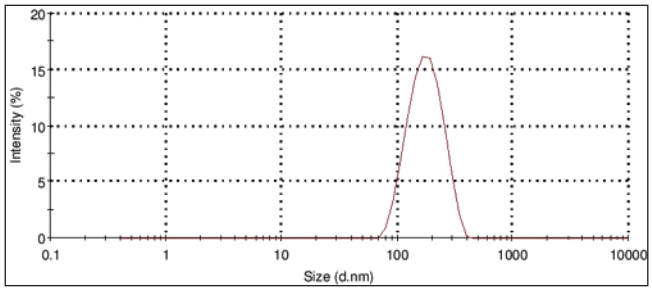


**Figure 3:** Size distribution of piroxicam nanoemulsion

**Table 2: Composition and physiochemical characterization of nanoemulsion**

| Formulation code | Components (%w/w) | | | Droplet size (nm) | PDI | Zeta potential (mV) | Viscosity (mPas) | Conductivity (μS/cm) |
|---|---|---|---|---|---|---|---|---|
| | Oil | Tween 80: ethanol in 1:3 | Water | | | | | |
| B1 | 5 | 55 | 40 | 128.2 | 0.173 | −31.2 | 11.5±0.24 | 101.21±0.34 |
| B2 | 5 | 45 | 50 | 126.3 | 0.185 | −29.4 | 10.8±0.43 | 111.2±0.65 |
| B3 | 5 | 35 | 60 | 125.1 | 0.166 | −28.6 | 9.5±0.32 | 159.2±0.18 |
| B4 | 10 | 55 | 35 | 138.1 | 0.168 | −53.4 | 27.7±0.61 | 99.20±0.58 |
| B5 | 10 | 45 | 45 | 136.2 | 0.170 | −51.7 | 25.4±0.32 | 101.3±0.45 |
| B6 | 10 | 35 | 55 | 135.4 | 0.164 | −55.8 | 23.6±0.27 | 139.6±0.33 |
| B7 | 15 | 55 | 30 | 147.6 | 0.192 | −41.8 | 38.0±0.55 | 98.70±0.24 |
| B8 | 15 | 45 | 40 | 145.4 | 0.172 | −38.5 | 34.5±0.45 | 99.2±0.45 |
| B9 | 15 | 35 | 50 | 143.6 | 0.183 | −36.4 | 31.2±0.44 | 129.2±0.41 |

PDI - Polydispersity index

stable.[27] Most appropriate zeta potential was of B6 formulation (−55.8 mV).

### Viscosity and conductivity determination

The viscosity of nanoemulsion was found to be low (9.5 ± 0.32-38.0 ± 0.55 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.

From viscosity determination it was observed that as the concentration of surfactant (tween 80) and cosurfactant (ethanol) increased viscosity of formulation also get increased[25,28] [Table 2].

The conductivities of nanoemulsion was 98.70 ± 0.24-159.2 ± 0.18 μS/cm, which was high and also confirmed formulation of o/w type of nanoemulsions as reported earlier.[20] According to the conductivity measurements, the investigated nanoemulsion can be separated as w/o or o/w.

In the region of low water contents, the w/o nanoemulsion was formed and the conductivity of the nanoemulsions remained at around 10 μS/cm. As the fraction of water volume was increased, the o/w nanoemulsion was formed and the conductivity was reached above 100 μS/cm. It has been previously reported that o/w nanoemulsions have relatively high conductivity as compared with w/o nanoemulsions.[29]

### Formulation of nanoemulgel

For transdermal application, piroxicam was formulated into nanoemulgel system containing carbopol 934 (0.5% w/w) as gel matrix. The thickened system was expected to offer good biophysical and sensorial benefits to skin for an effective and efficient transdermal delivery.[8] Carbopol 934 gel base is a cross linked polyacrylic acid, which has excellent mucoadhesive properties causing significant enhancement in transdermal bioavailability. It is reported that Carbopol 934 gel base showed higher release than other carbomer gel bases (carbomer 940, pluronic F-127 etc.), which is due to the fact that carbopol 934 exhibited a lower viscosity than other gel bases.[30] So carbopol 934 was used as a gel base for nanoemulgel formulation. Here, in this study, influence of order of addition of carbopol 934 on the formation of nanoemulgel was also investigated. In the first case, carbopol 934 was added directly to preformed nanoemulsion it took much more time to be swelled in nanoemulsion than in water. Even some tiny agglomerates of carbopol 934 were observed as it was not properly swollen.

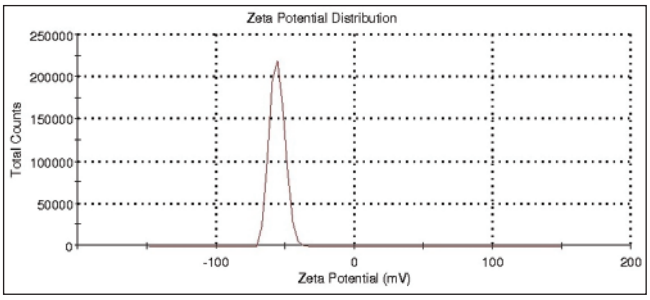


**Figure 4:** Zeta potential of piroxicam nanoemulsion

In the second case, carbopol 934 was swelled in the aqueous phase and its pH was adjusted with triethanolamine and then it was incorporated in the 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 carbopol 934 influenced homogenization of gel matrix. As here, the second case is found to have resulted in homogenous nanoemulgel. The gelling behavior of carbopol 934 is due to may be of non-covalent intermolecular associations deriving from forces such as coulombic, vander waals and hydrogen bond interaction. These physical interactions could lead to the formation of three-dimensional gel network and dispersed oil droplets were reasonably hosted within meshes of these networks.[31] Though, all the formulations (B1-B9) were found in nano size range but 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 increase by incorporating nanoemulsions into gel matrix of carbopol 934 resulting into nanoemulgel (BG1-BG9) 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 3] was in the range of 96.60 ± 0.52-99.6 ± 0.65%. The results showed that the drug was uniformly distributed throughout the formulation and drug loss was minimum while formulating nanoemulgel. The pH values of different nanoemulgel were found to be in a range of 6.2-6.7 (nearly neutral), permitting the safe 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 it spreads easily, exhibiting maximum slip and drag.[6] The spreadability of all formulations were found to be in the range of 3.17 ± 0.53-3.85 ± 0.32 $gcmS^{-1}$. The large diameter signifies better spreadability.

**Table 3: Physicochemical characterization of prepared nanoemulgel formulations**

| Formulation code | Drug content (%) | pH | Spreadability (gcm/S) | Viscosity (mPas) |
|---|---|---|---|---|
| BG1 | 96.9±0.38 | 6.2±0.34 | 3.32±0.21 | 13712.2±0.44 |
| BG2 | 96.7±0.57 | 6.5±0.430 | 3.24±0.32 | 12253.6±0.67 |
| BG3 | 96.6±0.52 | 6.7±0.66 | 3.17±0.53 | 11712.3±0.62 |
| BG4 | 98.4±0.34 | 6.5±0.32 | 3.61±0.22 | 21433.0±0.72 |
| BG5 | 98.8±0.45 | 6.4±0.76 | 3.57±0.42 | 19523.4±0.86 |
| BG6 | 99.6±0.65 | 6.7±0.84 | 3.44±0.11 | 17672.7±0.31 |
| BG7 | 97.3±0.43 | 6.5±0.32 | 3.85±0.32 | 24603.5±0.63 |
| BG8 | 97.4±0.32 | 6.4±0.45 | 3.79±0.15 | 23377.3±0.41 |
| BG9 | 97.7±0.65 | 6.4±0.44 | 3.68±0.21 | 22470.5±0.43 |

### *Rheological behavior and viscosity measurement*

The performance of topical formulation is monitored by its rheological behavior, which govern its flowability, spreadability and release of drug. The rheological evaluation of pharmaceutical semisolids is useful since it provides a method of quality control during and after the manufacturing process and information about the structure of the phase's information present in a product and the influence of various agents used in the formulation. Nanoemulgel was subjected to rheological examination. Farrow's constant ($N$) of the formulation BG6 was found to be 1.52. A value of $N>1$ indicates pseudoplastic flow or shear thinning and $N<1$ indicates dilatant or shear thickening flow [Figure 5]. The results confirmed that the nanoemulgel exhibited pseudoplastic 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 3] 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 aqueous phase. This is due to the concentration of water soluble surfactant in the system increased, the self-association of these amphiphilic molecules increased and formed different sizes and shape of micellar aggregates.[32] 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 phase, the higher the viscosity.

### Skin permeation studies

The *ex vivo* skin permeation studies were carried out to confirm as well as to compare the permeation potential of the nanoemulgel formulations (BG1-BG9) [Table 4]. Nanoemulgel, in particular, was known to enhance permeation rates in deep skin layers and decrease lag time when compared to conventional formulations. It was well-reported in previous works that nanoemulgel could perform as drug reservoir where drug was released from inner phase to outer phase and then further into the skin. The enhanced transdermal drug delivery might had resulted due to different mechanisms, which include the permeation enhancement potential of different components of nanoemulgel.

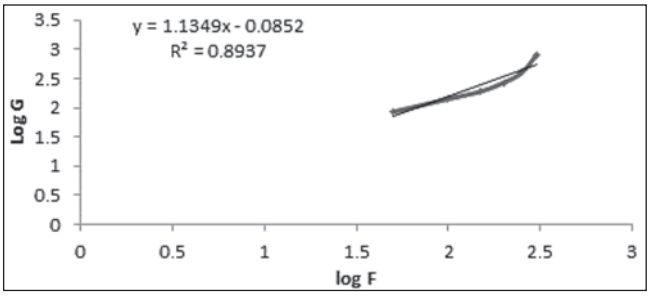


**Figure 5:** Rheogram depicting pseudoplastic behavior of nanoemulgel

In the present study, oleic acid (oil phase) was employed as an integral component which was widely known for increasing permeation the results revealed as the oleic acid in the formulation was increased from 5% w/w to 10% w/w, the flux (rate of permeation) was increased. This may be due to that oleic acid permeate within lipid bilayers of the stratum corneum by disrupting their order and management. Oleic acid had been commonly used as a powerful enhancer for many drugs,[33] including piroxicam.[34,35] The enhancing mechanism by oleic acid involves the increased fluidity of lipid portion of the stratum corneum.[36] [Figure 6] it was also observed that when the oleic acid concentration was further increased to 15% w/w, the flux gets decreased. This may well be correlated with aggregation of oil due to its higher concentration. Thus, formulation containing 10% w/w oil (BG4-BG6) attributed maximum permeation and less lag time in comparison to other nanoemulgel formulations.

Results also indicated that in nanoemulgel formulations (BG4-BG6) as the surfactant co-surfactant mixture concentration was decreased from 55% to 35% the skin permeation rate was increased to two-fold. The reason attributed to the situation

**Table 4: Permeation parameters of various nanoemulgel formulations**

| Formulation code | CADP (mg/cm²) | Flux (mg/cm²/h) | Lag time (h) | Drug retained (mg) |
|---|---|---|---|---|
| BG1 | 0.78±0.03 | 0.033±0.001 | 0.53±0.01 | 1.12±0.03 |
| BG2 | 0.80±0.06 | 0.034±0.002 | 0.51±0.01 | 1.04±0.02 |
| BG3 | 0.82±0.04 | 0.035±0.002 | 0.48±0.03 | 0.94±0.04 |
| BG4 | 0.87±0.06 | 0.036±0.003 | 0.46±0.02 | 1.69±0.05 |
| BG5 | 0.95±0.05 | 0.040±0.002 | 0.45±0.01 | 0.73±0.01 |
| BG6 | 1.00±0.04 | 0.042±0.003 | 0.41±0.03 | 0.09±0.001 |
| BG7 | 0.77±0.03 | 0.032±0.004 | 0.51±0.02 | 1.22±0.03 |
| BG8 | 0.79±0.05 | 0.034±0.002 | 0.54±0.04 | 1.12±0.06 |
| BG9 | 0.81±0.04 | 0.035±0.003 | 0.55±0.03 | 1.03±0.08 |
| BS1 | 0.61±0.04* | 0.020±0.002* | 0.77±0.04* | 1.79±0.05* |
| BS2 | 0.64±0.02* | 0.024±0.003* | 0.74±0.02* | 1.72±0.04* |
| BS3 | 0.68±0.05* | 0.027±0.004* | 0.71±0.03* | 1.68±0.03* |

CADP: Cumulative amount of drug permeated. *$P < 0.05$, Student unpaired $t$-test was used to compare BS batch (formulation prepared without co-surfactant) with BG6 (optimized formulation) for CADP, flux and lag time

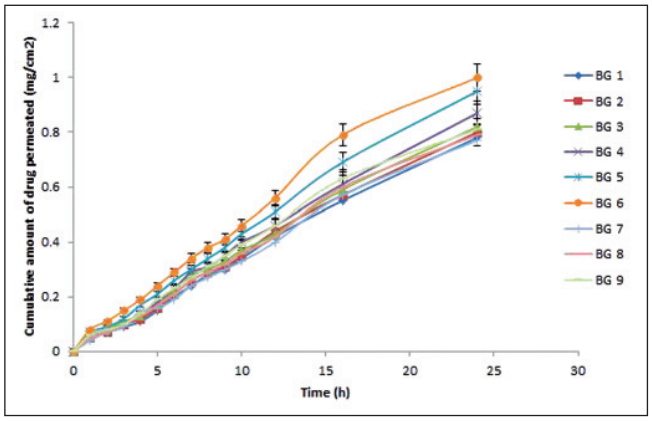


**Figure 6:** Permeation profile of nanoemulgel formulations (BG1-BG9)

could be an increase in thermodynamic activity of drug in nanoemulgel at lower content of surfactant.[15] It was well said that the thermodynamic activity of drug in the formulation was a sufficient driving force for the release and permeation of drug in to the skin.[5] The formulations (BG4-BG6) were also compared on the basis of concentration of aqueous phase, as water was the hydrophilic domain of nanoemulgel. When water content was increased, cumulative amount of drug permeated increased substantially. Furthermore, the flux calculated for formulation BG6 containing 55% of water was found to be significantly higher ($P < 0.05$, *t*-test) than formulation BG4 containing 35% 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.[3]

The cumulative amount of drug permeated, flux, enhancement ratio, lag time, skin retention and LAE were calculated for each formulation of nanoemulgel. The formulation BG6 having oil content 10% w/w, surfactant cosurfactant mixture 35% w/w, aqueous phase as 55% w/w showed highest CADP (1.0 ± 0.04 mg/cm$^2$) as compared to other formulations. Also a comparatively higher flux (0.042 ± 0.003) was observed for this formulation. In addition, lower lag time (0.41 ± 0.03 h) and less skin retention (0.09 ± 0.001 mg/cm$^2$) of BG6 than the other formulations tested, made it considerable for being selected as the optimized formulation.

### *Effect of ethanol as permeation enhancer*

In order to investigate the effect of ethanol on the permeation of drug through skin, three batches (BS1-BS3) were prepared without the use of cosurfactant in the same composition as of BG4, BG5 and BG6. The BS1, BS2 and BS3 formulations showed significantly ($P < 0.05$) less permeation when compared with BG6 (optimized formulation containing cosurfactant) [Figure 7].

The lag time and drug retained was significantly higher in case of BS batch compared to BG6 formulation which explained that BS formulations showed less permeation as more amount of the drug was retained in the skin and took more time to permeate through the skin. The results confirmed that ethanol (cosurfactant) itself acting as permeation enhancer. The presence of ethanol decreased the ending stress of interface and made the interfacial film sufficiently flexible to take up different curvatures required to form nanoemulsions over a wide range.[37]

### Comparison of permeation studies of marketed formulation, optimized nanoemulgel, plain nanoemulsion, plain drug gel and drug solution

The nanoemulgel formulation had higher flux (0.042 ± 0.003 mg/cm/h), than conventional marketed formulation (0.040 ± 0.02 mg/cm/h), plain drug gel (0.037 ± 0.02 mg/cm/h) and drug solution (0.032 ± 0.01 mg/cm/h) depicted in [Table 5]. The lower LAE of nanoemulgel which was found to be 0.09 elucidated the lower retention of drug in the skin and confirmed that the maximum amount of the drug has been permeated through skin. The LAE for drug solution was found to be highest, i.e., 1.10, showing that drug had permeated through skin to a negligible extent and retained in skin only.

Furthermore, low droplet size also accounts for high permeation potential of nanoemulgel. The number of droplets that could interact on fixed area of stratum corneum would increase as droplet size decrease. Also small droplets might embed into the stratum corneum without a transfer through hydrophilic phase of nanoemulsions and drug molecules were easily permeate into the skin. 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

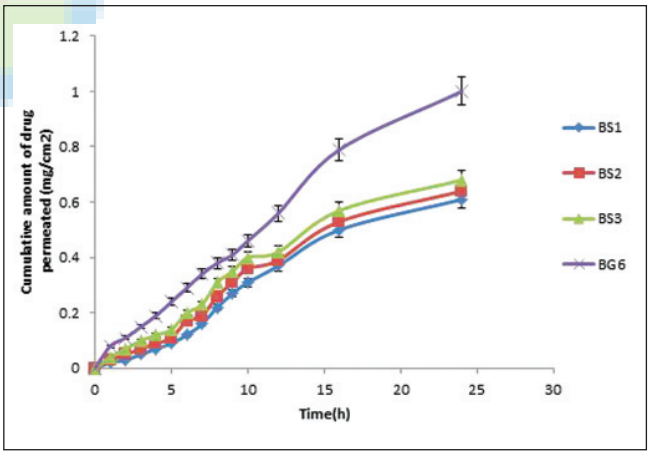


**Figure 7:** Comparison of permeation profile of BS (formulations without co-surfactant)

**Table 5: Comparative permeation parameters of different formulations**

| Formulation code | CADP | Drug permeated % | Flux | Lag time | $E_{pen}$ | Drug retained (mg) | LAE |
|---|---|---|---|---|---|---|---|
| Drug solution | 0.83±0.02 | 81.5 | 0.032±0.01 | 0.79±0.11 | 1 | 0.92±0.02 | 1.10 |
| Plain drug gel | 0.93±0.03 | 91.3 | 0.037±0.02 | 0.72±0.25 | 1.15 | 0.43±0.03 | 0.46 |
| Marketed formulation | 0.96±0.05 | 94.2 | 0.040±0.02** | 0.55±0.09 | 1.25 | 0.28±0.02 | 0.29 |
| BP6 | 1.01±0.06** | 99.1 | 0.044±0.04** | 0.57±0.05 | 1.37** | 0.04±0.02 | 0.03 |
| BG6 | 1.0±0.04* | 98.2 | 0.042±0.003* | 0.41±0.03* | 1.31* | 0.09±0.001* | 0.09* |

CADP: Cumulative amount of drug permeated, $E_{pen}$: Enhancement ratio, LAE: Local accumulation efficiency = piroxicam retained into skin/piroxicam permeated through skin. *$P < 0.05$, Student *t*-test was used to compare B6 (optimized formulation) with drug solution, plain drug gel; **$P > 0.05$, Student *t*-test was also used to compare BP6 (plain nanoemulsion) with BG6 and marketed formulation, no significant difference was found

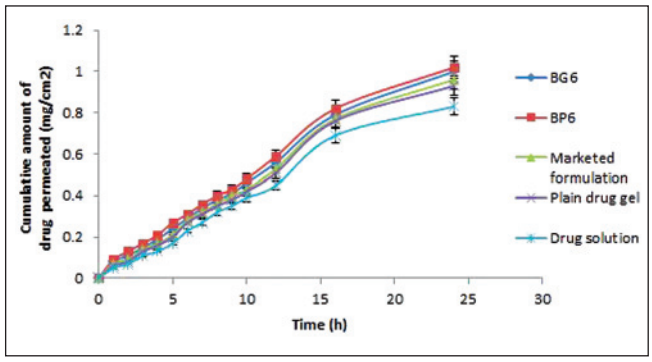


**Figure 8:** Comparison of permeation profile of optimized nanoemulgel BG6 with marketed formulation and plain nanoemulsion

viscosity of the formulation [Figures 8 and 9]. When the flux of plain nanoemulsion (BP6) was compared using unpaired Student *t*-test, no significant ($P > 0.05$) difference was observed [Table 5] though, the nanoemulgel had lower flux, it could 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. The best fit with highest $r^2$ value was found shown by zero order permeation for all nanoemulgel formulations. To analyze the release mechanism of drug from nanoemulgel, the data was fit to Korsmeyers peppas model, *n* value was obtained close to 0.5 suggesting that piroxicam 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 were physically stable. They showed no signs of breaking or cracking even when subjected to freeze thaw cycles. Nanoemulgels remained clear even after a period of 3 months at temperature 25 ± 2°C, 40 ± 0.1°C and 4 ± 0.2°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. Carbomer 934 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.[38]

## DISCUSSION

Nanoemulgel was proposed as a carrier for transdermal delivery of piroxicam due to its high solubilizing ability and permeation enhancing properties. Apart from this its transdermal route has the potential to bypass the problems associated with chronic oral delivery of piroxicam. The novel nanoemulgel system is drug loaded multi-component system containing oil, surfactant cosurfactant mixture, aqueous phase and gel base.

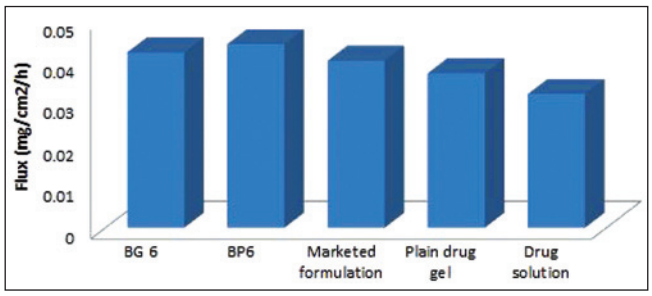


**Figure 9:** Comparative profile of flux for different formulations

The nanoemulsions were prepared by figuring out the concentration range of components.[5] All 9 nanoemulsions (B1-B9) formed were optimized for morphological structure, droplet size, viscosity and conductivity. The TEM image revealed spherical structure and all formulations were in nano size range (<200 nm) with low PDI value indicating uniformity of droplet size in formulation. The high conductivitry values confirmed the o/w structure of nanoemulsions. The viscosity values were found to be low which was not suitable for application on to the skin. Thus all nanoemulsions were incorporated in a gel base, carbomer 934 (0.5%), resulted 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 non-irritating nature of the formulation. The spreadability of all formulations exhibited slips and drag phenomenon with higher diameters. The viscosity was increased and rheogram displayed pseudoplastic behavior which ensures that the developed system will not flow by itself and when filled into a container viz. collapsible tube it will require some yield value for ejection.[22]

All nanoemulgels were subjected to permeation studies, nanoemulgel formulation showed significantly higher permeation which can be well correlated with the concentration of components. As the oil content was increased from 5% to 15% and content of surfactant cosurfactant mixture was decreased from 55% to 35% the permeation is enhanced. This synergistic effect was may be due to the oleic acid permeate 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 (ethanol) as permeation enhancer was also established by comparing the BG6 (optimized formulation) with BS1-BS3 (formulations prepared without cosurfactant) which showed BS formulations had significantly lower cumulative amount of drug permeated with lower flux. Furthermore, significantly higher lag time and skin retention than BG6, proved the permeation enhancing effect of cosurfactant. Furthermore, the optimized formulation (BG6) was compared for various permeation parameters with plain nanoemulsion (BP6), marketed formulation (Feldene®), plain drug gel and drug solution. Results clearly indicated that BG6 had higher cumulative amount of drug permeated, flux, enhancement ratio and lower skin retention, LAE than marketed

formulation, plain drug gel and drug solution. However, BP6 showed higher flux than BG6, 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 prololnged effect of nanoemulgel can be expected. Moreover, pseudoplastic flow behavior imparted by gel makes naoemulgel superior in terms of ease of applicability.[36]

The formulated nanoemulgel system was found to possess good permeation potential without incorporation of any chemical enhancers which are habitually irritants.[7] Hence, the novelty of this system lies here, as the components (oil, surfactant and especially cosurfactant) of nanoemulgel their selves 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 piroxicam for transdermal fungal infections without the use of chemical permeation enhancers.

## CONCLUSION

The novel nanoemulgel of piroxicam with suitable viscosity was successfully formulated for transdermal application. Nanoemulgel was formulated by addition of carbomer 934 into nanoemulsion, which resulted in increase in viscosity and had no significant influence on penetration of piroxicam. The contact of the nanoemulgel with skin and effect of oleic acid and ethanol 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 higher permeation rate and significantly less drug retained which justifies the nanoemulgel system to be a promising surrogate carrier for transdermal delivery of piroxicam.

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