

Nanoemulsions as Vehicles for Transdermal Delivery of Aceclofenac

Received: April 3, 2007; Final Revision Received: July 12, 2007; Accepted: July 13, 2007; Published: December 14, 2007

Faiyaz Shakeel,¹ Sanjula Baboota,¹ Alka Ahuja,¹ Javed Ali,¹ Mohammed Aqil,¹ and Sheikh Shafiq¹

¹Department of Pharmaceutics, Faculty of Pharmacy, Jamia Hamdard (Hamdard University), New Delhi – 110062, India

ABSTRACT

The aim of the present study was to investigate the potential of a nanoemulsion formulation for transdermal delivery of aceclofenac. Various oil-in-water nanoemulsions were prepared by the spontaneous emulsification method. The nanoemulsion area was identified by constructing pseudoternary phase diagrams. The prepared nanoemulsions were subjected to different thermodynamic stability tests. The nanoemulsion formulations that passed thermodynamic stability tests were characterized for viscosity, droplet size, transmission electron microscopy, and refractive index. Transdermal permeation of aceclofenac through rat abdominal skin was determined by Franz diffusion cell. The *in vitro* skin permeation profile of optimized formulations was compared with that of aceclofenac conventional gel and nanoemulsion gel. A significant increase in permeability parameters such as steady-state flux (J_{ss}), permeability coefficient (K_p), and enhancement ratio (E_r) was observed in optimized nanoemulsion formulation F1, which consisted of 2% wt/wt of aceclofenac, 10% wt/wt of Labrafil[®], 5% wt/wt of Triacetin[®], 35.33% wt/wt of Tween 80[®], 17.66% wt/wt of Transcutol P[®], and 32% wt/wt of distilled water. The anti-inflammatory effects of formulation F1 showed a significant increase ($P < .05$) in percent inhibition value after 24 hours when compared with aceclofenac conventional gel and nanoemulsion gel on carrageenan-induced paw edema in rats. These results suggested that nanoemulsions are potential vehicles for improved transdermal delivery of aceclofenac.

KEYWORDS: Aceclofenac, nanoemulsions, transdermal delivery, anti-inflammatory effects.

INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) are the most commonly used drugs to reduce pain and inflammation.¹ Aceclofenac, an NSAID, has been recommended orally for the treatment of rheumatoid arthritis and osteoarthritis.^{2,3}

It also has anti-inflammatory, antipyretic, and analgesic activities.⁴ The oral administration of aceclofenac causes gastrointestinal ulcers and gastrointestinal bleeding with chronic use.² Because of gastrointestinal bleeding, it also causes anemia. Using the transdermal route eliminates these side effects, increases patient compliance, avoids first-pass metabolism, and maintains the plasma drug level for a longer period of time. Therefore, an improved aceclofenac nanoemulsion formulation with a high degree of permeation could be useful in the treatment of locally inflamed skin and inflammatory and painful states of supporting structures of the body, such as bones, ligaments, joints, tendons, and muscles. There has been increased interest during recent years in the use of topical vehicle systems that could modify drug permeation through the skin. Many of the dermal vehicles contain chemical enhancers and solvents to achieve these goals.⁵ But use of these chemical enhancers may be harmful, especially in chronic application, as many of them are irritants. Therefore, it is desirable to develop a topical vehicle system that does not require 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 microemulsion or nanoemulsion. Nanoemulsions are thermodynamically stable transparent (translucent) dispersions of oil and water stabilized by an interfacial film of surfactant and cosurfactant molecules having a droplet size of less than 100 nm.^{6,7} Many studies have shown that nanoemulsion formulations possess improved transdermal and dermal delivery properties *in vitro*,⁸⁻¹⁶ as well as *in vivo*.¹⁷⁻¹⁹ Nanoemulsions have improved transdermal permeation of many drugs over the conventional topical formulations such as emulsions^{20,21} and gels.^{22,23} This article describes the potential of nanoemulsion systems in transdermal delivery of aceclofenac using nonirritating, pharmaceutically acceptable ingredients without using additional permeation enhancers, because excipients of nanoemulsions themselves act as permeation enhancers.

EXPERIMENTAL

Materials

Aceclofenac was a gift sample from Ranbaxy Research Labs (Gurgaon, India). Caprylic/capric triglyceride polyethylene glycol-4 complex (Labrafac[®]), caprylocaproyl macrogol-8-glyceride (Labrasol[®]), polyglyceryl-6-dioleate (Plurol Oleique[®]), and oleoyl macroglycerides EP (Labrafil) were gift samples from Gattefossé (Cedex, France). Isopropyl myristate

Corresponding Author: Sheikh Shafiq, Department of Pharmaceutics, Faculty of Pharmacy, Jamia Hamdard (Hamdard University), Hamdard Nagar, New Delhi – 110062, India. Tel: 0091-98-91433695; Fax: 0091-11-26059663; E-mail: shafiq_sheikh@fastmail.fm

Table 1. Formula for Preparation of Aceclofenac Gel*

Aceclofenac Gel	Ingredients (for 100 g of gel)
Aceclofenac (% wt/wt)	2
Carbopol 940 (% wt/wt)	1
IPA (% wt/wt)	10
PEG-400 (% wt/wt)	10
PG (% wt/wt)	10
TEA (g)	0.5
Distilled water (qs)	100

*IPA indicates isopropyl alcohol; PEG, polyethylene glycol; PG, propylene glycol; TEA, triethanolamine; qs, quantity sufficient.

(IPM), oleic acid, glycerol triacetate (Triacetin), olive oil, diethylene glycol monoethyl ether (Transcutol P), and ethanol were purchased from E-Merck (Mumbai, India). Tween 80 and polyoxy-35-castor oil (Cremophor EL[®]) were purchased from Sigma Aldrich (St. Louis, MO). All other chemicals used in the study were of analytical reagent grade.

Screening of Excipients

The solubility of aceclofenac in various oils (Triacetin, Labrafac, oleic acid, Labrafil, IPM, and olive oil), surfactants (Labrasol, Tween 80, and Cremophor EL), and cosurfactants (Transcutol P and Plurol Oleique) was determined by dissolving an excess amount of aceclofenac in 2 mL of each of the selected oils, surfactants, and cosurfactants in 5-mL-capacity stoppered vials separately. A combination of oils was also used for determination of solubility. An excess amount of aceclofenac was added to each 5-mL-capacity stoppered vial and mixed using a vortex mixer (Nickel-Electro Ltd, Oldmixon Crescent, UK). The mixture vials were then kept at 37°C ± 1.0°C in an isothermal shaker (Nirmal International, New Delhi, India) for 72 hours to get to equilibrium. The equilibrated samples were removed from the shaker and centrifuged at 3000 rpm for 15 minutes. The supernatant was taken and filtered through a 0.45-μm membrane filter. The concentration of aceclofenac was determined in each oil, surfactant, cosurfactant, and combination of oils by UV spectrophotometer at their respective λ_{max} .

Pseudoternary Phase Diagram Study

On the basis of the solubility studies, the combination of Labrafil and Triacetin (2:1) was selected as the oil phase. Tween 80 and Transcutol P were selected as surfactant and cosurfactant, respectively. Distilled water was used as an aqueous phase. Surfactant and cosurfactant (S_{mix}) were mixed in different weight ratios (1:0, 1:2, 1:3, 1:1, 2:1, 3:1, and 4:1). These S_{mix} ratios were chosen in increasing concentration of surfactant with respect to cosurfactant and increasing concentration of cosurfactant with respect to surfactant for detailed study of the phase diagrams needed for nanoemulsion

formation. For each phase diagram, oil and S_{mix} were combined in different weight ratios from 1:9 to 9:1 in different glass vials. Sixteen different combinations of oil and S_{mix} (1:9, 1:8, 1:7, 1:6, 1:5, 1:4, 1:3.5, 1:3, 1:2.3, 1:2, 1:1.5, 1:1, 1:0.7, 1:0.43, 1:0.25, and 1:0.1) were made so that maximum ratios were covered for the study to delineate the boundaries of phases precisely formed in the phase diagrams. Pseudoternary phase diagrams of oil, S_{mix} , and aqueous phase were developed using the aqueous titration method. Slow titration with the aqueous phase was done to each weight ratio of oil and S_{mix} , and visual observations were made for transparent and easily flowable oil-in-water (o/w) nanoemulsions. The physical state of the nanoemulsion was marked on a pseudo-3-component phase diagram with 1 axis representing the aqueous phase, 1 representing oil, and the third representing a mixture of surfactant and cosurfactant at fixed weight ratios (S_{mix} ratios).

Selection of Nanoemulsion Formulations

From each phase diagram constructed, different formulas were selected from the nanoemulsion region so that the drug could be incorporated into the oil phase. Exactly 2% wt/wt of aceclofenac, which was kept constant in all the selected formulations, was dissolved in the oil phase of the nanoemulsion formulation. Selected formulations were subjected to different thermodynamic stability tests.

Preparation of Conventional Aceclofenac Gel

Conventional aceclofenac gel (CG) was prepared by dispersing the 1 g of the Carbopol 940[®] in a sufficient quantity of distilled water.²⁴ After complete dispersion, the Carbopol 940 solution was kept in the dark for 24 hours for complete swelling. Then 2 g of aceclofenac was dissolved in a specified quantity of polyethylene glycol 400. This solution of drug was added slowly to the aqueous dispersion of Carbopol 940. Then other ingredients like isopropyl alcohol, propylene glycol, and triethanolamine were added to obtain a homogeneous dispersion of gel (Table 1).

Thermodynamic Stability Studies

To overcome the problem of metastable formulation, thermodynamic stability tests were performed.²⁵ Selected formulations were centrifuged at 822 g for 30 minutes. The formulations that did not show any phase separations were taken for the heating and cooling cycle. Six cycles between refrigerator temperature (4°C) and 45°C with storage at each temperature of not less than 48 hours were done. The formulations, which were stable at these temperatures, were subjected to a freeze-thaw cycle test. Three freeze-thaw cycles were done for the formulation between -21°C and 25°C.

The formulations that survived thermodynamic stability tests were selected for further study.

Characterization of Nanoemulsions

Transmission Electron Microscopy

Morphology and structure of the nanoemulsion were studied using transmission electron microscopy (TEM), with Topcon 002B operating at 200 kV (Topcon, Paramus, NJ) and capable of point-to-point resolution. To perform the TEM observations, a drop of the nanoemulsion was directly deposited on the holey film grid and observed after drying.

Nanoemulsion Droplet Size Analysis

Droplet size distribution of the nanoemulsion was determined by photon correlation spectroscopy that analyzes the fluctuations in light scattering due to Brownian motion of the particles,²⁶ using a Zetasizer 1000 HS (Malvern Instruments, Worcestershire, UK). Light scattering was monitored at 25°C at a 90° angle.

Viscosity Determination

The viscosity of the formulations (0.5 g) was determined using a Brookfield DV III ultra V6.0 RV cone and plate rheometer (Brookfield Engineering Laboratories, Middleboro, MA) using spindle # CPE40 at 25°C ± 0.3°C. The software used for the calculations was Rheocalc V2.6.

Refractive Index

The refractive index of placebo formulations and drug-loaded formulations was determined using an Abbe-type refractometer (Nirmal International).

In Vitro Skin Permeation Studies

In vitro skin permeation studies were performed on a Franz diffusion cell with an effective diffusional area of 0.636 cm² and 4 mL of receiver chamber capacity using rat abdominal skin. The automated transdermal diffusion cell sampling system (SFDC6, Logan Inst, Avalon, NJ) was used for these studies. The full-thickness rat skin was excised from the abdominal region, and hair was removed with an electric clipper. The subcutaneous tissue was removed surgically, and the dermis side was wiped with isopropyl alcohol to remove adhering fat. The cleaned skin was washed with distilled water and stored in the deep freezer at -21°C until further use. The skin was brought to room temperature and mounted between the donor and receiver compartment of the Franz diffusion cell, where the stratum corneum side faced the donor compartment and the dermal side faced the

receiver compartment. Initially the donor compartment was empty and the receiver chamber was filled with ethanolic phosphate-buffered saline (PBS) pH 7.4 (20:80% vol/vol). The receiver fluid was stirred with a magnetic rotor at a speed of 600 rpm, and the assembled apparatus was placed in the Logan transdermal permeation apparatus and the temperature maintained at 32°C ± 1°C. All the ethanolic PBS was replaced every 30 minutes to stabilize the skin. It was found that the receiver fluid showed negligible absorbance after 4.5 hours and beyond, indicating complete stabilization of the skin. After complete stabilization of the skin, 1 mL of nanoemulsion formulation (20 mg/mL aceclofenac) or 1 g of CG (20 mg/g) was placed into each donor compartment and sealed with paraffin film to provide occlusive conditions. Samples were withdrawn at regular intervals (0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 20, 22, and 24 hours), filtered through a 0.45-µm membrane filter, and analyzed for drug content by UV spectrophotometer at λ_{max} of 274 nm. The formulation F1 provided the highest release as compared with the other nanoemulsion formulations. The formulation F1 was also converted into nanoemulsion gel formulations by adding 1% wt/wt Carbopol 940 and was coded as NG1. The skin permeation profile of the optimized nanoemulsion formulation was compared with nanoemulsion gel (NG1) and CG using the Dunnett test of 1-way analysis of variance (ANOVA).

Permeation Data Analysis

The cumulative amount of drug permeated through the skin (mg/cm²) was plotted as a function of time (t) for each formulation. Drug flux (permeation rate) at steady state (J_{ss}) was calculated by dividing the slope of the linear portion of the graph by the area of the diffusion cell. The permeability coefficient (K_p) was calculated by dividing J_{ss} by the initial concentration of drug in the donor cell (C_0):

$$K_p = \frac{J_{ss}}{C_0} \quad (1)$$

Enhancement ratio (E_r) was calculated by dividing the J_{ss} of the respective formulation by the J_{ss} of the control formulation:

$$E_r = \frac{J_{ss} \text{ of formulation}}{J_{ss} \text{ of control}} \quad (2)$$

Skin Irritation Test

The skin irritation test was carried out on male Swiss albino mice weighing 20 to 25 g. The animals were kept under standard laboratory conditions, with temperature of 25°C ± 1°C and relative humidity of 55% ± 5%. The animals were housed in polypropylene cages, 6 per cage, with free access to a standard laboratory diet (Lipton feed, Mumbai, India)

and water ad libitum. A single dose of 10 μ L of the nanoemulsion was applied to the left ear of the mouse, with the right ear as a control. The development of erythema was monitored for 6 days using the method of Van-Abbe et al.²⁷

In Vivo Efficacy Study

Approval to carry out in vivo efficacy studies was obtained from the Institutional Animal Ethics Committee Jamia Hamdard (New Delhi, India), and the committee's guidelines were followed for the studies. The anti-inflammatory and sustaining action of the optimized formulation F1 was evaluated by the carrageenan-induced hind paw edema method developed by Winter et al in Wistar rats.²⁸ Young Wistar rats weighing 120 to 150 g were randomly divided into 4 groups: control, nanoemulsion (F1), nanoemulsion gel (NG1), and CG, each containing 6 rats. The animals were kept under standard laboratory conditions, with temperature of $25^{\circ}\text{C} \pm 1^{\circ}\text{C}$ and relative humidity of $55\% \pm 5\%$. The animals were housed in polypropylene cages, 6 per cage, with free access to a standard laboratory diet (Lipton feed) and water ad libitum. The dose for the rats was calculated based on the weight of the rats according to the surface area ratio.²⁹ The abdominal region of the rats was shaved 12 hours before the experiments started, except in the control group. F1, NG1, and CG were applied on the shaved abdominal region of all animals (except in control group) half an hour before subplanter injection of carrageenan in right paws. Paw edema was induced by injecting 0.1 mL of the 1% wt/wt homogeneous suspension of carrageenan in distilled water. The volume of paw was measured at 1, 2, 3, 6, 12, and 24 hours after injection using a digital plethysmometer. The amount of paw swelling was determined for 24 hours and expressed as percent edema relative to the initial hind paw volume. Percent inhibition of edema produced by each formulation-treated group was calculated against the respective control group. Results of anti-inflammatory activity were compared using the Dunnett test of 1-way ANOVA.

RESULTS AND DISCUSSION

Excipient Selection

The excipients selected needed to be pharmaceutically acceptable, nonirritating, and nonsensitizing to the skin and to fall into the GRAS (generally regarded as safe) category. Higher solubility of the drug in the oil phase was another important criterion, as it would help the nanoemulsion to maintain the drug in solubilized form.

Safety is a major determining factor in choosing a surfactant, as a large amount of surfactants may cause skin irritation. Non-ionic surfactants are less toxic than ionic surfactants. An important criterion for selection of the surfactants is that

the required hydrophilic lipophilic balance (HLB) value to form the o/w nanoemulsion be greater than 10. The right blend of low and high HLB surfactants leads to the formation of a stable nanoemulsion formulation.³⁰ In this study, we selected Tween 80 as a surfactant with an HLB value of 15. Transient negative interfacial tension and fluid interfacial film are rarely achieved by the use of single surfactant; usually, addition of a cosurfactant is necessary. The presence of cosurfactant decreases the bending stress of interface and allows the interfacial film sufficient flexibility to take up different curvatures required to form nanoemulsions over a wide range of composition.^{31,32} Thus, the cosurfactant selected for the study was Transcutol P, which has an HLB value of 4.2.

Aceclofenac is a highly lipophilic drug, and its physicochemical properties suggest that it has good potential for transdermal drug delivery.⁴ Therefore, in the present study different nanoemulsions were prepared for transdermal delivery of aceclofenac.

Screening of Excipients

The most important criterion for screening of excipients is the solubility of the poorly soluble drug in oil, surfactants, and cosurfactants. Since the aim of this study is to develop a transdermal formulation, it is important to determine the solubility of the drug in oils, surfactants, and cosurfactants. The solubility of aceclofenac was found to be highest in a 2:1 combination of Labrafil and Triacetin (48.95 ± 2.22 mg/mL) as compared with other oils and combinations of oils. Thus, this combination was selected as the oil phase for the development of the optimal formulation. The highest solubility of the drug was seen in Tween 80 (398.21 ± 2.89 mg/mL) and Transcutol P (292.42 ± 2.80 mg/mL). Therefore, Tween-80 and Transcutol P were selected as surfactant and cosurfactant, respectively, for the phase study (Table 2).

Pseudoternary Phase Diagram Study

Constructing phase diagrams is time-consuming, particularly when the aim is to accurately delineate a phase boundary.³¹ Care was taken to ensure that observations were not made on metastable systems—although the free energy required to form an emulsion is very low, the formation is thermodynamically spontaneous.³⁰ The relationship between the phase behavior of a mixture and its composition can be captured with the aid of a phase diagram.³³ Pseudoternary phase diagrams were constructed separately for each S_{mix} ratio (Figure 1), so that o/w nanoemulsion regions could be identified and nanoemulsion formulations could be optimized.

In Figure 1, the S_{mix} ratio 1:0 (Figure 1A) has a low nanoemulsion area. An o/w nanoemulsion region was found

Table 2. Solubility of Aceclofenac in Various Excipients (n = 3)*

Excipients	Solubility Mean ± SD (mg/mL) ^a	Excipients	Solubility Mean ± SD (mg/mL) ^a
Triacetin	8.22 ± 1.12	Labrafil + Triacetin (2:1)	48.95 ± 2.22
Labrafac	6.31 ± 0.52	Labrafil + Triacetin (3:1)	39.44 ± 1.98
Oleic acid	4.01 ± 0.92	Labrasol	386.45 ± 3.28
Labrafil	32.56 ± 2.43	Tween80	398.21 ± 2.89
IPM	2.97 ± 1.01	Cremophor EL	272.32 ± 2.94
Olive oil	1.69 ± 0.35	Transcutol P	292.42 ± 2.80
Labrafil + Triacetin (1:1)	35.24 ± 2.14	Plurol Oleique	110.52 ± 2.19

*IPM indicates isopropyl myristate.

toward the water-rich apex of the phase diagram. The maximum concentration of oil that could be solubilized in the phase diagram was only 16% wt/wt using 67% wt/wt of S_{mix} . As the surfactant concentration was increased in the S_{mix} ratio 1:1 (Figure 1B), a higher nanoemulsion region was observed, perhaps because of further reduction of the interfacial tension, increasing the fluidity of the interface, thereby increasing the entropy of the system. There may be greater penetration of the oil phase in the hydrophobic region of the surfactant monomers.^{31,32} The maximum concentration of oil that could be solubilized in the phase diagram was only 16% wt/wt using 67% wt/wt of S_{mix} . As we further increased surfactant concentration, S_{mix} 2:1 (Figure 1C), the nanoemulsion region increased as compared with the region in 1:0 and 1:1. The maximum concentration of oil that could be solubilized by this ratio was 22% wt/wt using 52% wt/wt of S_{mix} . When the S_{mix} ratio of 3:1 was studied (Figure 1D), the nanoemulsion region decreased slightly as compared with 1:1, which may have been due to the increased concentration of the surfactant, although the maximum oil that could be solubilized by this ratio of S_{mix} was 22% wt/wt with 52% wt/wt of S_{mix} . Similarly, when the S_{mix} ratio of 4:1 was studied (Figure 1E), the nanoemulsion area further decreased as compared with 3:1 and 2:1 but increased as com-

pared with 1:0 and 1:1. The maximum concentration of oil that could be solubilized by this ratio of S_{mix} was 17% wt/wt with 67% wt/wt of S_{mix} . When surfactant concentration increased as compared with cosurfactant, the nanoemulsion area increased up to the 2:1 ratio, but in the 4:1 ratio the nanoemulsion region decreased again, so there was no need to try an S_{mix} ratio of 5:1. No nanoemulsion regions were found in S_{mix} ratios of 1:2 and 1:3.

Thus, in the phase diagrams, it can be seen that the free energy of nanoemulsion formation can be considered to depend on the extent to which the surfactant lowers the surface tension of the oil-water interface and the change in dispersion entropy.³³ Thus, a negative free energy of formation is achieved when a large reduction in surface tension is accompanied by significant favorable entropic changes. In such cases, nanoemulsion formation is spontaneous and the resulting dispersions are thermodynamically stable.^{30,33} The surfactant or S_{mix} , which are able to increase the dispersion entropy, reduce the interfacial tension, increase the interfacial area, and thus lower the free energy of the system to a very low value with the minimum concentration (weight ratio), which is thermodynamically stable, and have the potential for the transdermal drug delivery.

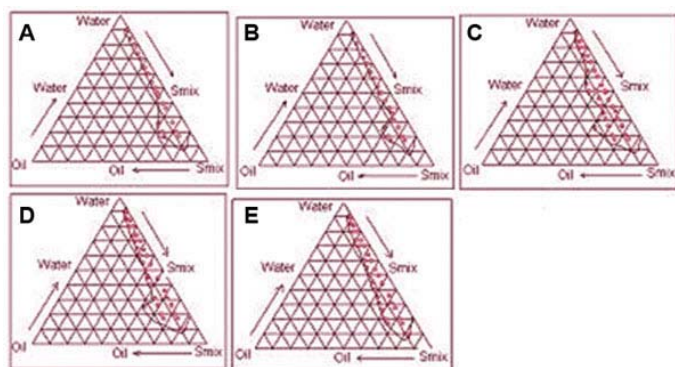


Figure 1. Pseudoternary phase diagrams indicating oil-in-water nanoemulsion (shaded area) region of Labrafil and Triacetin (oil), Tween 80 (surfactant), and Transcutol P (cosurfactant) at different S_{mix} ratios indicated in parts A (S_{mix} 1:0), B (S_{mix} 1:1), C (S_{mix} 2:1), D (S_{mix} 3:1), and E (S_{mix} 4:1).

Selection of Nanoemulsion Formulations

It is well known that large amounts of surfactants cause skin irritation³³⁻³⁵; therefore, it is important to determine the

Table 3. Composition of Selected Nanoemulsion Formulations

S_{mix} Ratio	% Wt/Wt of Components in Nanoemulsion Formulation			Oil: S_{mix} Ratio	Code
	Oil	S_{mix}	Water		
2:1	15	53	32	1:3.53	F1
2:1	20	53	27	1:2.65	F2
3:1	15	50	35	1:3.33	F3
3:1	20	51	29	1:2.55	F4
4:1	15	50	35	1:3.33	F5
4:1	20	51	29	1:2.55	F6

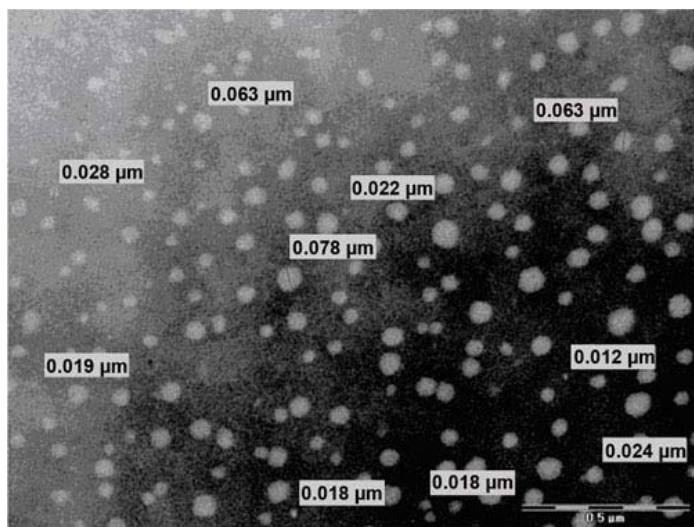


Figure 2. Transmission electron microscopic positive image of aceclofenac nanoemulsion showing the size of some oil droplets.

surfactant concentration properly and use the optimum concentration of surfactant in the formulation. From pseudoternary phase diagrams, the formulations in which the amount of oil phase completely solubilized the drug and which could accommodate the optimum quantity of S_{mix} and distilled water were selected for the study.

Thermodynamic Stability Studies

Nanoemulsions are thermodynamically stable systems and are formed at a particular concentration of oil, surfactant, and water, making them stable and not subject to phase separation, creaming, or cracking. It is the thermostability that differentiates nano- or microemulsions from emulsions that have kinetic stability and eventually phase-separate.^{33,36} Thus, the formulations were tested for their thermodynamic stability by using centrifugation, a heating-cooling cycle, and a freeze-thaw cycle. Only formulations that survived the thermodynamic stability tests were selected for further study. The compositions of selected formulations are given in Table 3.

Characterization of Nanoemulsions

TEM

In the TEM positive image, the nanoemulsion appeared dark and the surroundings were bright (Figure 2). Some droplet sizes were measured, as TEM is capable of point-to-point resolution. These sizes were in agreement with the droplet size distribution measured using photon correlation spectroscopy (Table 4).

Nanoemulsion Droplet Size Analysis

The droplet size increased with the increase in concentration of oil in the formulations (Table 4). The droplet size of formulation F1, containing 15% oil, was lowest (35.20 ± 1.24 nm). The droplet size of formulation F6 was highest (68.3 ± 5.26 nm). All the formulations had droplets in the nano range, which is very well evident from the low polydispersity values. Polydispersity is the ratio of standard deviation to mean droplet size, so it indicates the uniformity of droplet size within the formulation. The higher the polydispersity, the lower the uniformity of the droplet size in the formulation. Although the polydispersity values of all formulations were very low, indicating uniformity of droplet size within each formulation, the polydispersity of formulation F1 was lowest (0.035).

Viscosity Determination

The viscosity of the selected formulations was determined (Table 4). The viscosity of formulation F1 (92.2 ± 1.41 cP) was lower than that of any other formulation, and this difference was significant ($P < .05$). The viscosity of formulation F6 was highest (125.3 ± 2.75 cP), but it was observed that the viscosity of the nanoemulsion formulations generally was very low. This was expected, because one of the characteristics of nanoemulsion formulations is lower viscosity.³³

Refractive Index

The mean values of the refractive index of drug-loaded formulations and placebo formulations are given in Table 5.

Table 4. Droplet Size, Polydispersity Values, and Viscosity of the Nanoemulsion Formulations (n = 3)

Formulation Code	Droplet Size Mean \pm SD (nm)	Polydispersity	Viscosity Mean \pm SD (cP)
F1	35.20 ± 1.24	0.035	92.20 ± 1.41
F2	46.50 ± 2.96	0.063	103.40 ± 1.87
F3	41.70 ± 3.15	0.075	107.60 ± 2.35
F4	59.30 ± 4.23	0.071	115.40 ± 2.45
F5	54.60 ± 4.09	0.074	117.20 ± 2.56
F6	68.30 ± 5.26	0.077	125.30 ± 2.75

Table 5. Refractive Index of Selected Nanoemulsions and Placebo Nanoemulsion Formulations (n = 6)

Sample Code	Refractive Index \pm SD	
	Fresh Formulation	Placebo Formulation
F1	1.401 \pm 0.007	1.405 \pm 0.005
F2	1.403 \pm 0.008	1.406 \pm 0.009
F3	1.404 \pm 0.009	1.407 \pm 0.052
F4	1.409 \pm 0.014	1.411 \pm 0.012
F5	1.407 \pm 0.013	1.402 \pm 0.021
F6	1.411 \pm 0.015	1.412 \pm 0.015

When the refractive index values for formulations were compared with those of the placebo, it was found that there were no significant differences between the values. Therefore, it can be concluded that the nanoemulsion formulations were not only thermodynamically stable but also chemically stable and remained isotropic; thus, there were no interactions between nanoemulsion excipients and drug.

In Vitro Skin Permeation Studies

In vitro skin permeation studies were performed to compare the release of drug from 6 different nanoemulsion formulations (F1-F6), NG1, and CG, all having the same quantity (2% wt/wt) of aceclofenac. In vitro skin permeation was highest in formulation F1 and lowest for CG (Figures 3 and 4). The formulation NG1 showed an intermediate skin permeation profile. The skin permeation profile of F1 was significantly different when compared with that of CG and NG1 ($P < .05$). The significant difference in aceclofenac permeation between nanoemulsion formulations, NG1, and CG was probably due to the mean size of internal phase droplets, which were significantly smaller in nanoemulsions. The

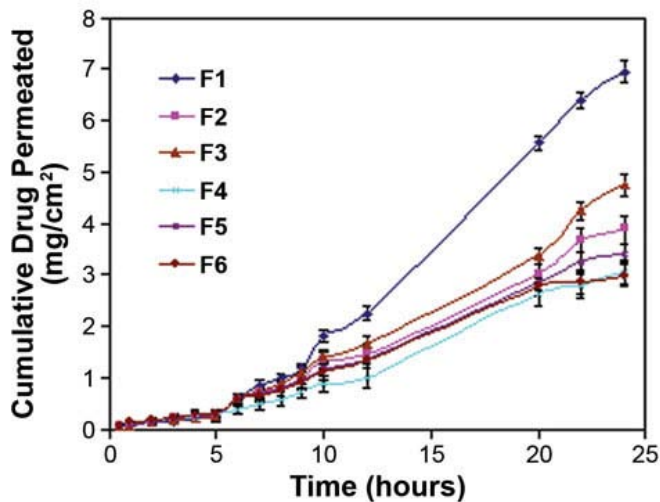


Figure 3. In vitro skin permeation profile of aceclofenac from 6 different nanoemulsion formulations (F1-F6).

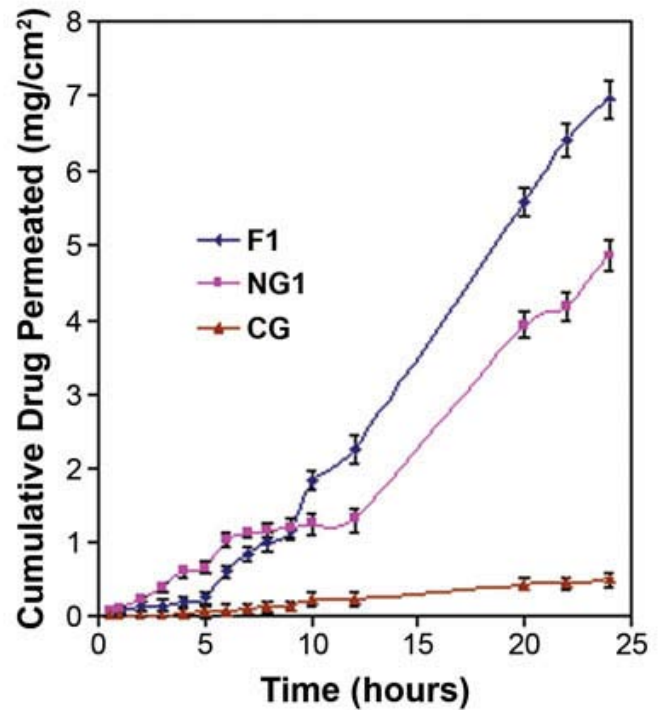


Figure 4. Comparative in vitro skin permeation profile of aceclofenac from F1, NG1, and CG. NG1 indicates nanoemulsion gel; CG, conventional aceclofenac gel formulation.

maximum release in F1 could be due to having the lowest droplet size and lowest viscosity of all the nanoemulsions.

Permeation Data Analysis

Permeability parameters like steady-state flux (J_{ss}), permeability coefficient (K_p), and enhancement ratio (E_r) were significantly increased in nanoemulsions and the NG1 formulation as compared with CG ($P < .05$). This is because nanoemulsions and NG1 excipients contain permeation enhancers like Labrafil, Triacetin, Tween 80, and Transcutol P. The permeability parameters of different formulations are given in Table 6.

Table 6. Permeability Parameters of Different Formulations (n = 3)*

Formulation Matrices	$J_{ss} \pm$ SD (mg/cm ² /h)	$K_p \pm$ SD (cm/h) $\times 10^{-2}$	E_r
CG	0.021 \pm 0.012	0.109 \pm 0.091	—
F1	0.313 \pm 0.096	1.565 \pm 0.120	14.360
F2	0.170 \pm 0.085	0.853 \pm 0.130	7.830
F3	0.202 \pm 0.068	1.014 \pm 0.161	9.300
F4	0.134 \pm 0.031	0.671 \pm 0.103	6.150
F5	0.152 \pm 0.110	0.762 \pm 0.098	6.990
F6	0.134 \pm 0.130	0.674 \pm 0.113	6.180
NG1	0.199 \pm 0.230	0.997 \pm 0.161	9.140

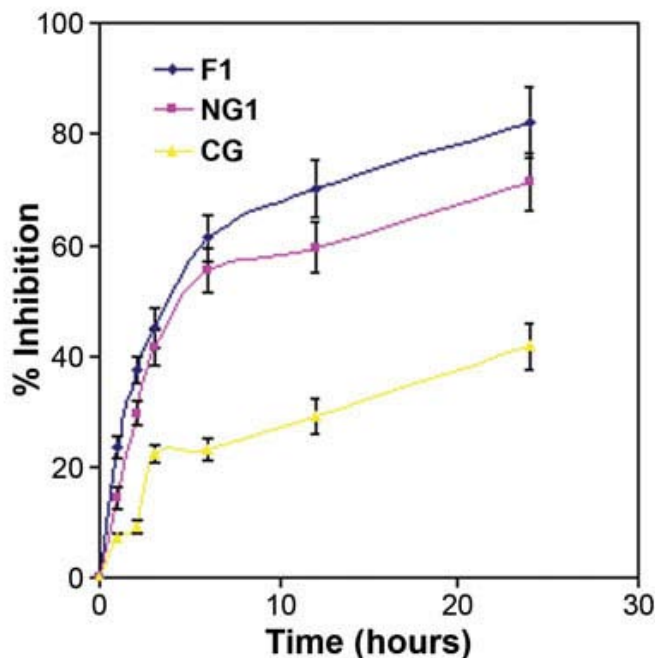


Figure 5. Anti-inflammatory effects of F1, NG1, and CG. NG1 indicates nanoemulsion gel; CG, conventional aceclofenac gel formulation.

Skin Irritation Test

The skin irritation test was performed to confirm the safety of the optimized nanoemulsion formulation. Van-Abbe et al²⁷ mentioned that a value between 0 and 9 indicates that the applied formulation is generally not an irritant to human skin. The mean skin irritation score for formulation F1 was 2.12 ± 0.45 . From this it was concluded that the optimized nanoemulsion formulation was safe to be used for transdermal drug delivery.

In Vivo Efficacy Study

Based on higher drug permeation, lowest droplet size, lowest viscosity, and lowest polydispersity index, formulation F1 was selected for the study of in vivo anti-inflammatory effects. The anti-inflammatory and sustaining action of the optimized formulation was evaluated by the carrageenan-induced hind paw edema method developed by Winter et al²⁸ in female Wistar rats. The percent inhibition value after 24 hours of administration was found to be high for F1—that is, 82.2% as compared with 41.8% for CG; this difference was extremely significant ($P < .01$). The percent inhibition value for formulation NG1 was 71.4% (Figure 5), and the difference between F1's and NG1's percent inhibition was significant ($P < .05$). The enhanced anti-inflammatory effects of formulation F1 could be due to the enhanced permeation of aceclofenac through the skin.

CONCLUSION

On the basis of highest drug permeation, lowest droplet size, lowest polydispersity, lowest viscosity, and optimum surfactant and cosurfactant concentration, we selected formulation F1 of aceclofenac, which contained Labrafil (10% wt/wt), Triacetin (5% wt/wt), Tween 80 (35.33% wt/wt), Transcutol P (17.66% wt/wt), and distilled water (32% wt/wt), for use in in vivo studies. The in vivo studies revealed a significant increase in the anti-inflammatory effects as compared with aceclofenac gel and nanoemulsion gel. From in vitro and in vivo data it can be concluded that the developed nanoemulsions have great potential for transdermal drug delivery.

ACKNOWLEDGMENT

The authors are grateful to the University Grant Commission (New Delhi, India) for providing financial support for this project. The authors are also thankful to Gattefossé (Cedex, France) for providing the gift samples of oils, surfactants, and cosurfactants.

REFERENCES

- Escribano E, Calpena AC, Queralt J, Obach R, Domenech J. Assessment of diclofenac permeation with different formulations: anti-inflammatory study of a selected formula. *Eur J Pharm Sci.* 2003;19:203–210.
- Yamazaki R, Kawai S, Mastsuzaki T, et al. Aceclofenac blocks prostaglandin E2 production following its intracellular conversion into cyclooxygenase inhibitors. *Eur J Pharmacol.* 1997;329:181–187.
- Gonzalez E, Cruz C, Nicolas R, Egido J, Herrero-Beaumont G. Long-term effects of nonsteroidal anti-inflammatory drugs on the production of cytokines and other inflammatory mediators by blood cells of patients with osteoarthritis. *Agents Actions.* 1994;41:171–178.
- Yang JH, Kim Y, Kim KM. Preparation and evaluation of aceclofenac microemulsion for transdermal delivery system. *Arch Pharm Res.* 2002;25:534–540.
- Walters KA. Penetration enhancers and their use in transdermal therapeutic systems. In: Hadgraft J, Guy RH, eds. *Transdermal Drug Delivery, Developmental Issues and Research Initiatives.* New York, NY: Marcel Dekker; 1989:197–246.
- Shafiq S, Faiyaz S, Sushma T, Ahmad FJ, Khar RK, Ali M. Design and development of oral oil in water ramipril nanoemulsion formulation: in vitro and in vivo evaluation. *J Biomed Nanotech.* 2007;3:28–44.
- Shafiq S, Faiyaz S, Sushma T, Ahmad FJ, Khar RK, Ali M. Development and bioavailability assessment of ramipril nanoemulsion formulation. *Eur J Pharm Biopharm.* 2007;66:227–243.
- Osborne DW, Ward AJ, Neil KJ. Microemulsions as topical delivery vehicles: in-vitro transdermal studies of a model hydrophilic drug. *J Pharm Pharmacol.* 1991;43:450–454.
- Trotta M, Pattarino F, Gasco MR. Influence of counter ions on the skin permeation of methotrexate from water-oil microemulsions. *Pharm Acta Helv.* 1996;71:135–140.
- Delgado-Charro MB, Iglesias-Vilas G, Blanco-Mendez J, Lopez-Quintela MJ, Marty MA, Guy JP. Delivery of a hydrophilic solute through the skin from novel microemulsion systems. *Eur J Pharm Biopharm.* 1997;43:37–42.

11. Dreher F, Walde P, Walter P, Wehrli E. Interaction of a lecithin microemulsion gel with human stratum corneum and its effect on transdermal transport. *J Control Rel.* 1997;45:131–140.
12. Schmalfus U, Neubart R, Wohlrab W. Modification of drug penetration into human skin using microemulsions. *J Control Rel.* 1997;46:279–285.
13. Kreilgaard M, Pedersen EJ, Jaroszewski JW. NMR characterization and transdermal drug delivery potentials of microemulsion systems. *J Control Rel.* 2000;69:421–433.
14. Alvarez-Figueroa MJ, Blanco-Mendez J. Transdermal delivery of methotrexate: iontophoretic delivery from hydrogels and passive delivery from microemulsions. *Int J Pharm.* 2001;215:57–65.
15. Rhee YS, Choi JG, Park ES, Chi SC. Transdermal delivery of ketoprofen using microemulsions. *Int J Pharm.* 2001;228:161–170.
16. Lee PJ, Langer R, Shastri VP. Novel microemulsion enhancer formulation for simultaneous transdermal delivery of hydrophilic and hydrophobic drugs. *Pharm Res.* 2003;20:264–269.
17. Kemken J, Ziegler A, Muller BW. Influence of supersaturation on the pharmacodynamic effect of bupranolol after dermal administration using microemulsions as vehicle. *Pharm Res.* 1992;9:554–558.
18. Kreilgaard M. Dermal pharmacokinetics of microemulsion formulations determined by *in-vitro* microdialysis. *Pharm Res.* 2001;18:367–373.
19. Kreilgaard M, Kemme MJB, Burggraaf J, Schoemaker RC, Cohen AF. Influence of a microemulsion vehicle on cutaneous bioequivalence of a lipophilic model drug assessed by microdialysis and pharmacodynamics. *Pharm Res.* 2001;18:593–599.
20. Ktistis G, Niopas I. A study on the *in-vitro* percutaneous absorption of propranolol from disperse systems. *J Pharm Pharmacol.* 1998;50:413–419.
21. Gasco MR, Gallarate M, Pattarino F. In vitro permeation of azelaic acid from viscosized microemulsions. *Int J Pharm.* 1991;69:193–196.
22. Kriwet K, Muller-Goymann CC. Diclofenac release from phospholipid drug systems and permeation through excised human stratum corneum. *Int J Pharm.* 1995;125:231–242.
23. Trotta M. Influence of phase transformation on indomethacin release from microemulsions. *J Control Rel.* 1999;60:399–405.
24. Baboota S, Shakeel F, Kohli K. Formulation and evaluation of once a day transdermal gels of diclofenac diethylamine. *Methods Find Exp Clin Pharmacol.* 2006;28:109–114.
25. Shafiq-un-Nabi S, Shakeel F, Talegaonkar S, et al. Formulation development and optimization using nanoemulsion technique: a technical note. *AAPS PharmSciTech [serial online].* 2007;8:E28.
26. Attwood D, Mallon C, Ktistis G, Taylor CJ. A study on factors influencing the droplet size in nonionic oil-in-water microemulsions. *Int J Pharm.* 1992;88:417–422.
27. Van-Abbe NJ, Nicholas P, Boon E. Exaggerated exposure in topical irritancy and sensitization testing. *J Soc Cosmet Chem.* 1975;26:173–187.
28. Winter CA. Anti-inflammatory testing methods: comparative evaluation of indomethacin and other agents. *Nonsteroid Anti-inflammatory Drugs.* 1965;52:190–202.
29. Gosh MN. *Fundamentals of Experimental Pharmacology.* Kolkata, India: Hilton and Company; 2005:192.
30. Craig DQM, Barker SA, Banning D, Booth SW. An investigation into the mechanisms of self-emulsification using particle size analysis and low frequency dielectric spectroscopy. *Int J Pharm.* 1995;114:103–110.
31. Eccleston J. Microemulsions. In: Swarbrick J, Boylan JC, eds. *Encyclopedia of Pharmaceutical Technology.* vol. 9. New York, NY: Marcel Dekker; 1995:375–421.
32. Kawakami K, Yoshikawa T, Moroto Y, et al. Microemulsion formulation for enhanced absorption of poorly soluble drugs, I: prescription design. *J Control Rel.* 2002;81:65–74.
33. Lawrence MJ, Rees GD. Microemulsion-based media as novel drug delivery systems. *Adv Drug Deliv Rev.* 2000;45:89–121.
34. Warisnoicharoen W, Lansley AB, Lawrence MJ. Light scattering investigations on dilute non-ionic oil-in-water microemulsions. *AAPS PharmSciTech [serial online].* 2002;2:E12.
35. Li P, Ghosh A, Wagner RF, Krill S, Joshi YM, Serajuddin ATM. Effect of combined use of nonionic surfactant on formation of oil-in-water microemulsions. *Int J Pharm.* 2005;288:27–34.
36. Shinoda K, Kunieda H. Phase properties of emulsions: PIT and HLB. In: Schuster D, ed. *Encyclopedia of Emulsion Technology.* New York, NY: Marcel Dekker; 1983:337–367.