Available Online at www.ijppronline.com
International Journal Of Pharma Professional's Research

Research Article

Evaluate in vivo and in vitro model on Nanoemulsion-Based Gel Formulation Aceclofenac

JIGNESH D. MODI [1]*JAYVADAN K. PATEL[2]
Jodhpur National University Jodhpur, Rajshathan, India

ISSN NO:0976-6723

Abstract

The aim of the present study was to investigate the potential of a nanoemulsion formulation for topical 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. Topical 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 (Jss), permeability coefficient (Kp), and enhancement ratio (Er) was observed in optimized nanoemulsion formulation consist of 2% wt/wt of aceclofenac, 10 % wt/wt of Labrafac, 45% wt/wt surfactant mixture (Cremophor EL: Ethanol), and 43 % wt/wt of distilled water. The anti-inflammatory effects of formulation showed a significant increase 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, topical 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 anaemia. 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 nanoemulsion or nanoemulsion. Nanoemulsions are thermodynamically stable transparent (translucent) dispersions of oil and water

Correspondence Address:
JIGNESH D. MODI
Jodhpur National University Jodhpur, Rajshathan, India
Email: vision_jignesh@yahoo.co.in

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

MATERIAL AND METHOD

In vitro permeation studies

In vitro skin permeation studies were performed on a Franz diffusion cell with an effective diffusion area of 0.636 cm 2 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 leaned 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 ± 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-membrane filter, and analyzed for drug content by UV spectrophotometer at λmax of 274 nm.

Permeation Data Analysis

The cumulative amount of drug permeated through the skin (mg/cm^2) 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 Jss by the Initial concentration of drug in the donor cell (C_0):

$$K_p = \frac{J_{ss}}{C_0}$$

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

$$E_r = \frac{J_{ss} \text{ of formulation}}{J_{ss} \text{ of control}}$$

In Vivo Efficacy Study

Approval to carry out in vivo efficacy studies was obtained from the Technocrafts Institute of Technology – Pharmacy (Bhopal, India), and the committee's guidelines were followed for the studies. The anti-inflammatory and sustaining action of the optimized formulation B5 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°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) 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) .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.

RESULT AND DISCUSSION

In vitro permeation study

The *in-vitro* permeation profiles of aceclofenac through excised dorsal part of rat skin were shown in Figure 2. A steady increase of aceclofenac in the receptor chambers with time was observed. Statistical evaluation of the flux throughout 24h showed that among the all preparations, the nanoemulsion preparations B5 provided maximum permeation 81.24 % after 24h. Cumulative permeation of aceclofenac from nanoemulsion based gel of B5 and marketed commercial cream of containing same amount of aceclofenac were respectively 66.81% and 43.8% at 24h post-application shown in Figure 3.

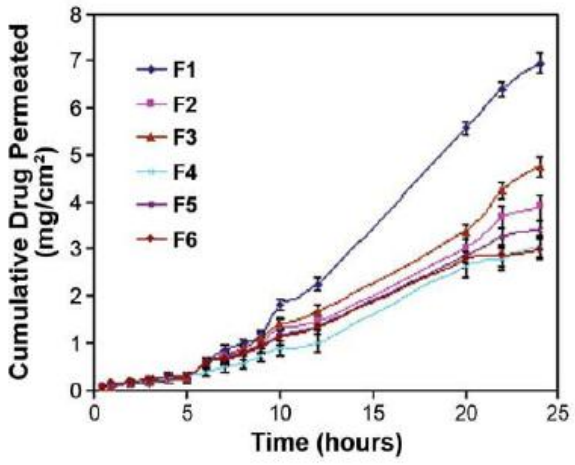

Figure 3. In vitro skin permeation profile of aceclofenac from 6 different nanoemulsion formulations (F1-F6).

Measurement of rheological properties of gels:

However, most of the nanoemulsions possess a very low viscosity and therefore their application, especially in pharmaceutical industry may be restricted due to inconvenient application. In order to overcome this disadvantage, some gelling agents such as Carbopol 934 have been used to increase the viscosity of nanoemulsion and form nanoemulsion base gel which are more suitable for topical application when compared with nanoemulsion as a vehicle for drug delivery. Suitable viscosity of Carbomer 934 could enhance the skin permeation of drug because of the tight contact of drug preparation with skin and delayed release time. Observed viscosities of all formulation were in range from 105×10^5 to 154×10^5 cps which made the preparation more suitable for topical administration shown in table 4.

Permeability parameters of different formulation (n=3)*

Formulation Matrices	Jss ± SD (mg/cm2/h)	Kp ± SD (cm/h) 10-2	Er
CG	0.021 ± 0.012	0.109 ± 0.091	----
B1	0.170 ± 0.096	0.853 ± 0.130	7.830
B2	0.202 ± 0.068	1.014 ± 0.161	9.300
B3	0.134 ± 0.031	0.671 ± 0.103	6.150
B4	0.152 ± 0.110	0.762 ± 0.098	6.990
B5	0.313 ± 0.096	1.565 ± 0.120	14.360
B6	0.302 ± 0.068	0.014 ± 0.161	6.300
B7	0.234 ± 0.031	1.671 ± 0.153	7.150
B8	0.102 ± 0.110	1.762 ± 0.198	6.920
B9	0.134 ± 0.130	0.674 ± 0.113	6.180

Permeation Data Analysis

Permeability parameters like steady-state flux (Jss), permeability coefficient (Kp), and enhancement ratio (Er) 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 Labrafac, Cremophor EL, and Ethanol. The permeability parameters of different formulations are given in Table 6.

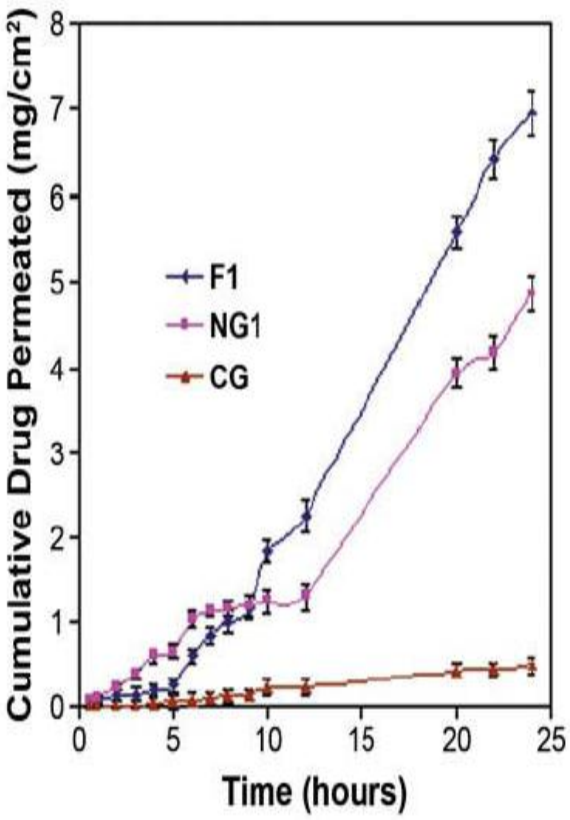

Figure 4. Comparative in vitro skin permeation profile of aceclofenac from F1, NG1, and CG. NG1 indicates nanoemulsion gel; CG, conventional aceclofenac gel formulation.

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 G .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 G .05). The enhanced anti-inflammatory effects of formulation F1 could be due to the enhanced permeation of aceclofenac through the skin.

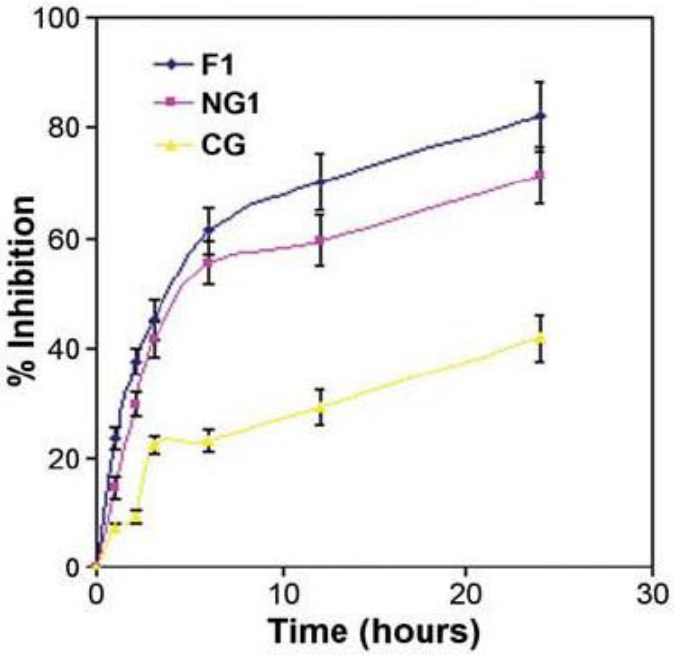

Figure 5. Anti-inflammatory effects of F1, NG1, and CG. NG1 indicates nanoemulsion gel; CG, conventional aceclofenac gel formulation.

CONCLUSION

In this work, nanoemulsion base gel with suitable viscosity was constructed to deliver aceclofenac for topical administration. The nanoemulsion base gel formulation of aceclofenac containing 10% of oil phase (Labrafac), 45% of surfactant mixture (Cremophor EL and Ethanol) and 43 % of distilled water has been optimized. From *in vitro* data it can be concluded that the developed nanoemulsion- based gel have great potential for topical drug delivery.

REFERENCES

1).Lee PJ, Langer R, Shastri VP. Novel microemulsion enhancer formulation for simultaneous transdermal delivery of hydrophilic and hydrophobic drugs. Pharm Res. 2003;20:264Y269.

2).Lehmann L, Keipert S, Gloor M. Effects of nanoemulsions on the stratum corneum and hydrocortisone penetration, Eur. J. Pharm. Biopharm. 2001; 52: 129– 136.

3).Li P, Ghosh A, Wagner RF, Krill S, Joshi YM, Serajuddin

ATM. Effect of combined use of nonionic surfactant on formation of oil-inwater microemulsions. Int J Pharm. 2005;288:27Y34.

4).Osborne DW, Ward AJ, Neil KJ. Microemulsions as topical delivery vehicles: in-vitro transdermal studies of a model hydrophilic drug. J Pharm Pharmacol. 1991;43:450Y454.

5).Peltola S, Saarinen P, Kiesvaara J, Suhonen T, Urtti A. Nanoemulsions for topical delivery of estradiol. Int. J. Pharm. 2003; 254: 99–107.

6).Rhee YS, Choi JG, Park ES, Chi SC. Transdermal delivery of ketoprofen using microemulsions. Int J Pharm. 2001;228: 161Y170.

7).Rhee YS, Choi JG, Park ES, Chi SC. Transdermal delivery of ketoprofen using nanoemulsions. Int. J. Pharm. 2001; 228: 161–170.

8).Sanjula B, Faiyaz S, Alka A, Javed A, Sheikh S. Design, development and evaluation of novel nanoemulsion formulations for transdermal potential of celecoxib**.** Acta Pharm. 2007; 57: 315–332.

9).Schmalfus U, Neubart R, Wohlrab W. Modification of drug penetration into human skin using microemulsions. J Control Rel. 1997;46:279Y285.

10).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;3:28Y44.

11).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;66:227Y243.

12).Shafiq S, Faiyaz S, Sushma T, Farhan J, Khar R, Ali M. Design and development of ramipril nanoemulsion formulation: In vitro and in vivo assessment, J. Biomed. Nanotechnol. 2007; 2: 28–44.

13).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.

14). Shinoda K, Kunieda H. Phase properties of emulsions: PIT and HLB. In: Schuster D, ed. Encyclopedia of Emulsion Technology. New York, NY: Marcel Dekker; 1983:337Y367.

15).Sintov AC, Shapiro L. New nanoemulsion vehicle facilitates percutaneous penetration in vitro and cutaneous drug bioavailability in vivo; J. Pharm. Pharmacol. 1991; 43: 450–454.

16).Spiclin P, Homar M, Zupancic-Valant A, Gasperlin M. Sodium ascorbyl phosphate in topical nanoemulsions. Int. J. Pharm. 2003; 256: 65–73.

17).Tenjarla S. Nanoemulsions: an overview and pharmaceutical applications. Crit. Rev. Ther. Drug Carrier Syst. 1999; 16: 461–521.

18).Trotta M, Pattarino F, Gasco MR. Influence of counter ions on the skin permeation of methotrexate from water-oil microemulsions. Pharm Acta Helv. 1996;71:135Y140.

19).Trotta M. Influence of phase transformation on indomethacin release from microemulsions. J Control Rel. 1999;60:399Y405.

20).Van-Abbe NJ, Nicholas P, Boon E. Exaggerated exposure in topical irritancy and sensitization testing. J Soc Cosmet Chem. 1975;26: 173Y187.

21).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; 1989:197Y246.

22).Warisnoicharoen W, Lansley AB, Lawrence MJ. Light scattering investigations on dilute non-ionic oil-in-water microemulsions. AAPS PharmSciTech [serial online]. 2002;2:E12.

23).Winter CA. Anti-inflammatory testing methods: comparative evaluation of indomethacin and other agents. Nonsteroid Anti-inflammat Drugs. 1965;52:190Y202.

24).Yamazaki R, Kawai S, Mastsuzaki T, et al. Aceclofenac blocks prostaglandin E2 production following its intracellular conversion into cyclooxygenase inhibitors. Eur J Pharmacol. 1997;329:181Y187.

25).Yang JH, Kim Y, Kim KM. Preparation and evaluation of aceclofenac microemulsion for transdermal delivery system. Arch Pharm Res. 2002;25:534Y540.