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Original Article

Formulation and evaluation of microemulsion system for transdermal delivery of nimodipine

Prashant Khade, Ganesh Chailang, Gurauraj Shahabade, Sujit Kakade, Sachin kotwal

PDEA's Shankarrao Ursal College of Pharmaceutical Sciences and Research Center, Kharadi, Pune-14, India

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ABSTRACT

The purpose of this study was to construct novel o/w microemulsion formulation for transdermal delivery of Nimodipine. Capmul PG 8 as the oil, Tween 20 as the surfactant, polyethylene glycol 400 as the cosurfactant screened as phases of microemulsions, due to a good solubilizing capacity of the microemulsion systems and excellent skin permeation rate of Nimodipine. The pseudo-ternary phase diagrams for microemulsion regions were constructed using Capmul PG 8 as the oil, Tween 20 as the surfactant, polyethylene glycol 400 as the cosurfactant. Various microemulsion formulations were prepared from Surfactant and cosurfactant ratio 2:1 and the abilities of various microemulsions to deliver Nimodipine through the skin were evaluated in vitro using Keshary-Chein diffusion cells fitted with rat skins. The in vitro permeation data showed that microemulsions increased the permeation rate of Nimodipine over the control solution of Nimodipine. The optimum formulation consisted of 1.5 % Nimodipine, 5% Capmul PG 8, 25 % Tween 20/PEG (2:1) and 68.5 % water, showed a high permeation rate of 0.407 mg/cm^2/h. These results indicate that the optimized formulation of Nimodipine microemulsion may be used as a promising vehicle for transdermal delivery of Nimodipine.

Key words: Microemulsion, Transdermal delivery, Nimodipine, Permeation rate.

INTRODUCTION

Nimodipine (NM), isopropyl-2-methoxyethyl-1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridine dicarboxylate, is a dihydropyridine calcium antagonist with therapeutic indications for cerebrovascular spasm, stroke and migraine (1, 2). Recently, NM has been shown to be effective in ameliorating memory degeneration and preventing senile dementia in the old age (3, 4). However, the clinical usefulness of NM is limited by its high first-pass effect in liver (80 %) and which leads to low oral bioavailability (13 %) (5-7). Nimodipine occurs in two polymorphic forms. The intrinsic solubilities of both modifications are 0.86 and 0.44 µg/ml at 37 ^{0}C, respectively [8]. In view of its physicochemical and pharmacokinetic characteristics, it seems that there is potential for investigating the ability of Nimodipine to permeate human epidermis [9]. Various studies have demonstrated that the transdermal pathway may be a suitable alternative to the oral route in the administration of drugs with systemic activity. The potential advantages associated with transdermal drug delivery are well documented and include avoidance of first-pass effect, administration of lower doses, potentially decreased side effects, constant plasma levels and improved patients compliance (9).

A microemulsion is defined as a dispersed system consisting of an oil, surfactant, co-surfactant and an aqueous phase, which is a single optically isotropic and thermodynamically stable solution, with a droplet diameter usually within the range of 10~100 nm (10). Microemulsions have several advantages as drug delivery systems, such as enhanced drug solubility, good thermodynamic stability, ease of manufacturing and enhancement of drug permeation effects upon transdermal administration (11, 12). Recently more attention has focused on microemulsion for transdermal delivery of drug. The transdermal delivery of aceclofenac, triptolide, dexamethasone, using microemulsion has been reported (12-14). In transdermal delivery, the key point of dosage design was to solubilize the drug in microemulsion and improved the permeability (15).

***Corresponding Author Address: Prashant Khade,** *PDEA's Shankarrao Ursal College of Pharmaceutical Sciences and Research Center, Kharadi, Pune-14, India; E-mail: phkhade@gmail.com*

In this study, O/W microemulsions containing 1.5% Nimodipine have been developed after screening of oils, surfactants and cosurfactants obtaining optimum concentration ranges of components for microemulsion formation to provide maximal skin permeation rate of Nimodipine.

MATERIALS AND METHODS

Materials: Nimodipine was received as a gift sample from USV Pharma Ltd (Mumbai, India), Capmul PG-8 and Capmul MCM was received as a gift sample from Abitec Corporation (US). Labrafac CC, Labrafac Lipophile WL 1349, Labrafil M 1944 CS and Labrafil M 2125 CS were received as a gift sample from Gattefosse India Pvt Ltd (Mumbai, India). Eucalyptus oil, Tween 80, Tween 20, PEG 200, PEG 400, carbitol and ethanol were purchased form Research lab (Mumbai, India). Water was purified by double distillation in a glass apparatus. All other chemicals and solvents were of analytical reagent grade.

Screening of Components: The most important criterion for the screening of components for microemulsion is the solubility of poorly soluble drug in oils, surfactants and cosurfactants. The solubility of Nimodipine in various oils, surfactants, cosurfactants and water was determined by adding an excess amount of drug in 2 mL of selected oils, surfactants, cosurfactants and distilled water separately in 5 mL capacity stopper vials, and mixed using a vortex mixer. The mixtures in vials were then kept at 25 ± 1.0 ^{o}C in an isothermal shaker for 72 h to reach equilibrium. The equilibrated samples were removed from shaker and centrifuged at 3000 rpm for 15 min. The supernatant was taken and filtered through a 0.45 µm membrane filter. The concentration of Nimodipine was determined in oils, surfactants, cosurfactants and water using UV spectrophotometer at 355 nm. For each excipient determine standard calibration curve for determination of concentration of Nimodipine in excipients (16).

Pseudo Ternary Phase Diagram Studies: On the basis of the solubility studies of drug, select the oil phase, surfactants and cosurfactants. Water was used as an aqueous phase for the construction of phase diagrams. Surfactant and cosurfactant (Smix) are mixed in different weight ratios 1:0, 1:1, 1:2, 2:1, 3:1. These Smix 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 for formulation of microemulsion. For each phase diagram, oil and specific Smix ratio was mixed thoroughly in different weight ratios from1:9 to 9:1 in different glass vials. Sixteen different combinations of oil and Smix, 1:9, 1:8, 1:7, 1:6, 1:5, 2:8(1:4), 1:3.5, 1:3, 3:7(1:2.3), 1:2, 4:6(1:1.5), 5:5(1:1), 6:4(1:0.7), 7:3 (1:0.43), 8:2(1:0.25), 9:1(1:0.1), were made so as to cover possible combinations for the study to delineate the boundaries of phases precisely formed in the phase diagrams. Pseudo ternary phase diagrams were developed using aqueous titration method. Slow titration with aqueous phase was done to each weight ratio of oil and Smix and visually observed for transparent and easily flowable o/w microemulsions. The physical state of the microemulsion was marked on a pseudo-three-component phase diagram with one axis representing aqueous phase, the other representing oil and the third representing a mixture of surfactant and cosurfactant at fixed weight ratios (Smix ratio) (16). Based on the results, appropriate percentages of oil, surfactant and co-surfactant were selected and correlated in the phase diagram and then were used for preparation of microemulsion of Nimodipine.

Preparation of the Nimodipine loaded microemulsion: Nimodipine was added to the mixtures of oil and Smix in selected formulation as reported in **Table 2,** and then an appropriate amount of water was added to the mixture drop by drop and the microemulsion containing Nimodipine was obtained by stirring the mixtures. All microemulsions were stored at 30 ± 2^{o}C. Nimodipine at 1.5 %w/w was incorporated in all formulations (14). Compositions of Nimodipine loaded microemulsions are reported in Table 3. Saturated solution of Nimodipine as control was prepared by dissolving sufficient Nimodipine in ethanol (14).

Characterization of Nimodipine loaded microemulsion:

pH measurement: The pH of formulated microemulsion was determined using pH meter (Model GMPH, Labindia, (Mumbai). The electrode was immersed in microemulsion and pH was recorded.

Viscosity Determination: The viscosity of microemulsions were measured at 25 ^{0}C using a Brookfield Viscometer (DV-II + Pro) with small sample adapter spindle No. C–18, at 60 rpm.

Droplet Size: The droplet size distribution of the prepared microemulsion was determined by using a photon correlation spectrometer (Zetasizer 3000 HAS, Malvern Ltd., UK). The measurements were performed at 25 ^{o}C using a He-Ne laser (13).

In-vitro skin permeation study through excised rat skin[19]: Dorsal skin, excised from male albino Wistar rats (7-8 weeks old, 140-160 g) was

mounted in diffusion cells. After the hair on the dorsal skin had been removed with animal hair clippers, the subcutaneous tissue was surgically removed, and the dermis side wiped with isopropyl alcohol to remove the residually adhered fat. A Keshary-Chein diffusion cell apparatus was used for this purpose with a diffusional surface area of 2.83 cm^2 and a volume of receptor cell of 70 ml. The skin piece was mounted over diffusion cells with the dermal side in contact with the receptor phase, equilibrated for 1h and then air bubbles were removed. Subsequently, donor compartments were filled with 2 g of microemulsion formulation equivalent to 30 mg of drug. Then, it was covered with aluminium foil to prevent evaporation of vehicle. The receptor compartments were filled with phosphate buffer pH 7.4:ethanol (70:30 v/v) and stirred with a small magnetic bar for uniform mixing of the contents. The receptor compartment was surrounded by water jacket by using diffusion cell apparatus (Orchid Scientifics, Mumbai) for maintaining the temperature at 37˚C ± 0.5˚C and was provided with a sampling port. Samples (1 mL) were withdrawn at every 2 h intervals over a period of 24 hours from the receptor compartment and replenished with fresh receptor medium. At the end of study the concentration of drug in receptor samples analyzed by U.V. spectrophotometric method. All experiments for each sample were carried out in triplicate and results were presented as the mean ± S.D (12).

Data analysis of skin permeation: **Flux:** The skin flux Can be experimentally determined from the following equation (12):

$$\mathbf{J_{ss} = (dQ/dt)_{ss} \times 1/A}$$

Where, $\mathbf{J_{ss}}$ **is** steady-state flux (mg/cm^2 per h), **A is** area of skin tissue (cm^2) through which drug permeation takes place and $\mathbf{(dQ/dt)_{ss}}$ **is** the amount of drug passing through the skin per unit time at a steady-state (µg/h). The cumulative amount of Nimodipine permeating through the rat skin was plotted as a function of time. The permeation rate of Nimodipine through rat skin at a steady-state (J_{ss}, mg/cm^2 /h) was calculated from the slope of the linear portion of the plot (12).

Permeability coefficient: The following equation was used to calculate the permeability coefficient, K_p (cm/h).

$$\mathbf{K_p = J_{ss}/C_o}$$

where, $\mathbf{K_p}$ is the permeability coefficient and $\mathbf{C_0}$ is represents the initial drug concentration, which remain constant in the vehicle (12).

Stability of microemulsions: Physical stabilities of blank and drug-loaded microemulsions were evaluated by centrifugation at l0000 rpm for 30 min. Stabilities of the microemulsions incorporated with Nimodipine were studied by clarity and phase separation observation (17).

Skin irritation study: The albino Wistar rats were housed in polypropylene cages, with free access to standard laboratory diet and water. Animals were acclimatized for at least 7 days before experimentation. The dorsal abdominal skin of rats was shaved 24 h before study. Microemulsion was applied and side of application was occluded with gauze and covered with a nonsensitizing microporous tapes. A 0.8 %v/v aqueous solution of formalin was applied as standard skin irritant. The formulation was removed after 24 h and score of erythema was recorded and it compared with standard. Score of erythema recorded as follows:
Score 0: no erythema; **Score 1:** Mild erythema (barely perceptible- light pink); **Score 2:** Moderate erythema (dark pink); **Score3:** Severe erythema (Extreme redness) (14, 18).

RESULTS AND DISCUSSION

Screening of Components: The most important criterion for screening of excipients is the solubility of the poorly soluble drug in oil, surfactants, and co-surfactants. Since the aim of this study is to develop a transdermal formulation, it is important to determine the solubility of the drug in different oils, surfactants, and co-surfactants. The solubility of Nimodipine in different oils surfactants, cosurfactants and water was determined (Table 1). The solubility of Nimodipine was found to be highest in Capmul PG 8 (85.98 ± 3.2 mg/mL) and Eucalyptus oil (82.93 ± 2.3 mg/mL) as compared to other oils while in water it was 0.07 ± 0.08 mg/mL. Tween 20 which was show highest solubility of Nimodipine of 325.00 ± 2.09 mg/mL. PEG-400, Ethanol and Carbitol show the solubility of Nimodipine 99.2 ± 1.1 mg/mL, 85.22 ± 0.07 mg/mL and 253.3 ± 0.07 mg/mL respectively. Capmul PG 8 and Tween 20 selected as oil and surfactant respectively. P. Santos et al. reported medium chain triglycerides of caprylic acid and capric acid have been employed as the oil phase in a number of transdermal microemulsion formulations (19). Because of Capmul PG 8 is selected as oil phase and Nimodipine show highest solubility in Capmul PG 8. Tween 20 selected as surfactant because HLB value 16.7, Non-ionic surfactants are less toxic than ionic surfactants, good biological acceptance, powerful permeate enhancers and highest solubility of Nimodipine (20). PEG 400 selected as cosurfactant because highest solubility of Nimodipine. On the other hand, in ethanol highest solubility of Nimodipine but alcohols and other volatile co-solvents have the disadvantage of evaporation there may be chances drug precipitation (21).

Pseudo Ternary Phase Diagram Studies: Constructing phase diagrams is time consuming, particularly when the aim is to accurately delineate a phase boundary. Care was taken to ensure that observations are not made on metastable systems, although the free energy required to form an emulsion is very low, the formation is thermodynamically spontaneous. The relationship between the phase behavior of a mixture and its composition can be captured with the aid of a phase diagram.

Pseudo ternary phase diagrams were constructed separately for different Smix ratios, so that o/w microemulsion regions could be identified and microemulsion formulations could be optimized (16). In Figure 1 (S mix ratio1:0) it can be observed that when Tween 20 was used alone without cosurfactant, 17 % w/w oil could be solubilized at a low concentration 41 %w/w of surfactant. As the concentration of surfactant decreased solubilization of oil decreased. When cosurfactant was added with surfactant in equal amount [Smix ratio1:1(Figure 2)], the microemulsion region in the phase diagram decreased and the very low amount of oil 16 %w/w could be solubilized at the concentration 58 %w/w of surfactant. When cosurfactant concentration was further increased to S mix ratio 1:2 (Figure 3), it was observed that the microemulsions area is a decreased as compared to Smix ratio 1:1. Only 10 %w/w oil is solubilized in high concentration of surfactant 65 % w/w.

When surfactant concentration was increased with respect to cosurfactant [Smix ratio 2:1 (Figure 4)], it was seen that 20 %w/w oil could be solubilized with a surfactant concentration of 57 % w/w. When the surfactant concentration was further increased to 3 parts is to 1 part of cosurfactant (Figure 5), the microemulsion area decreased further and maximum amount of oil that could be solubilized was 10 %w/w and that too at a lower concentration of Smix (21 %w/w). It can be observed that the formulations prepared from phase diagrams in which the microemulsion area was extended towards aqueous rich apex could be diluted to a larger extent. Smix ratio 2:1 shows maximum solubility of oil in minimum surfactant concentration. This Smix ratio select for the preparation of Nimodipine loaded microemulsion. Five formulations selected for preparation of Nimodipine loaded microemulsion from microemulsion region of Smix ratio 2:1 (Table 2) Also Smix ratio 1:0 is shows maximum solubility of oil in minimum amount of surfactant mixture, but Smix ratio 1:0 not selected because there is Smix without cosurfactant.

Characterization of Nimodipine loaded microemulsion:

pH measurement: The human skin has a pH in between 4 to 6. The pH of microemulsions was found in between 4 to 6 indicating suitability for skin application. The pH of microemulsion formulations are given in Table 4.

Viscosity Determination: The microemulsion of Nimodipine showed viscosities in the range of 25 - 32 cP (small sample adapter spindle No. C–18; at 60 rpm). Which indicates that as concentration of water increases the viscosity of the formulation decreases. The viscosity of microemulsion formulations are given in Table 4.

Droplet Size: The droplet sizes of different optimized formulations are presented in Table 4 Particle size measurement indicated that the mean droplet sizes of the formulations were within the 69 to 82 nm range. The droplet sizes were increase with increasing water content (12). There for a maximum droplet size of 82 nm with formulation M5 contain 68.5 %w/w water and smaller droplet size of 69 nm with formulation M1 contain 28.5 %w/w water. The droplet sizes were decrease with increasing Smix content, due to the effective interfacial activity (13). In case of formulation M1 concentration of Smix (54 %w/w) due to droplet size is less as compare to other. Advantage of small droplet sizes are very much prerequisite for drug delivery as the oil droplet tends to fuse with skin thus providing channel for delivery (14). Droplet sizes of the formulations are show in Table 4.

In-vitro skin permeation study through excised rat skin: The permeation ability of the various microemulsions was evaluated using the in vitro permeation experiments. The permeation parameters and percentage drug permeated of the tested microemulsion and control formulations were presented in Table 5. The permeation profiles of Nimodipine through rat skins from various vehicles are shown in Figure 6. A steady increase of Nimodipine in the receptor chambers with time was observed. The two principal factors govern the penetration of Nimodipine from the microemulsion water content and the Smix ratio. It is clear from the Figure 6 that the M1 shows the lowest drug permeation (8.01 ± 1.27 mg/cm^2 at 24 h) and lowest flux (0.329 ± 0.16 mg/cm^2/h), because the concentration of Smix highest (54 %w/w) and concentration of water lowest (28.5 %w/w). On the other side M4 and M5 shows highest drug permeation (9.79 ± 1.11 and 10.32 ± 1.54 mg/ cm^2 at 24h, respectively) and highest flux (0.386 ± 0.12 and 0.407 ± 0.09 mg/cm^2/h respectively), because of the lowest amount of Smix (32 and 25 %w/w respectively) and highest amount of the water (58.5 and 68.5 %w/w respectively). As the content of Smix was decreased from 54 to 25 %w/w, the skin permeation rate of Nimodipine was increased.

This may be due to an increased thermodynamic activity of the drug in the microemulsion at the lower content of Smix (14, 22, 23). Nimodipine can be released from the interfacial film to external phase, and then from the external phase to the skin; therefore, the reduction of the content of surfactant might become a driving force of drug release to the skin (17). Meanwhile, high content of surfactant will increase the irritation to skin (15). As the content of water was decreased from 68.5 to 28.5 %w/w, the skin permeation rate of Nimodipine was decreased. When the aqueous fluid in the microemulsion enters the polar pathway of the stratum corneum, the water in the microemulsion can hydrate the stratum corneum to a great extent, and the interlamellar volume of the stratum corneum lipid bilayers will be increased. This results in the disruption of bilayer structure, which then swells enough to expand drug tunnels, and thereby promoting the permeation (23). Compared the Nimodipine permeation after 24 h and flux of microemulsion formulations and control Nimodipine solution using permeation study. The drug permeation of all microemulsion formulations have in between 8.01 ± 1.27 to 10.32 ± 1.54 mg/ cm^2 and flux of of all microemulsion formulations have in between 0.329 ± 0.16 to 0.407 ± 0.09 mg/cm^2/h. The drug permeation after 24 h of control Nimodipine solution have 04.81 ± 0.32 mg/cm^2 and flux of of control Nimodipine solution have 0.186 ± 1.3 mg/cm^2/h. These differences clearly indicate that the microemulsion good permeation enhancer.

Stability of microemulsions: The microemulsion vehicles were isotropic transparent dispersions and after centrifugation no phase separation was observed. This demonstrated the good physical stability of the tested microemulsions.

Skin irritation study: The microemulsion was applied (24h) to the albino Wistar rats skin and erythema was compared with by skin sensitization visual score. No erythema was found after 24 h, when response of animal under test were observed visually (Figure 7 & 8) and compared with standard irritant animal. This indicated that the microemulsion was response of nonirritant and never produced erythema after application. Thus the formulations were safe for transdermal application.

CONCLUSION

An O/W microemulsion containing Nimodipine was formulated for transdermal application. Different microemulsion formulations were designed using pseudo-ternary phase diagrams. pH, Droplet sizes and viscosity data of the microemulsions confirmed the continuous structural transitions during increases in the water phase volume fraction in the oil/surfactant/cosurfactant mixture selected in this study. The permeation rates of the drug in the microemulsion formulations studied were increased compared to that the control. Also maximum permeation rate achieved formulation M5 shows maximum permeation rate. All microemulsions formulations were physically stable and also not show any skin irritation.

Table (1): Solubility of Nimodipine in various oils, Surfactant, Cosurfactants & Water at 25±1 ^{0}C

Sr. No	Components	* Solubility (mg/mL)
1	Capmul PG-8	**85.98 ± 3.2**
2	Capmul MCM	**39.23 ± 1.7**
3	Capmul MCM (C8)	**38.78 ±.12**
4	Labrafac CC	**55.54 ± 0.5**
5	Labrafac Lipophile WL 1349	**36.95 ± 2.6**
6	Labrafil M 1944 CS	**45.56 ± 0.8**
7	Labrafil M 2125 CS	**45.64 ± 0.7**
8	Eucalyptus oil	**82.93 ± 2.3**
9	Tween 80	**117.5 ± 1.23**
10	Tween 20	**325.0 ± 2.09**
11	PEG 200	**073.2 ± 0.35**
12	PEG 400	**099.2 ± 1.1**
13	Ethanol	**146. 5 ± 1.9**
14	Carbitol	**85.22 ± 0.07**
15	water	**0.07 ±0.08**

* Represents mean ± S.D. (n = 3**)**

Table (2): Selected formulations from Smix 2:1 phase diagrams.

Sr. No	Microemulsions	Oil (%w/w)	Smix (%w/w)	Water (%w/w)
1	M1	16	54	30
2	M2	14	46	40
3	M3	10	40	50
4	M4	8	32	60
5	M5	5	25	70

Table (3): The composition of the different microemulsions from Smix 2:1

Sr. No	Microemulsions	Nimodipine (%w/w)	Oil (%w/w)	Smix (%w/w)	Water (%w/w)
1	M1	1.5	16	54	28.5
2	M2	1.5	14	46	38.5
3	M3	1.5	10	40	48.5
4	M4	1.5	8	32	58.5
5	M5	1.5	5	25	68.5

Table (4): pH, viscosity and Droplet size of the microemulsions.

Sr. No.	Microemulsions	*pH	*Viscosity (cP)	Droplet Size (nm)
1	M1	4.2	31.22±0.12	69
2	M2	4.4	29.44±0.31	71
3	M3	4.7	27.92±0.90	74
4	M4	4.6	26.56±0.65	78
5	M5	4.8	25.47±0.46	82

* Represents mean ± S.D. (n = 3**)**

Table 5. *In vitro* permeation parameters for the transmission of Nimodipine contained within the microemulsions through excised rat skin.

Formulation	*Flux (Jss) (mg/cm2 / h)	*Permeability coefficient (Kp) (cm/h)	*Q_{24} (mg/cm^2)
M1	0.329 ± 0.16	10.97 ± 0.92	8.01 ± 1.27
M2	0.353 ± 0.55	11.78 ± 0.54	8.77 ± 0.76
M3	0.368 ± 0.03	12.27± 0.23	9.03 ± 0.54
M4	0.386 ± 0.12	12.88 ± 0.15	9.79 ± 1.11
M5	0.407 ± 0.09	13.56 ± 1.43	10.32 ± 1.54
CONTROL	0.186 ± 1.34	06.21 ± 1.11	04.81 ± 0.32

* Represents mean ± S.D. (n = 3), **Q_{24}:** Nimodipine permeated after 24h in mg/cm^2.

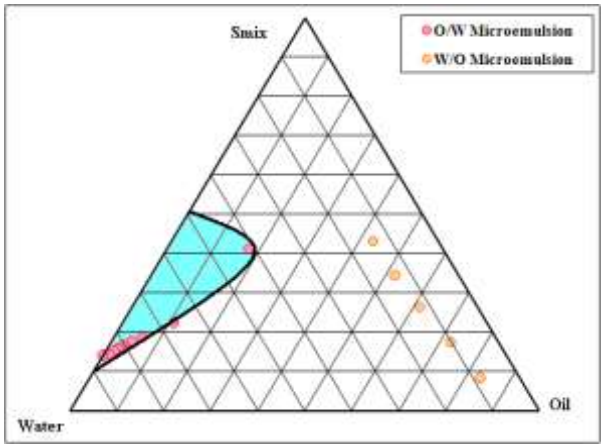

Figure 1: Microemulsion region of Smix ratio 1:0

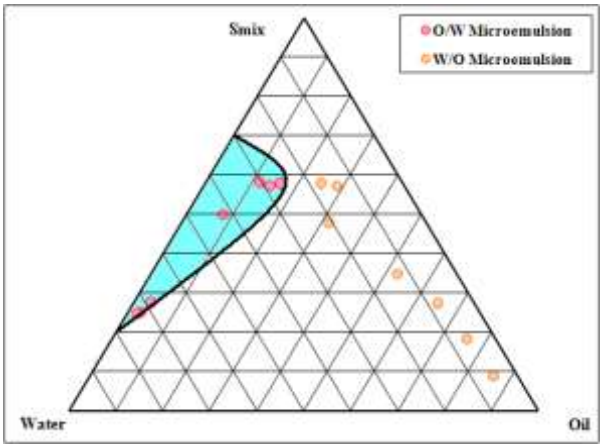

Figure 2: Microemulsion region of Smix ratio 1:1

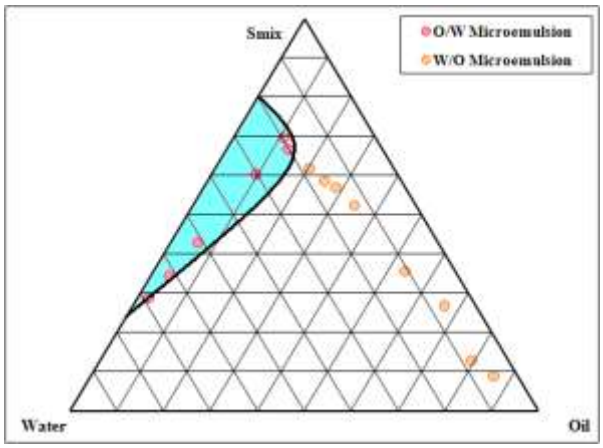

Figure 3: Microemulsion region of Smix ratio 1:2

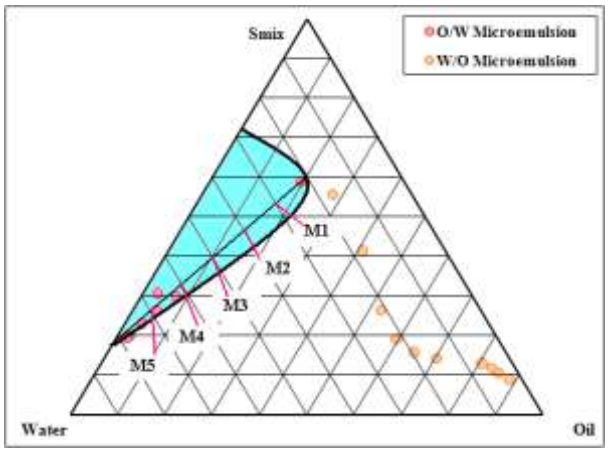

Figure 4: Microemulsion region of Smix ratio 2:1

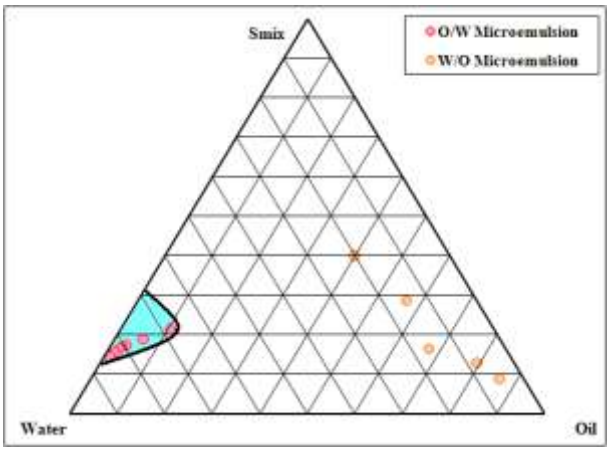

Figure 5: Microemulsion region of Smix ratio 3:1

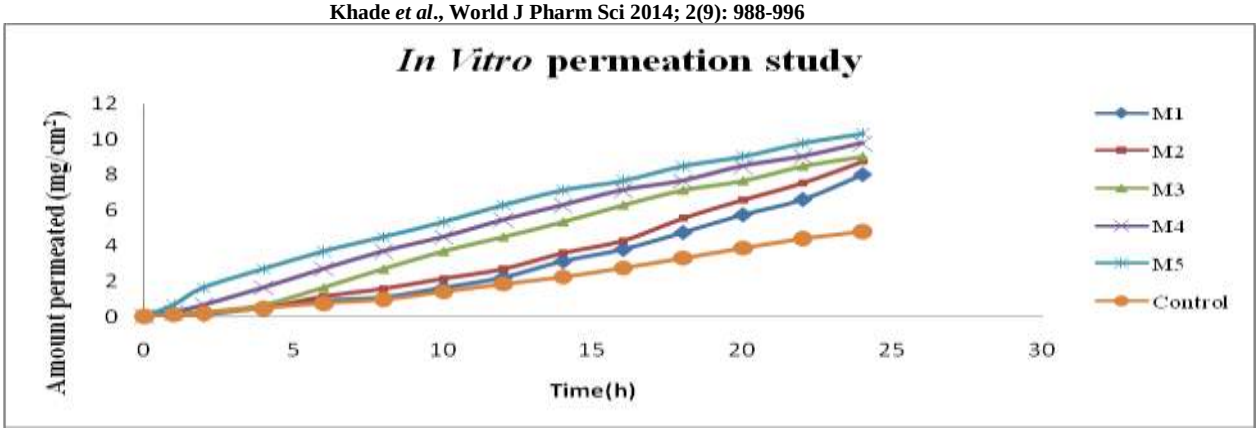

Figure 6. *In vitro* Permeation profile of Nimodipine through the excised rat skin from microemulsion formulations. The control solution was prepared by solubilizing Nimodipine in ethanol (1.5 %w/v) (mean±S.D., *n*=3).

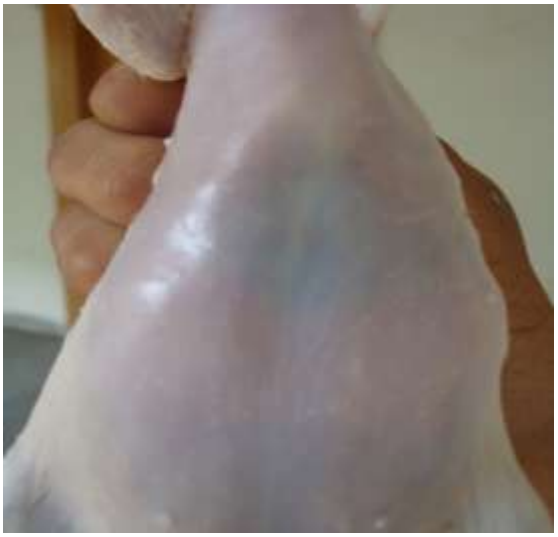

Figure 7: Tested animal (Microemulsion), Score 0: no erythema.

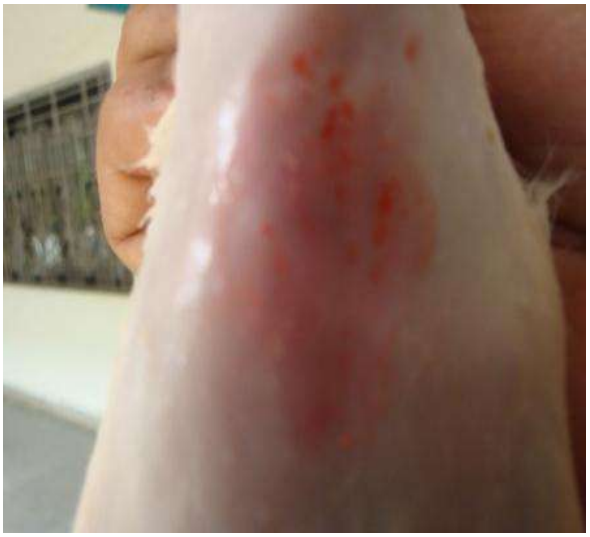

Figure 8: Standard irritant animal (0.8 %v/v formalin solution) Score3: Severe erythema (Extreme redness).

REFERENCE

1. Gelmers HJ. Calcium-channel blockers in the treatment of Migraine. Am J Cardiol 1985; 55:139-143.
2. Langley MS, Sorkin EM. Nimodipine-A review of its pharmacodynamic and pharmacokinetic properties and therapeutic. Drugs 1989; 37:669–699.
3. Zhang JT. Altered calcium homeostasis in brain aging and senile dementia, new approach to treat Alzheimer's disease. Acta Pharmacol Sin 1993; 28: 641–646.
4. Pantoni L et al. Efficacy and safety of nimodipine in subcortical vascular dementia: a subgroup analysis of the Scandinavian Multi-Infarct Dementia Trial. J Neurosci 2000; 175: 124–134.
5. Muck W et al. The influence of age on the pharmacokinetics of nimodipine. Int J Clin Pharmacol 1996; 34: 293–298.
6. Blardi P et al. Nimodipine: drug pharmacokinetics and plasma adenosine levels in patients affected by cerebral ischemia. Clin Pharmacol Ther 2002; 72:556–561.
7. Qizhi Zhangn et al. Preparation of nimodipine-loaded microemulsion for intranasal delivery and evaluation on the targeting efficiency to the brain. Int J Pharm 2004; 275: 85–96
8. Grunenberg A et al. Polymorphism in binary mixture, as exemplified by nimodipine. Int J Pharm 1995; 118: 11–21.
9. Giannakou SA et al. In vitro evaluation of nimodipine permeation through human epidermis using response surface methodology. Int J Pharm 2002; 241: 27–34.
10. Tenjarla S. Microemulsions: an overview and pharmaceutical applications. Drug Carrier Syst 1999; 16: 461–521.
11. Lawrence MJ, Rees GD. Microemulsion-based media as novel drug delivery systems. Adv Drug Delivery Rev 2000; 45: 89–121.
12. Jaehwi L et al. Formulation of microemulsion systems for transdermal delivery of aceclofenac. Arch Pharm Res 2005; 28(9): 1097-1102.
13. Huabing Chen et al. Hydrogel-thickened microemulsion for topical administration of drug molecule at an extremely low concentration. Int J Pharm 2007; 41: 78-84.
14. Chandra A et al. Mcroemulsion-based hydrogel formlation for transdermal delivery of dexamethasone. Int J Pharma 2009; 30-36.
15. Yue Yuan et al. Investigation of microemulsion system for transdermal delivery of meloxicam. Int J Pharm 2006; 321: 117–123.

16. Sheikh S et al. Development and bioavailability assessment of ramipril nanoemulsion formulation. Eur J Pharm and Biopharm 2007; 66: 227–243.
17. Yuan Yuel et al. Physicochemical Properties and evaluation of microemulsion systems for transdermal delivery of meloxicam. Chem. Res. Chin. Univ. 2007; 23(l): 81-86.
18. Mutalik S, Udupa N. Formulation development, *in vitro* and *in vivo* evaluation of membrane controlled transdermal system of gibenclamide. J pharm sci 2005; 8(1): 26-38.
19. Santos P et al. Application of microemulsions in dermal and transdermal drug delivery. Skin Pharmacol Physiol 2008; 21: 246–259.
20. Wade A, Weller P. *Handbook of pharmaceutical excipients*, 2nd ed.; The Pharmaceutical Press: 1534 -1550.
21. Gursoy NR, Simon B. Self-emulsifying drug delivery systems (SEDDS) for improved oral delivery of lipophilic drugs. Biomed & Pharmacother 2004; 58; 173–182.
22. Huabing Chen et al. Microemulsion-based hydrogel formulation of ibuprofen for topical delivery. Int J Pharm 2006; 315: 52–58.
23. Yun-Seok Rhee et al. Transdermal delivery of ketoprofen using microemulsions. Int J Pharm2001; 228: 161–170.