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Formulation and Evaluation of Microemulsion Gel for Transdermal Delivery of Nifedipine

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ABSTRACT

The objective of this research was to design a new microemulsion gel for transdermal delivery of Nifedipine. For its good solubilizing capacity and skin penetration capability, a microemulsion-based system was selected. Castor oil, Tween 20, and PEG 400 were screened as oil, surfactant, and cosurfactant respectively from the solubility study. From pseudo ternary phase diagrams, the concentration of oil phase, Smix, and distilled water were determined and further processed for the formulation of Nifedipine microemulsion. Nifedipine loaded microemulsion optimized by stability study and in vitro permeation study. 1 percent w/w Carbopol was used to develop a microemulsion gel formulation. Using Keshary Chien diffusion cells, the efficacy of several microemulsion formulations to transport Nifedipine through the rat skin was studied in vitro. The in vitro permeation data showed that microemulsion gel increased the permeation rate (0.373 mg/cm2/h) of Nifedipine compared with the control solution (0.129 mg/cm2/h). All microemulsion formulations had droplet sizes ranging from 69 to 82 nm. The studied microemulsion gels were stable toward all stability conditions. A skin irritation study suggested that Nifedipine loaded microemulsion and microemulsion gel formulation were nonirritating and did not cause erythema. Thus, microemulsion gel systems are a promising formulation for the transdermal delivery of Nifedipine.

1. INTRODUCTION:

Hypertension, a disease that affects both developed and developing countries, required long-term therapy. As per the data, cardiovascular disease is the leading cause of death, and moderate hypertension may be the precursor to fatal cardiovascular diseases [1]. Patients with hypertension must take long-term medication, and in some cases, lifelong treatment is recommended. As a response, therapy non-compliance is a big issue, particularly in cases where dose frequency is high. Transdermal delivery is believed to be the ideal method for avoiding the difficulties of first-pass metabolism, ensuring absolute elimination of GIT toxic effects, maintaining a constant plasma level of the drug for a long time, and delivering the drug at a predetermined rate without the risks associated with intravenous infusion [2]. Transdermal formulations are more popular than oral dose forms because they provide a higher quality of life [3].

Nifedipine is a drug that is used to treat high blood pressure and angina (chest pain). Nifedipine is a Calcium-channel blocker. It lowers blood pressure by relaxing blood vessels, allowing the heart to pump more efficiently. It relieves chest pain by boosting blood and oxygen supply to the heart [4].

Existing Nifedipine dosage formulations require thrice-daily dosing and twice-daily dosing in the case of sustained-release formulations. Its low water solubility, high first-pass metabolism [5], and rapid clearance rate may cause significant intersubjective pharmacokinetic variability, as well as a short elimination half-life [6]. Adverse side effects such as headache, flushing, dizziness, lethargy, bradycardia, and ankle edema are common.

Several studies have shown that the transdermal method may be a viable alternative to the oral route for the delivery of systemic medicines. Transdermal drug delivery has several potential benefits, including avoiding the first-pass effect, administering smaller dosages, potentially reducing side effects, maintaining stable plasma levels, and improving patient compliance [7, 8, 9]. Nifedipine's low molecular weight, low daily dose, very poor aqueous solubility [10], and elimination of first-pass metabolism [11] are all important factors in its development as an effective transdermal system. Ensure more consistent plasma levels, limit side effects, and aid in the design and development of a successful transdermal system with a 3- to 5-day dosage interval and improved patient compliance [12].

Microemulsion gel for transdermal drug administration has recently received much interest. The major goal of dosage design in microemulsion gel for transdermal administration was to solubilize the medication in microemulsion and increase permeability [13].

In this study, O/W microemulsions gel containing 0.5 % Nifedipinehas been developed after the screening of oils, surfactants, and cosurfactants obtaining optimum concentration ranges of components for microemulsion gel formation to provide maximal skin permeation rate of Nifedipine.

2. MATERIALS AND METHODS

2.1 MATERIALS

Nifedipine was purchased from Aarti Distributor (Mumbai, India), Captex 500, Capmul PG-8, and Capmul MCM were received as a gift sample from Abitec Corporation (US). Labrafil M 1944 CS and Labrafil M 2125 CS were received as a gift samples from Gattefosse India Pvt Ltd (Mumbai, India). Oleic Acid, Isopropyl Myristate, Castor Oil, Coconut Oil, Lemon OilArachis Oil, Span 20, Span 60, Span 80, Tween 20, Tween 60, Tween 80, Cremophor RH 40, PEG 200, PEG 400, PEG 600, and Carbitol were purchased from the Research lab (Mumbai, India). Water was purified by double distillation in a glass apparatus. All other chemicals and solvents were of analytical reagent grade.

2.2 Screening of Components [14]:

The most important criterion for the screening of components for microemulsion is the solubility of a poorly soluble drug in oils, surfactants, and cosurfactants. The solubility of Nifedipine in various oils, surfactants, cosurfactants, and water was determined by adding an excess amount of the 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 the 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 Nifedipine was determined in oils, surfactants, cosurfactants, and water using a UV spectrophotometer at 237 nm.

2.3 Pseudo Ternary Phase Diagram Studies [14]**:**

Oil, Surfactants, and cosurfactants were chosen based on the drug's solubility tests. For the development of phase diagrams, water was employed as an aqueous phase. Surfactant and cosurfactant (Smix) are combined in various weight ratios such as 1:0, 1:1, 2:1, and 3:1. For a full examination of the phase diagrams for the formulation of the microemulsion, these Smix ratios were chosen in increasing the concentration of surfactant about cosurfactant. Oil and certain Smix ratios were completely mixed in different weight ratios from 1:9 to 9:1 in different glass vials for each phase diagram. Sixteen different oil and Smix combinations were created to cover all conceivable combinations: F1:9, 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). The aqueous titration approach was used to produce pseudo ternary phase diagrams. Slow titration with an aqueous phase was carried out for each weight ratio of oil and Smix, and translucent and easily flowable o/w microemulsions were visually seen. A pseudo-three-component phase diagram was used to depict the physical condition of the microemulsion, with one axis representing the aqueous phase, the other showing oil, and the third showing a mixture of surfactant and cosurfactant at fixed weight ratios (Smix ratio). Based on the findings, the proper percentages of oil, surfactant, and co-surfactant were chosen and associated in the phase diagram and then employed to make a Nifedipine microemulsion.

2.4 Selection Microemulsion Formulations from Phase Diagrams [14]**:**

Different formulations from the microemulsion area were chosen (Table 2) from each generated phase diagram so that the drug may be introduced into the oil phase based on the following criteria.

- The oil concentration should be such that it completely solubilizes the single dose of a drug.
- The additional drug should not affect the phase behavior or microemulsion area of the phase diagram.
- The lowest concentration of Smix was recommended.

2.5 Preparation of the Nifedipine loaded microemulsion, and control solution:

Microemulsion: Nifedipine (0.5 %w/w) was added to the mixtures of oil and Smix in the 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 Nifedipine was obtained by stirring the

mixtures. All microemulsions were stored at 30 ± 2°C [15]. Compositions of Nifedipine-loaded microemulsions are reported in Table 2.

Control solution: The control solution of Nifedipine was prepared by dissolving 0.5 gm in 100 mL of ethanol [16].

2.6. Optimization of microemulsion formulation:

Optimization of formulations was done by performing the following study:

1. Stability study and
2. *In Vitro* permeation study.

2.6.1. Stability studies[14]**:**

2.6.1.1. Heating cooling cycle:

Six cycles were performed between refrigerator temperatures of 4°C and 45°C, with storage at each temperature for at least 48 hours. Centrifugation tests were performed on the formulations that were stable at these temperatures.

2.6.1.2. Centrifugation:

The formulations that passed the heating-cooling cycling test were centrifuged for 30 minutes at 3500 rpm. The freeze-thaw stress test was performed on those formulations that did not produce any phase separation.

2.6.1.3 Freeze-thaw cycle:

The formulations were exposed to three freeze-thaw cycles at temperatures ranging from -21°C to +25°C, with storage at each temperature for at least 48 hours. Table 2 shows the results of the stability testing.

The formulations that survived these stability trials were next subjected to an in vitro permeation test.

2.6.2. *In-vitro* skin permeation study:

2.6.2.1. Preparation of skin [16]**:**

Male albino Wistar rats (7-8 weeks old, 140-160 g) had their dorsal skin removed and mounted in diffusion cells. Animal hair clippers were used to remove the hair on the dorsal skin, the subcutaneous tissue was surgically removed, and the dermis side was wiped with isopropyl alcohol to eliminate any remaining fat.

2.6.2.2 Skin permeation study[16]**:**

Skin penetration studies play an important role in the optimization of drug and formulation design in transdermal delivery. *In vitro* permeation studies across rats, the skin was carried out using a permeation set-up using rat skin. 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 cells 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 ml of microemulsion formulation equivalent to 10 mg of the drug. Then, it was covered with aluminum foil to prevent evaporation of the 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 a 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 24 hours from the receptor compartment and replenished with a fresh receptor medium. At the end of the study, the concentration of drugs in receptor samples was 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. The results of the drug permeability study are reported in Figures 5&6.

2.6.2.3. Data analysis of skin permeation :

Flux [16]**:** Theskin flux Can be experimentally determined from the following equation:

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

Where,

- **J_{ss}:** Steady-state flux (mg/cm^2 per h),

- **A:** Area of skin tissue (cm^2) through which drug permeation takes place,
- **$(dQ/dt)_{ss}$:** The amount of drug passing through the skin per unit time at a steady-state (µg/h).

The cumulative amount of Nifedipine permeating through the rat skin was plotted as a function of time. The permeation rate of Nifedipine 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.

Permeability coefficient [16]**:** The following equation was used to calculate the permeability coefficient, K_p (cm/h).

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

where,

- **K_p:** The permeability coefficient
- **C_0:** Represents the initial drug concentration.

Enhancement ratio (Er)[15]: The enhancement ratio was calculated by dividing the Jss of the respective formulation by the Jss of the control formulation.

$$Er = J_{ss} \text{ of formulation} / J_{ss} \text{ of control}$$

Flux, Permeability coefficient, and Enhancement ratio of different formulations are shown in Table 3.

2.7. Preparation of microemulsion gel:

2.7.1. Optimization of concentration of Carbopol 934 (Gelling agent) [15, 17, 18]:

Concentration of Carbopol 934 was 0.5, 0.75, 1.0 and 1.5 %w/w taken for preparation of microemulsion gel. An appropriate amount of Carbopol 934 was slowly mixed with a small amount of water and was kept overnight to obtain a highly viscous solution. Then Nifedipine loaded microemulsion was slowly added to the viscous solutions of Carbopol 934. The pH values were subsequently adjusted to 4-6 by using Triethanolamine solution (0.5 %v/v), and microemulsion gel was obtained. The concentration of Carbopol 934 was optimized based on the consistency of the microemulsion gel. Results are reported in the result and discussion Section 3.5.1.

2.7.2. Preparation of microemulsion gel of Nifedipine and control Nifedipine gel [15, 17, 18]**:**

The method of preparation is reported in section 2.7.1.

2.7.3. Preparation of control Nifedipine gel [18]**:**

Control Nifedipine gel was prepared by dispersing the 1 %w/w of the carbopol 934 in a sufficient quantity of distilled water. After complete dispersion, the carbopol 934 solution was kept overnight for complete swelling. Then, 0.5 gm of Nifedipine was dissolved in a sufficient quantity of ethanol. This solution of the drug was added slowly to the aqueous dispersion of carbopol 934 to get a final concentration of 0.5 % w/w Nifedipine. Then pH was subsequently adjusted to 4-6 using triethanolamine solution (0.5 %v/v) and to obtain Nifedipine gel.

2.8 Characterization of Nifedipine loaded microemulsion and microemulsion gel:

2.8.1 pH measurement:

The pH of formulated microemulsion and microemulsion gel was determined using a pH meter (Model GMPH, Labindia, Mumbai.). The electrode was immersed in microemulsion and microemulsion gel and pH was recorded. Results are reported in Table 5.

2.8.2 Viscosity Determination:

The viscosity of microemulsions was measured at 25 ^{0}C using a Brookfield Viscometer (DV-II + Pro) with a small sample adapter spindle No. C – 18, at 60 rpm. Viscosities of the microemulsion gels were determined using Brookfield Viscometer (DV-II + Pro) with a T-Shaped spindle set with spindle no.92at 2 rpm at 25° C. Results are reported in Table 5.

2.8.3 Droplet Size [19]**:**

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. Results are reported in Table 6.

2.8.4. Spreadability [20]**:**

Spreadability is an important property of transdermal formulations from a patient compliance point of view. Application of the formulation would be more comfortable if the base spreads easily exhibiting maximum ‘slip’ and ‘drag’. The spreadability of the microemulsion gel formulations was determined by placing 0.5 g of the respective formulations within a circle of diameter 1 cm,

premarked on a glass plate over which a second glass plate was placed. A weight of 500 g was allowed to rest on the upper glass plate for about 15 seconds. The diameter due to the spreading of the gel was noted. Results are reported in Table 7.

2.8.5 Stability study [19]:

Physical stabilities of blank and drug-loaded microemulsions wereevaluatedbycentrifugationat l0000 rpmfor 30 min. Stabilities ofthe microemulsions incorporated with Nifedipine were studied by clarity and phase separation observation.

2.8.6 Skin irritation study [15, 21]:

The albino Wistar rats were housed in polypropylene cages, with free access to a 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 the study. The microemulsion was applied and the side of the application was occluded with gauze and covered with a non-sensitizing microporous tape. A 0.8 %v/v aqueous solution of formalin was applied as a standard skin irritant. The formulation was removed after 24 h and a score of erythema was recorded and compared with the standard. A score of erythema was 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).

2.8.7 *In-vitro* skin permeation study through excised rat skin:

Reported in section 2.6.2.

3. RESULTS AND DISCUSSION:

3.1 Screening of Components:

The solubility of Nifedipine in oils, surfactants, and cosurfactants was determined and reported in Table 1. To preparation of o/w microemulsion, screening, and selection of an oil phase influence the selection of surfactant and cosurfactants. The oil was selected on basis of the maximum solubility of a selected drug in the oil phase to increase drug loading capacity in microemulsion and get maximum microemulsion area [21]. The solubility of Nifedipine was found to be highest in Castor Oil (75.07±1.83 mg/mL) as compared to other oils. Castor oil was selected oil phase because of the maximum solubility of Nifedipine in it and greatly miscible with other microemulsion components. Also, it was selected because it is skin nonirritant, has a good

fragrance, anti-oxidant property, and antiseptic characteristics. Castor oil is monounsaturated chemically that is used as a permeation enhancer; it is good for enhancing the permeation rate of Nifedipine [22]. The solubility of Nifedipine was found to be highest in Tween 20 which was 87.46±2.74 mg/mL. Tween 20 was selected as a surfactant because HLB value of 16.7, Non-ionic surfactants are less toxic than ionic surfactants, have good biological acceptance, powerful permeate enhancers and the highest solubility of Nifedipine [23, 24]. Polyethylene Glycol 400 (PEG 400) was selected as a co-surfactants because of its potential to solubilize the drug. Nifedipine was show highest solubility in PEG-400 as compared to other cosurfactants (68.18±5.39 mg/mL). It allows interfacial film with suitable flexibility to undertake various curvatures, needed to prepare microemulsion over a broad concentration range and decrease the interface bending stress [25]. Castor oil, Tween 20, and PEG-400 were selected as oil, surfactant, and cosurfactant respectively.

Table 1: Solubility study of Nifedipine in Various Excipients

Sr. No.	**Excipients**	***Solubility of Nifedipine (mg/ml) at 25 º C)**
Oils		
1.	Capmul PG-8	45.23±1.23
2.	Labrafil M 1944 CS	29.34±1.03
3.	Labrafil M 2125 CS	54.00±0.93
4.	Oleic Acid	44.58±0.01
5.	Isopropyl Myristate	21.48±1.39
6.	**Castor Oil**	**75.07±1.83**
7.	Coconut Oil	33.92±1.40
8.	Lemon Oil	29.24±1.68
9.	Arachis Oil	25.49±0.24
Surfactants		
10.	Span 20	70.56±1.42
11.	Span 60	64.82±2.33
12.	Span 80	58.87±1.02
13.	**Tween 20**	**87.46±2.74**
14.	Tween 60	62.45±1.74
15.	Tween 80	69.42±1.25
16.	Cremophor RH 40	70.90±0.65
Cosurfactants		
17.	PEG 200	14.29±5.75
18.	**PEG 400**	**68.18±5.39**
19.	PEG 600	25.21±4.75
20.	Capmul MCM	34.34±3.34
21.	Captex 500	29.39±4.44
22.	Carbitol	50.19±4.44

* Represents mean ± S.D. (n = 3)

3.2 Pseudo Ternary Phase Diagram Studies:

The Pseudo ternary phase diagrams are constructed separately for the different concentrations of Smix so that o/w microemulsion areas may be recognized and microemulsion formulations can be optimized [14]. The shaded areas in the pseudo-ternary phase diagrams shown in Figures 1 to 4 represent the presence of a stable, clear, and transparent O/W microemulsion field containing Castor oil such as oil and the Tween 20: PEG 400 fixed mixing ratio as Smix. At Smix ratio 1: 0 (Figure 1), it can be noted that when Tween 20 was used alone a small microemulsion region was obtained as compared with another Smix ratio. Smix ratio 1: 1, when the cosurfactant is added in equal proportions with surfactant (Figure 2), the microemulsion region in the phase diagram increases compared to the Smix ratio of 1: 0. At Smix 2:1, the concentration of the surfactant increased for the cosurfactant (Figure 3), reflecting the presence of a broad microemulsion in the phase diagram. When the concentration of the surfactant is increased to 3 to 1 part of the cosurfactant (Figure 4), the microemulsion content increases and the maximum amount of oil can be dissolved. It can be noted that the composition formed from the ternary phase diagrams where the microemulsion area was expanded to a rich aqueous apex can be diluted to a large extent.

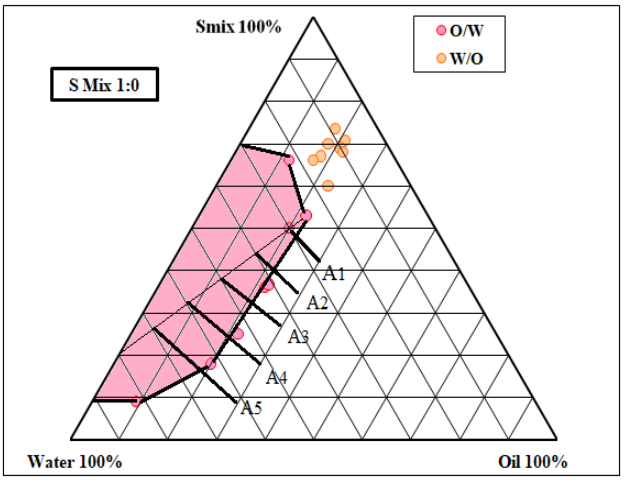


Figure 1: Microemulsion regions of ratio 1:0

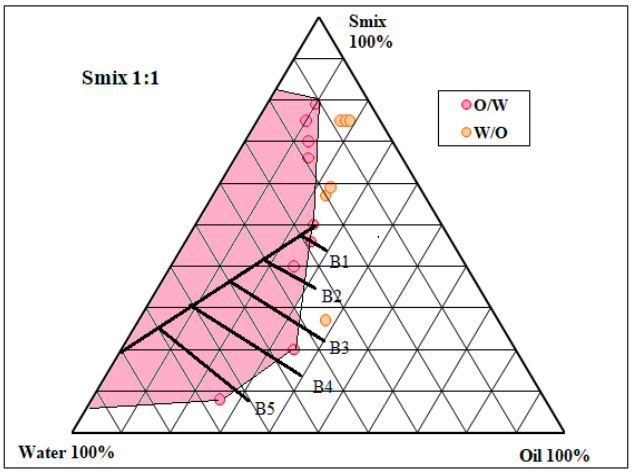


Figure 2: Microemulsion regions of ratio 1:1

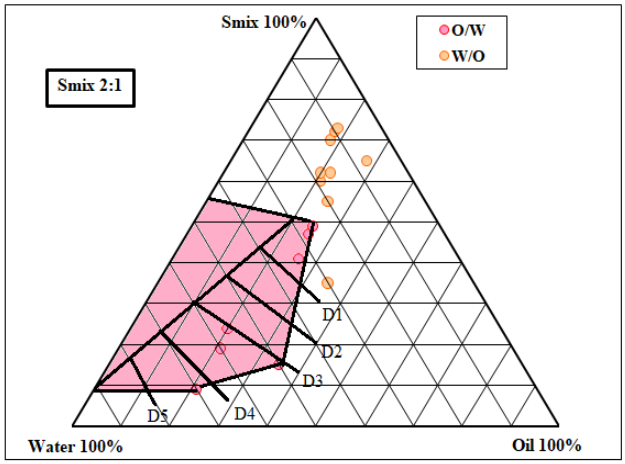


Figure 3: Microemulsion regions of ratio 2:1

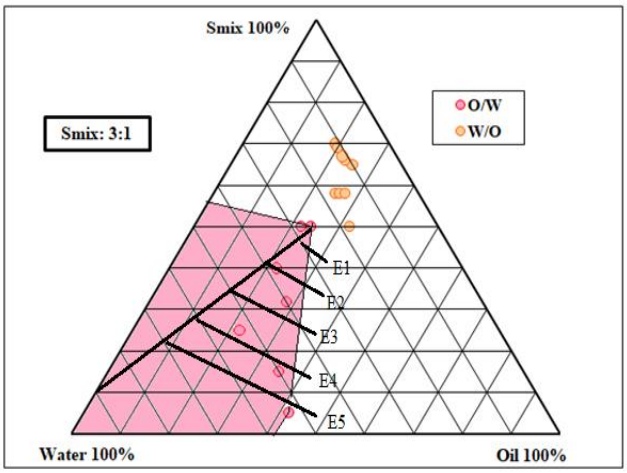


Figure 4: Microemulsion regions of ratio 3:1

3.3. Selection of formulations from phase diagrams:

The selected concentration of the oil phase is between 4 % w/w to 23 % w/w; so that maximum formulations could be selected covering the microemulsion area of the phase diagram (Tables 2). To avoid skin irritation and also avoid the stability problem of the microemulsion, oil phase, and surfactant were selected at minimum concentration. When Nifedipine (10 mg) was added to the formulations, there was no effect seen in the phase behavior and microemulsion area of phase diagrams. This was expected as nonionic components containing formation was shows good stability of microemulsions and were not affected by the pH and or ionic strength [14].

3.4. Optimization of microemulsion formulation:

The Nifedipine (0.5 %w/w) can be loaded in selected formulations (Table 2); then these selected formulations are optimized by the following study:

1. Stability study and
2. *In Vitro* permeation study

3.4.1. Stability studies:

According to thermodynamic stability study results, all selected formulations from Smix ration 1:0 are thermodynamically unstable, it may be the only surfactant used in the formulation. In Smix ratio 1:1, B1 and B2 formulation was found to be stable but the remaining formulations are unstable. In Smix ration 2:1, C1 to C3 formulations were passed thermodynamic stability studied and C4 and C5 were thermodynamically unstable. In Smix ratio 3:1, D1 and D2 formulations were found to be stable and the remaining formulations are thermodynamically unstable. Those

formulations, which survived stability tests, are reported in Table 2; were taken for *in vitro* permeation study.

Table 2: Data of selected composition and stability study.

Formulation	**Formulation (% w/w)**				**Observations- Thermodynamic stability studies.**			**Inference**
Smix ratio: 1:0								
Batch	**NF.**	**Oil**	**S_{mix}**	**Aq.**	**H/C**	**Cent.**	**Freez. Tha.**	
A_1	0.5	20	50	30	√	×	-	Failed
A_2	0.5	17	43	40	√	×	-	Failed
A_3	0.5	12	38	50	√	√	×	Failed
A_4	0.5	08	32	60	√	√	×	Failed
A_5	0.5	05	25	70	×	-	-	Failed
Smix ratio: 1:1								
B_1	**0.5**	**22**	**48**	**30**	√	√	√	**Passed**
B_2	**0.5**	**18**	**42**	**40**	√	√	√	**Passed**
B_3	0.5	13	37	50	×	-	-	Failed
B_4	0.5	10	30	60	×	-	-	Failed
B_5	0.5	05	25	70	×	-	-	Failed
Smix ratio: 2:1								
C_1	**0.5**	**16**	**44**	**40**	√	√	√	**Passed**
C_2	**0.5**	**14**	**36**	**50**	√	√	√	**Passed**
C_3	**0.5**	**10**	**30**	**60**	√	√	√	**Passed**
C_4	0.5	07	23	70	√	×	-	Failed
C_5	0.5	04	16	80	√	×	-	Failed
Smix ratio: 3:1								
D_1	**0.5**	**23**	**47**	**30**	√	√	√	**Passed**
D_2	**0.5**	**20**	**40**	**40**	√	√	√	**Passed**
D_3	0.5	15	35	50	×	-	-	Failed
D_4	0.5	12	28	60	×	-	-	Failed
D_5	0.5	09	21	70	×	-	-	Failed

NF. : Nifedipine, **Cent.:** Centrifugation. **Freez. Tha. :** Freeze-thaw cycle. **H/C:** Heating cooling cycle.

3.4.2. *In-vitro* skin permeation study:

The penetration capacity of various microemulsions was tested using *in vitro* skin permeation tests. The permeation parameters and percentage of drug permeation of the tested microemulsion and control formulations were presented in Figures 5 and 6. The steady concentration of Nifedipine increases in the receptor chamber was observed over time. The two principal factors govern the penetration of Nifedipine from the microemulsion water content and the Smix ratio. It is clear from figures5 and 6, the B1 formulation shows the lowest % of drug permeation through the skin (75.65 ± 2.33 % at 24 hrs.) and lowest flux (0.328 ± 1.00 mg/cm^2/h) because of the high concentration of Smix (48 %w/w) in B1 formulation and low concentration of water (30 %w/w). On the other side, C3 and C2 show the highest % of drug permeation (97.14 ± 1.26 and 95.63±2.30 % at 24 hrs., respectively) and highest flux (0.408 ± 0.08 and 0.397 ± 0.08 mg/cm^2/h respectively), because of the lowest amount of Smix (30 and 36 % w/w respectively) and the highest amount of the water (60 and 50 %w/w respectively). As the content of Smix was decreased from 48 to 30 %w/w, the skin permeation rate of Nifedipine was increased. The increased permeation rate of the drug because of decreased concentration of Smix in the microemulsion and increased thermodynamic activity of the drug in the microemulsion. [15]. The % of drug permeation of control Nifedipine solution has 45.45 ± 2.49 % and the flux of control Nifedipine solution has 0.185 ± 0.34 mg/cm2/h. These differences indicate that the microemulsion good permeation enhancer.

From the results B2, C2, C3, and D2 show the highest % permeation of Nifedipine (Figure 5 & 6), based on the objective of the research, preparation of microemulsion in a minimum amount of Smix concentration and % of Nifedipine permeation, those formulations show the highest permeation, these are selected for preparation of microemulsion gel, these formulations are B2, C2, C3, and D2 and further coded as M1, M2, M3, and M4 respectively.

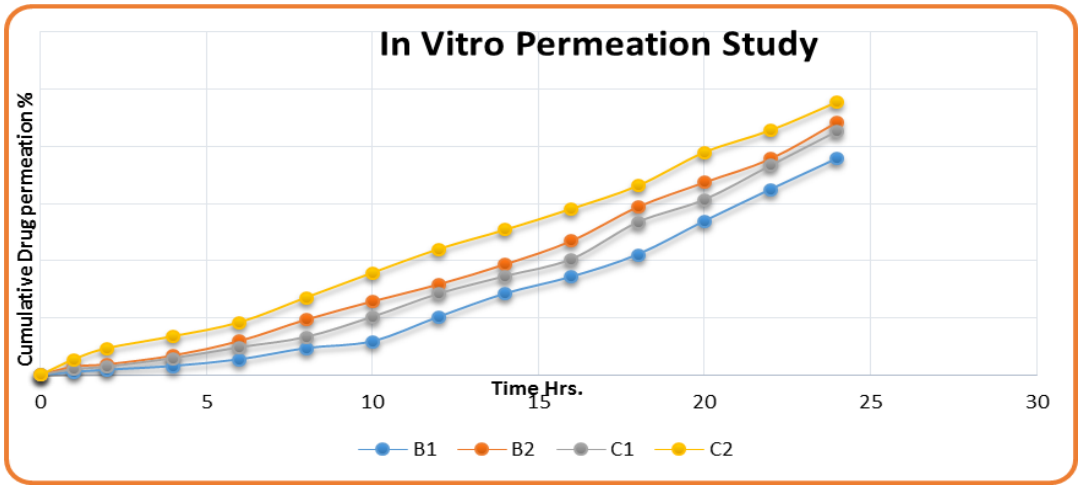


Figure 5: *In vitro* permeation study of formulations B1, B2, C1, and C2.

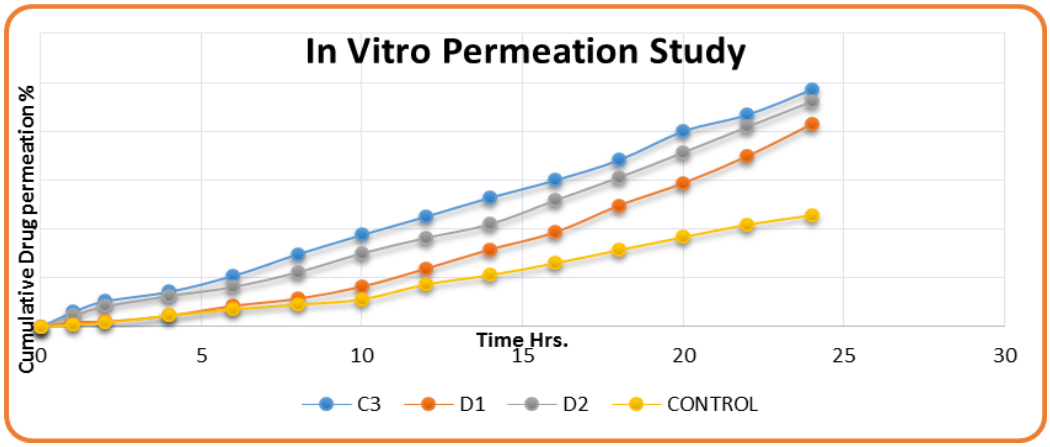


Figure 6: *In vitro* permeation study of formulations C3, D1, D2, and Control Solution.

Table 3: Data of Permeation parameter.

Formulation	***Flux (Jss) ($mg/cm^2/h$)**	***Permeability coefficient (Kp) (cm/h)**	***Enhancement ratio**
B1	0.328 ± 1.00	0.0328 ± 0.02	1.77
B2	0.382 ± 0.34	0.0382 ± 0.04	2.06
C1	0.370 ± 0.11	0.0370± 0.04	2.00
C2	0.397 ± 0.08	0.0397 ± 0.06	2.14
C3	0.408 ± 0.08	0.0408 ± 0.02	2.20
D1	0.354 ± 0.56	0.0354 ± 0.01	1.91
D2	0.388 ± 0.11	0.0388 ± 0.04	2.09
CONTROL	0.185 ± 0.34	0.0185 ± 0.01	-

*Represents mean ± S.D. (n = 3**)**

3.5 Preparation of microemulsion gel:

3.5.1 Optimization of concentration of carbopol 934:

Carbopol 934 was selected as the gel matrix to prepare a micro emulsion-based hydrogel formulation. The concentration of Carbopol 934 was optimized based on the consistency of the microemulsion gel. Microemulsion gel containing 0.5 and 0.75 % w/w carbopol 934 had relatively high fluidity. However, 1.5 %w/w carbopol 934 resulted in a highly viscous microemulsion gel. Microemulsion gel containing 1 %w/w carbopol 934 had almost optimum consistency for transdermal application. Hence 1 %w/w Carbopol 934 was selected as the gel matrix to prepare the microemulsion gel formulation.

3.5.2 Preparation of microemulsion gel of Nifedipine and control Nifedipine gel:

Microemulsion gel and control gel of Nifedipine is prepared as per the reported procedure in Section 2.7.1 and 2.7.1. Formulation of microemulsion gel is reported in table no 4.

Table 4: Optimize microemulsion and microemulsion gel formulation.

%w/w of different components in the formulation										
Microemulsion					**Microemulsion gel**					
	NF	**Oil**	**Smix**	**Aq.**		**NF**	**oil**	**Smix**	**Aq.**	**carbopol**
M1	0.5	5	35	59.5	**MG1**	1.5	5	35	58.5	1
M2	0.5	4	26	69.5	**MG2**	1.5	4	26	68.5	1
M3	0.5	5	45	49.5	**MG3**	1.5	5	45	48.5	1
M4	0.5	5	25	69.5	**MG4**	1.5	5	25	68.5	1

NF: Nifedipine; Aq**:** Water.

3.6 Characterization of Nifedipine loaded microemulsion and microemulsion gel:

3.6.1 pH measurement:

The human skin has a pH between 4 to 6. The pH of microemulsion and microemulsion gels was found in between 4 to 6 indicating suitability for skin application. The pH of microemulsion and microemulsion gel formulations are given in Table 5.

3.6.2 Viscosity Determination:

The microemulsion of Nifedipine showed viscosities in the range of 20 - 32 cP. This indicates that as the concentration of water increases the viscosity of the formulation decreases. The microemulsion gel of Nifedipine showed viscosities in the range of 16890 - 17724 cP which indicates that the concentration of carbopol 934 (1 %w/w) increases the viscosity of formulation as compared to microemulsion; which made the preparation more suitable for transdermal administration. The viscosity of microemulsions and gel is shown in Table 5.

Table 5: pH and viscosity of formulations

❖ **Microemulsion**			❖ **Microemulsion Gel**		
Formulation	***pH**	***Viscosity (cP)**	**Formulation**	***pH**	***Viscosity (cP)**
M_1	4.5 ± 0.04	31.22 ± 1.25	**MG_1**	5.6 ± 0.07	17724 ± 0.88
M_2	4.7 ± 0.10	21.46 ± 1.54	**MG_2**	5.9 ± 0.04	16930 ± 1.67
M_3	4.1 ± 0.05	20.55 ± 0.99	**MG_3**	5.3 ± 0.12	16890 ± 1.11
M_4	4.7 ± 0.09	27.84 ± 1.05	**MG_4**	5.8 ± 0.06	17250 ± 1.23

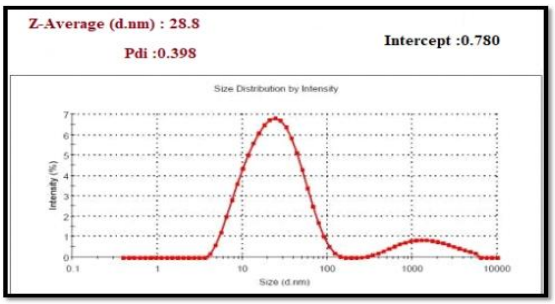
*Represents mean ± S.D. (n = 3**)**

3.6.3 Droplet Size:

Determination of the droplet size of the microemulsion is very important because the stability of a formulation depends on the droplet size of the formulation. Also, the advantage of the small droplet size is very important in drug delivery as the oil droplet tends to come into contact with the skin thus providing a delivery pathway through the skin [15]. Droplet sizes of the formulations are shown in Table 6 and Figures 7 to 10. According to the above results, the mean droplet size of the formulation increases with an increase in water content in the formulation and decreases Smix concentration due to the effective interfacial activity [18].

Table 6: Data of droplet size

Microemulsion	
Formulation	**Average droplet size (nm)**
M1	28.8
M2	37.6
M3	42.6
M4	34.4



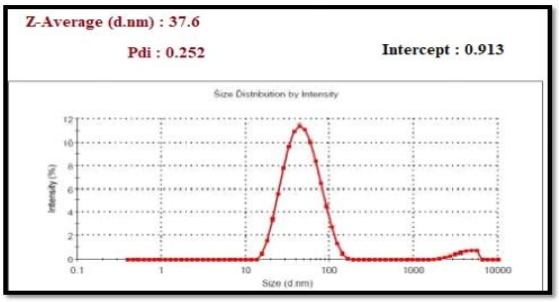
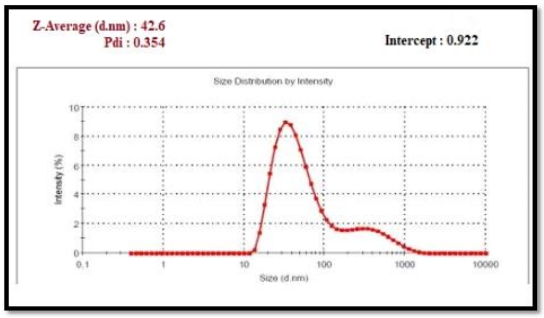


Figure 7: Droplet size of formulation M1.**Figure 8**: Droplet size of formulation M2



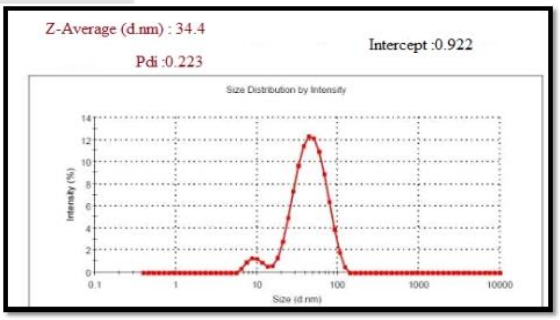


Figure 9: Droplet size of formulation M3.**Figure 10**: Droplet size of formulation M4.

3.6.4 Spreadability:

A large diameter indicates better spreadability. The developed formulations had adequate spreadability indicating ease of application [19]. From the spreadability study, it was noticed that as the viscosity of the formulation increases the spreadability decreases. The spreadability of the formulation gel is shown in **Table 7.**

Table 7: Result of spreadability study.

Microemulsion Gel	
Formulation	***Spreadability (cm)**
MG1	7.2 ± 0.87
MG2	6.1 ± 0.34
MG3	5.7 ± 1.65
MG4	6.8 ± 0.99

*Represents mean ± S.D. (n = 3**)**

3.6.5. Stability studies of Microemulsion gel [26]**:**

All microemulsion gel was stable at 30 ^{0}C in the presence of Nifedipine (Table 8). No significant change in drug content, no phase separation, and clarity were observed during 3 months. The centrifuge tests showed that all microemulsions gel had good physical stability. Microemulsion gel still maintains good thermodynamic stability similar to microemulsion.

Table 8: Data of stability study.

Stability of Microemulsion gel determination 30 ◦C for up to 3 months.					
Formulation	**Clarity**	**Phase separation**	**Drug content (%)**	**Centrifuge test**	**Inference**
MG1	√	No	98.99	√	Passed
MG2	√	No	97.00	√	Passed
MG3	√	No	97.24	√	Passed
MG4	√	No	98.33	√	Passed

3.6.6. Skin irritation study

The microemulsion and microemulsion gel was applied (24hrs.) to the albino Wistar rat's skin and erythema was compared with skin sensitization visual score. No erythema was found after 24 hrs. when responses of the animal under test were observed visually and compared with standard irritant animals (Figure 11). This indicated that the microemulsion and microemulsion gels were nonirritant and never produced erythema after application. Thus the formulations were safe for transdermal application.

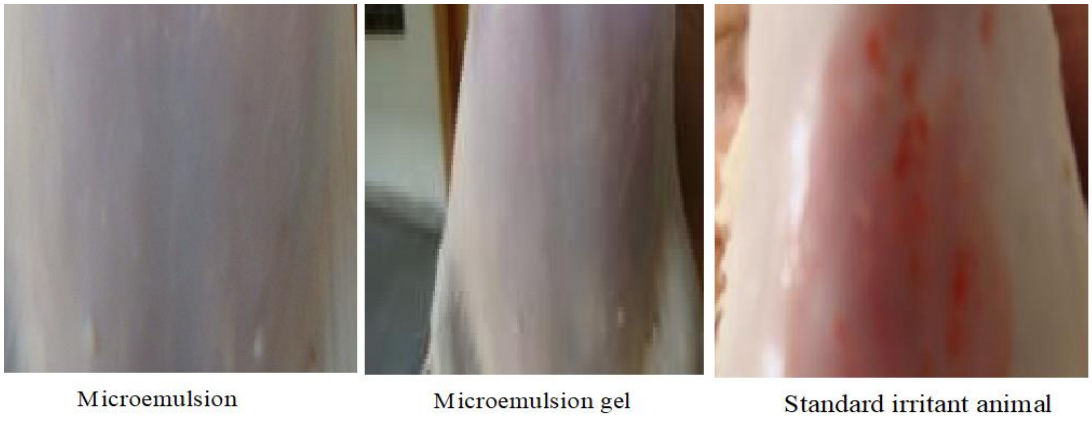


Figure 11: Skin Irritation Study

3.6.7.*In-vitro* skin permeation study of microemulsion gel:

The concentration of Nifedipine in the receptor chambers increased steadily with time. That the Control gel shows the lowest % of drug permeation (31.45 ± 2.81 % at 24 h) and lowest flux (0.129 ± 0.55 mg/cm^2/h), on the other side MG3 and MG2 shows highest % drug permeation (80.99 ± 1.39 and 77.23 ± 2.48 % at 24 h respectively) and highest flux (0.373 ± 0.75 and 0.347 mg/cm^2/h respectively). As compared to microemulsion formulation to microemulsion gel % of drug permeation and flux are significantly changed. In microemulsion (C3 Formulation) highest % of drug permeation is 97.14 ± 1.26 %This is because adding carbopol 934 to a microemulsion reduced Nifedipine permeability, which might be due to increased viscosity and the transition from a microemulsion to a lamellar structure or a highly ordered microstructure [18].

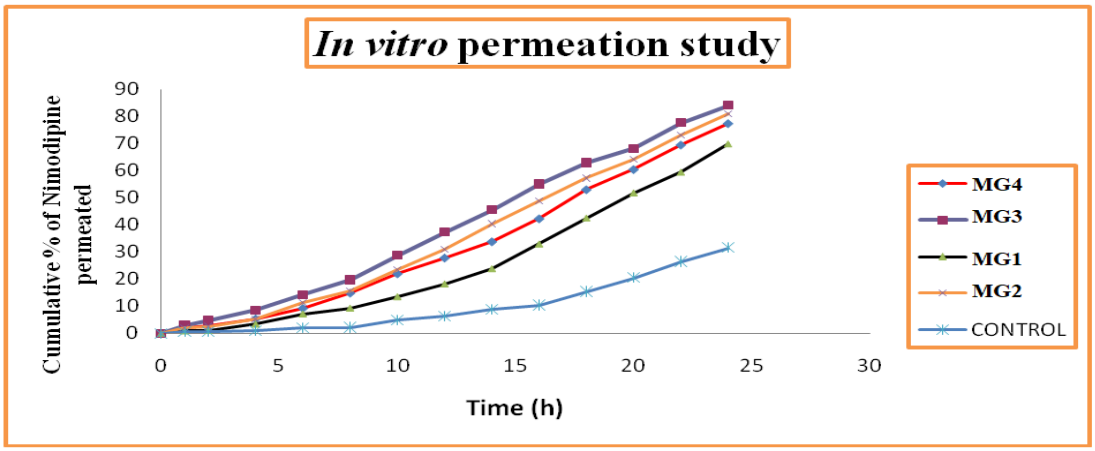


Figure 12: In vitro permeation study of microemulsion gel formulations.

Table 9: Permeation parameter of microemulsion gel.

Formulation	*Q_{24} (mg)[A]	*Flux (Jss) (mg/cm^2 / h)	*Permeability coefficient (Kp) (cm^2/h)	Enhancement ratio (Er)
MG1	6.434±0.65	0.295±0.49	0.0295±0.49	2.16
MG2	7.723 ±0.45	0.369±0.28	0.0369±0.28	2.71
MG3	8.099±0.31	0.390±0.54	0.0390±0.54	2.86
MG4	6.988 ±0.32	0.315±0.22	0.0315±0.22	2.31
CONTROL	3.145 ±1.01	0.136±0.99	0.0136±0.99	

*****Represents mean ± S.D. (n = 3**).**

4. CONCLUSION

Suitable excipients were selected from the solubility study. From pseudo ternary phase diagrams, the concentration of oil phase; Smix, and distilled water were determined. Formulated Nifedipine microemulsion optimized by stability study and in vitro permeation study and further processed for the formulation of Nifedipine microemulsion gel. It was found that the pH of all the formulations was in the range of 4 to 6 which suits the skin pH, indicating skin compatibility. Droplet Size of Nifedipine microemulsion formulations was found between 28 to 35 nm Stability studies suggested that Nifedipine loaded microemulsion and microemulsion gel formulations were stable at all stability conditions. A skin irritation study suggested that Nifedipine microemulsion and microemulsion gel formulation were nonirritating and did not cause erythema. In Nifedipine loaded microemulsion gel, MG3 and MG2 show the highest % drug permeation,

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6. REFERENCES:

1. Veronica R C, Gary K D, Sereima C, Parshu R, Paul Z Z. High Mortality from Cardiovascular Disease and Analysis of Risk Factors in Indian and Melanesian Fijians.Int. J. Epidemiol. 1996; 25(1): 59–69.
2. Chong S and Fung H L, In Hadgraft J and Guy R H. Transdermal Drug Delivery: Developmental Issues and Research Initiatives. 3rd ed. Marcel Dekker, New York, 1989.
3. R Payne, S D Mathias, D J Pasta , L A Wanke , R Williams , R Mahmoud. Quality of life and cancer pain: satisfaction and side effects with transdermal fentanyl versus oral morphine. J. Clin. Oncol. 1998; 16(4): 1588-1593.
4. https://medlineplus.gov/druginfo/meds/a684028.html

5. Hermann P, Morselli PL. Pharmacokinetics of diltiazem and other calcium channel entry blockers. Acta Pharmacol Toxicol. 1985; 579(2): 10–20.
6. Robertson DR, Walker DG, Renwick AG, George C F. Agerelated changes in the pharmacokinetics and pharmacodynamics of nifedipine. Br J Clin Pharmacol. 1988; 25(3):297–305.
7. S A Giannakou, P P Dallas, D M Rekkas, N H Choulis. Development and in vitro evaluation of nimodipine transdermal formulations using factorial design.Pharm Dev Technol. 1998; 3(4), 517–525.
8. Berba J, Benakar U.Clinical Efficacy of Current Transdermal Drug Delivery Systems: A Retrospective Evaluation: Basic and clinical research on this technology shows that the system development process needs serious review and revision. APhA. 1990;30(11): 33–41.
9. R C Wester 1, H I Maibach, L Sedik, J Melendres, S DiZio, M Wade. In vitro percutaneous absorption of cadmium from water and soil into human skin. Fundam Appl Toxicol. 1992; 19(1), 1-5.
10. Kohri N, Miyazaki K, K IchiMori, Arita T. Release Characteristics of Nifedipine Sustained-Release. Chem Pharm Bull. 1987; 35: 2504–2504.
11. Prausnitz MR, Mitragotri S, Langer R. Current status and future potential of transdermal drug delivery. Nat Rev Drug Discov. 2004; 3: 115–24.
12. McDaid D M, Deasy P B. An investigation into the transdermal delivery of Nifedipine. Pharmaceutics Acta Helvetiae. 1996; 71:253–258.
13. Yue Yuan, San-ming Li, Feng-kui Mo, Da-fang Zhong. Investigation of microemulsion system for transdermal delivery of meloxicam. Int. J. Pharm. 2006; 321(1): 117–123.
14. Sheikh Shafiq., Faiyaz Shakeel., Sushma Talegaonkar., Farhan J. Ahmad., Roop K. Khar., Mushir Ali. Development and bioavailability assessment of ramipril nanoemulsion formulation. Eur. J. Pharm. and Biopharm. 2007; 66: 227–243.
15. Amrish Chandra., pramod kumar Sharma., Raghuveer Irchhiaya. Microemulsion-based hydrogel formulation for transdermal delivery of dexamethasone. Int. J. Pharma. 2009; 30-36.
16. Lee, J., Lee, Y., Kim, J., Yoon, M., Choi, Y.W. Formulation of microemulsion Systems for Transdermal Delivery of Aceclofenac. Arch Pharm Res.2005;28(9):1097 -1102.
17. Weiwei Zhua., Chenyu Guoa., Aihua Yua., Yan Gaoa., Fengliang Caoa., Guang Xi Zhai. Microemulsion-based hydrogel formulation of penciclovir for topical delivery. Int. J. Pharm. 2009; 378:152–158.
18. Huabing Chen, Dongsheng Mou, Danrong Du, Xueling Chang, Dandan Zhu, Jie Liu, Huibi Xu, Xiangliang Yang.Hydrogel-thickened microemulsion for topical administration of drug molecule at an extremely low concentration. Int. J. Pharm;2007;341(1-2):78-84
19. Joshi, M, Patravale V. Formulation and Evaluation of Nanostructured Lipid Carrier (NLC)-based Gel of Valdecoxib. Drug Dev Ind Pharm. 2006;32(8):911-918.
20. Srinivas Mutalik., Nayanabhirama Udupa. Formulation development, in vitro and in vivo evaluation of membrane controlled transdermal system of gibenclamide. J Pharm Pharm Sci. 2005;8(1):26-38.
21. Brajesh Kumar, Sanjay Kumar Jain, Sunil Kumar Prajapati, Alok Mahor and Ajay Kumar.Development and Characterization of Transdermal Microemulsion Gel for an Antiviral Drug.Int. j. pharm. sci. 2010;1(6);57-74.
22. Lee J.M., Park K.M., Lim S.J., Lee M.K., Kim C.K. Microemulsion formulation of clonixic acid: solubility enhancement and pain reduction. J Pharm Pharmacol. 2002 Jan;54(1):43-9.
23. Wade A, Weller P. Handbook of pharmaceutical excipients. 2nd ed.The pharmaceutical press; 1994.