IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
April 2022 Vol.:24, Issue:1

Design and Development of Amlodipine Microemulsion Gel for Transdermal Delivery

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Prashant Khade[*1]

[1]*Research Scholar, Institute of Pharmacy, Shri JJT University, Jhunjhunu, Rajasthan, India.*

[*1]*Assistance Professor, Pune District Education Association's, Shankarrao Ursal College of Pharmaceutical Sciences and Research Centre, Kharadi, Pune-14. India.*

Submitted: 23 March 2022
Accepted: 28 March 2022
Published: 30 April 2022

Keywords: Microemulsion, Transdermal delivery, Amlodipine, Permeation rate

ABSTRACT

The objective of this research was to design a new microemulsion gel for transdermal delivery of Amlodipine. For its good solubilizing capacity and skin penetration capability, Labrafil M 2125, Tween 60, and PEG-400 were screened as oil, surfactant, and co-surfactant respectively from the solubility study. From pseudo ternary phase diagrams, the concentration of oil phase, Smix, and distilled water was determined and further processed for the formulation of Amlodipine microemulsion and gel. Amlodipine loaded microemulsion optimized by stability study and *in vitro* permeation study. 0.75 percent w/w Carbopol was used to develop a microemulsion gel formulation. The in vitro permeation data showed that microemulsion gel increased the permeation rate (0.179±0.30 mg/cm2/h) of Amlodipine compared with the control solution (0.060±0.07 mg/cm2/h). The studied microemulsion gels were stable toward all stability conditions. A skin irritation study suggested that Amlodipine loaded microemulsion and microemulsion gel formulations were nonirritating and did not cause erythema. Thus, microemulsion gel systems are a promising formulation for the transdermal delivery of Amlodipine.

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].

Amlodipine belongs to the 1,4-dihydropyridine categories of Calcium Channel Blockers and is structurally related to Nifedipine. The clinical pharmacology of the antihypertensive action of amlodipine involves a direct relaxant effect on vascular smooth muscle. Amlodipine is given orally (5 mg daily), with peak plasma concentration occurring after 6-12 h. It has a favorable logP of 2.22 and oral bioavailability of 60-65% due to extensive hepatic metabolism. The above limitation associated with conventional formulations of Amlodipine can be circumvented by transdermal delivery [4, 5, 6].

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 some 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].

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 [10].

In this study, O/W microemulsion gel containing 0.25 % Amlodipinehas 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 Amlodipine.

2. MATERIALS AND METHODS

2.1 MATERIALS

Amlodipine 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 Carbitolwere 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 [11]:

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 Amlodipine 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°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 Amlodipine was determined in oils, surfactants, cosurfactants, and water using a UV spectrophotometer at 237 nm.

2.3 Pseudo Ternary Phase Diagram Studies [11]:

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 an Amlodipine microemulsion.

2.4 Selection Microemulsion Formulations from Phase Diagrams [11]**:**

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 addition of a 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 Amlodipine loaded microemulsion, and control solution:

Microemulsion: Amlodipine (0.25 %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 Amlodipine was obtained by stirring the mixtures. All microemulsions were stored at 30 ± 2^{o}C [15]. Compositions of Amlodipine-loaded microemulsions are reported in Table 2.

Control solution: The control solution of Amlodipine was prepared by dissolving 0.25 gm in 100 mL of ethanol [12].

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[11]**:**

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 [13]**:**

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[13]**:**

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 5 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 cells 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 Figure 5.

2.6.2.3. Data analysis of skin permeation :

Flux [13]**:** 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 Amlodipine permeating through the rat skin was plotted as a function of time. The permeation rate of Amlodipine 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 [13]**:** 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)[12]: The enhancement ratio was calculated by dividing the Jss of the respective formulation by the Jss of the control formulation.

Er = J_{ss} of formulation / J_{ss} 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) [12, 14, 15**]:**

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 Amlodipine 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 Amlodipine and control Amlodipine gel [12, 14, 15]**:**

The method of preparation is reported in section 2.7.1.

2.7.3. Preparation of control Amlodipine gel:

Control Amlodipine 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, 0.25 gm of Amlodipine 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 0.25 % w/w Amlodipine. Then pH

was subsequently adjusted to 4-6 using triethanolamine solution (0.5 %v/v) to obtain Amlodipine gel.

2.8 Characterization of Amlodipine 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 [16]**:**

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 [17]**:**

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 [16]:

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

2.8.6 Skin irritation study [12, 17]:

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 Amlodipine 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 [17]. Table 1 demonstrated that solubility of the lipophilic drug – amlodipine – was found to be highest in Labrafil M 2125 CS (Linoleoyl macrogol- 6 glycoside) followed by Isopropyl Myristate and Labrafil M 1944 CS (Oleoyl macrogol- 6 glycoside). Solubility of the drug in these oils was significantly high than in Capmul PG-8 and Oleic acid. All the surfactants showed good solubility of the drug (Table 1). The solubility of Amlodipine was found to be highest in Tween 60. Tween 60 was selected as a surfactant because of the HLB value of 14.9, Non-ionic surfactants are less toxic than ionic surfactants, have good biological acceptance, powerful permeate enhancers, and highest solubility of Amlodipine [18,19]. Polyethylene Glycol 400 (PEG 400) was selected as a co-surfactant because of its potential to solubilize the drug. Amlodipine was show highest solubility in PEG-400 as compared to other cosurfactants. 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 [20]. Labrafil M 2125, Tween 60, and PEG-400 were selected as oil, surfactant, and cosurfactant respectively.

Table 1: Solubility study of Amlodipine in Various Excipients

Sr. No.	**Excipients**	***Solubility of Amlodipine (mg/ml) at 25 º C)**
Oils		
1.	Labrafil M 1944 CS	10.24 ±1.23
2.	Isopropyl Myristate	11.83 ±1.40
3.	**Labrafil M 2125 CS**	**17.00 ±2.68**
4.	Capmul PG 8	9.2±1.23
5.	Oleic acid	6.16 ±2.24
6.	Castor Oil	7.00 ±1.54
7.	Coconut Oil	10.02 ±2.01
8.	Lemon Oil	06.92 ±1.74
9.	Arachis Oil	11.75 ±0.99
Surfactants		
10.	Span 20	115.93 ±3.42
11.	Span 60	69.34 ±2.06
12.	Span 80	74.5 ±2.33
13.	Tween 20	90.12 ±6.74
14.	**Tween 60**	**120.68 ±7.25**
15.	Tween 80	97.34 ± 0.25
16.	Cremophor RH 40	54.87 ± 3.25
Cosurfactants		
17.	Ethanol	160.95 ±5.04
18.	PEG 200	204.82 ±5.75
19.	**PEG 400**	**228.95 ±5.39**
20.	PEG 600	154.16 ±4.49
21.	Capmul MCM	64.46 ±4.66
22.	Captex 500	11.66 ± 1.53

* 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 [11]. 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 Labrafil M 2125 CS such as oil and the Tween 60: PEG 400 fixed mixing ratio as Smix. It can be also seen that the microemulsion region exists at S_{mix} ratio 1:0 (i.e.

without co-surfactant). However, an equal mixture of surfactant and co-surfactant decreases the microemulsion region. Increasing the concentration of surfactant (2:1) resulted in an even larger area of the microemulsion region. Further increasing surfactant concentration from 2:1 to 3:1 resulted in no influence on the microemulsion region.

The existence of a large or small microemulsion region depends on the capability of a particular surfactant or surfactant mixture to solubilize the oil phase. The extent of solubilization resulted in a greater area with a clearer and homogenous solution. It was seen that when the surfactant (Tween 60) was used alone, the oil phase was solubilized to a lesser extent at a higher concentration of surfactant implying that surfactant alone was not able to reduce the interfacial tension of oil droplet to a sufficiently low level and thus was not able to reduce the free energy of the system to an ultra-low level desired to produce microemulsions. When a co-surfactant was added, the interfacial tension was reduced to a very low level and very small free energy was achieved which helps in the increased solubilization capacity of oil.

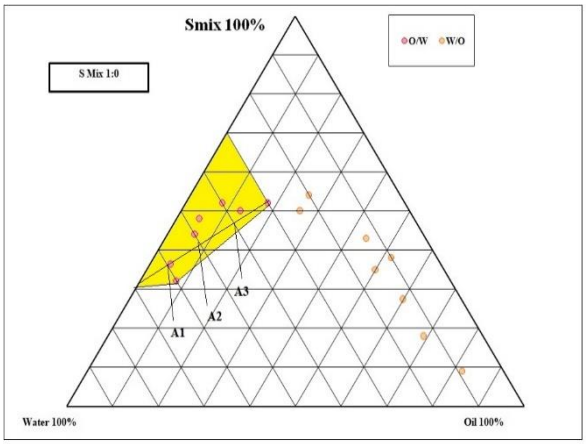


Figure 1: Microemulsion regions of ratio 1:0

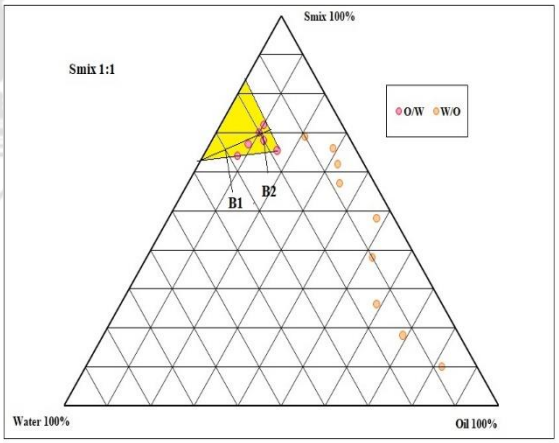
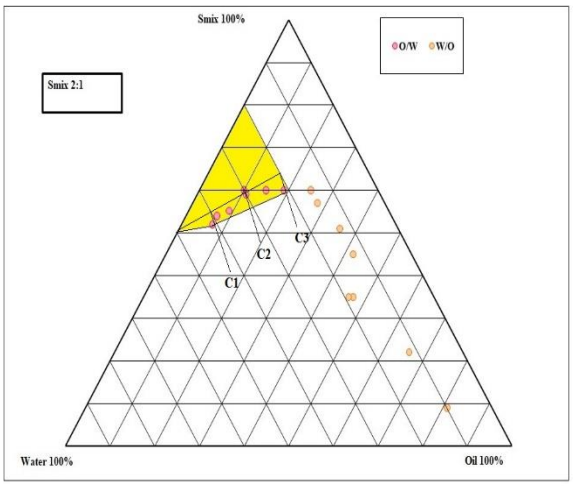


Figure 2: Microemulsion regions of ratio 1:1



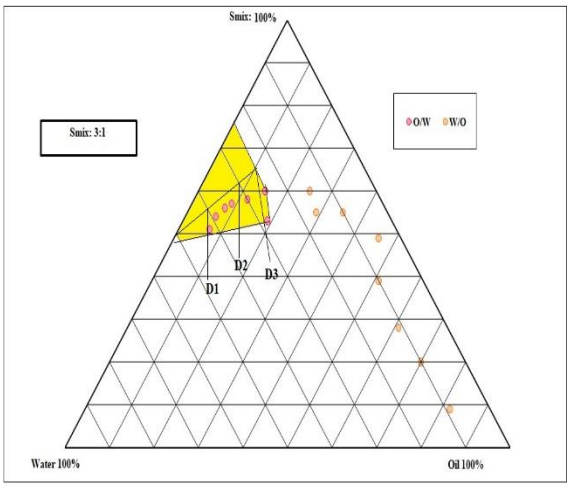


Figure 3: Microemulsion regions of ratio 2:1**Figure 4:** Microemulsion regions of ratio 3:1

3.3. Selection of formulations from phase diagrams:

The selected concentration of the oil phase is between 7 % w/w to 20 % 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 Amlodipine (5 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 [11].

3.4. Optimization of microemulsion formulation:

The Amlodipine (0.25 %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:

Microemulsions are thermodynamically stable systems and are formed at a particular concentration of oil, surfactant, and water, with no phase separation, creaming, or cracking. It is the thermostability that differentiates microemulsion from emulsions that have kinetic stability and will eventually phase separate [21]. Thus, the selected formulations were

subjected to different thermodynamic stability testing by using heating-cooling cycle, centrifugation, and freeze-thaw cycle stress tests. Those formulations, which passed thermodynamic stability tests, were taken for *in vitro* permeation study. Those formulations that survived the stability study are reported in Table 2.

Thus it was concluded that the efficiency of the surfactant and co-surfactant mixture was affected after exposure to extreme conditions. In Smix ratios 1:0 and 1:1, all selected formulations were found to be thermodynamically unstable as compared to Smix ratios 2:1 and 3:1. In Smix ratios 2:1 and 3:1, all selected formulations are thermodynamically stable and these are selected for further studies.

Table 2: Data of selected composition and stability study.

Formulation	**Formulation (% w/w)**				**Observations-Thermodynamic stability studies.**			**Inference**
Smix ratio: 1:0								
Batch	**AM.**	**Oil**	**S_{mix}**	**Aq.**	**H/C**	**Cent.**	**Freez. Tha.**	
A_1	0.25	5	35	59.75	√	×	-	Failed
A_2	0.25	8	42	49.75	√	-	-	Failed
A_3	0.25	15	45	39.75	×	-	-	Failed
Smix ratio: 1:1								
B_1	0.25	5	65	29.75	√	×	-	Failed
B_2	0.25	10	70	19.75	√	×	-	Failed
Smix ratio: 2:1								
C_1	0.25	5	55	39.75	√	√	√	**Passed**
C_2	0.25	10	60	29.75	√	√	√	**Passed**
C_3	0.25	15	65	19.75	√	√	√	**Passed**
Smix ratio: 3:1								
D_1	0.25	5	55	39.75	√	√	√	**Passed**
D_2	0.25	8	62	29.75	√	√	√	**Passed**
D_3	0.25	10	65	24.75	√	√	√	**Passed**

AM.: Amlodipine, **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 Figure 5. The steady concentration of Amlodipine increases in the receptor chamber was observed over time. The two principal factors govern the penetration of Amlodipine from the microemulsion water content and the Smix ratio. It is clear from figure 5, the C3 formulation shows the lowest % of drug permeation through the skin (79.77±2.28 % at 24 hrs.) and lowest flux (0.1614 mg/cm^2/h) because of the high concentration of Smix(65 %w/w) and low concentration of water (20 %w/w).On the other side, C1 and D1 show the highest % of drug permeation(96.49±2.38 and 94.00±2.67 % at 24 hrs., respectively)and highest flux (0.2004 and 0.1904 mg/cm^2/h respectively), because of the lowest amount of Smix (55 % w/w) and the highest amount of the water (40 %w/w). As the content of Smix was decreased from 65 to 55 %w/w, the skin permeation rate of Amlodipine 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[12]. The % of drug permeation of control Amlodipine solution has 44.23±1.67 and the flux of control Amlodipine solution has 0.0931 mg/cm2/h. These differences indicate that the microemulsion good permeation enhancer.

From the results C1, C2, D1, and D2 show the highest % permeation of Amlodipine (Figure 5), based on the objective of the research, preparation of microemulsion in a minimum amount of Smix concentration and high % of Amlodipine permeation, those formulations show the highest permeation, these are selected for preparation of microemulsion gel.

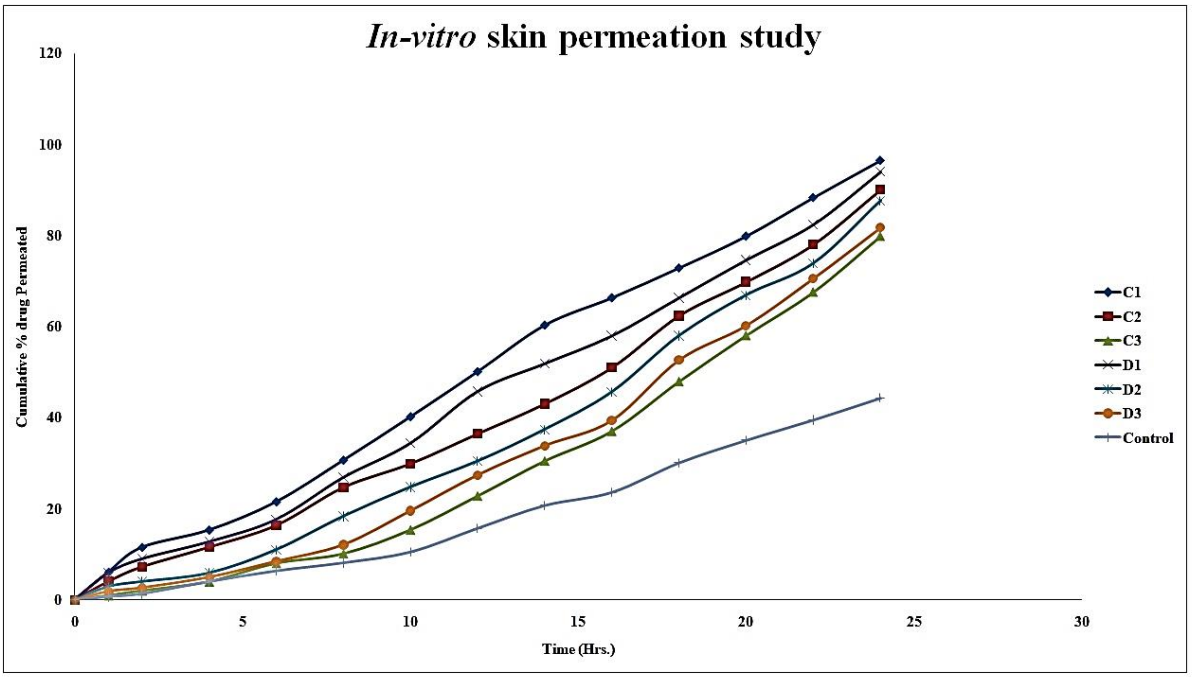


Figure 5: *In vitro* permeation study of formulations and control solution.

Table 3: Data of Permeation parameter.

Formulation	Flux (Jss) ($mg/cm^2/h$)	Permeability coefficient (Kp) (cm/h)	Enhancement ratio
C1	0.2004	0.040	2.15
C2	0.1802	0.036	1.93
C3	0.1614	0.032	1.73
D1	0.1904	0.038	2.04
D2	0.1778	0.0035	1.90
D3	0.1672	0.033	1.79
CONTROL	0.0931	0.018	-

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 carbopol 934 had relatively high fluidity. However,1 %w/w and 1.5 %w/w carbopol 934 resulted in a highly viscous microemulsion gel. Microemulsion gel containing 0.75 % w/w carbopol 934 had almost

optimum consistency for transdermal application. Hence 0.75 % w/w Carbopol 934 was selected as the gel matrix to prepare the microemulsion gel formulation.

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

Microemulsion gel and control gel of Amlodipine 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**					
	AM	**Oil**	**Smix**	**Aq.**		**AM**	**oil**	**Smix**	**Aq.**	**Carbopol**
C1	0.25	5	55	39.75	**MC1**	0.25	5	55	39	0.75
C2	0.25	10	60	29.75	**MC2**	0.25	10	60	29	0.75
D1	0.25	5	55	39.75	**MD1**	0.25	5	55	39	0.75
D2	0.25	8	62	29.75	**MD2**	0.25	8	62	29	0.75

AM: Amlodipine; Aq**:** Water.

3.6 Characterization of Amlodipine 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 Amlodipine showed viscosities in the range of 33 to 39 cP. This indicates that as the concentration of water increases the viscosity of the formulation decreases. The microemulsion gel of Amlodipine showed viscosities in the range of 18255 - 19373 cP which indicates that the concentration of carbopol 934 (0.75 %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)**
C1	4.9 ± 0.04	33.27 ± 1.05	**MC1**	6.1 ± 0.07	18255 ± 2.38
C2	4.3 ± 0.10	37.78 ± 1.28	**MC2**	5.8 ± 0.04	19001 ± 3.81
D1	4.7 ± 0.05	35.95 ± 1.36	**MD1**	6.0 ± 0.12	18876 ± 2.82
D2	4.2 ± 0.09	38.98 ± 0.98	**MD2**	5.3 ± 0.06	19373 ± 3.49

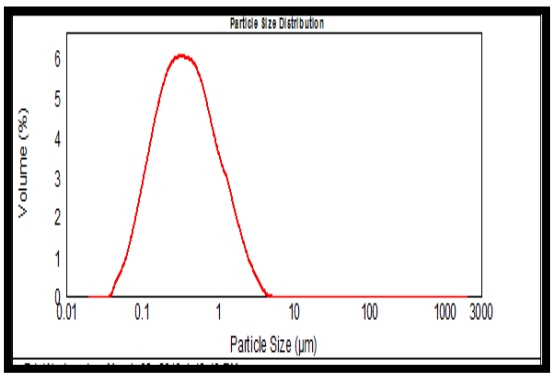
*Represents mean ± S.D. (n = 3**)**

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 [12]. Droplet sizes of the formulations are shown in Table 6 and Figures 6 to 9. 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 [15].

Table 6: Data of droplet size

Microemulsion	
Formulation	**Average droplet size (µm)**
C1	0.366
C2	0.315
D1	0.348
D2	0.306



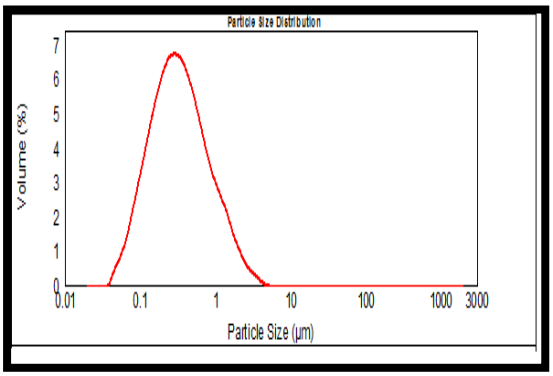
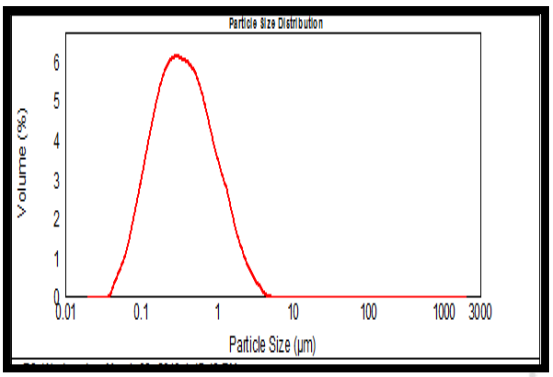


Figure 6: Droplet size of formulation C1.**Figure 7**: Droplet size of formulation C2



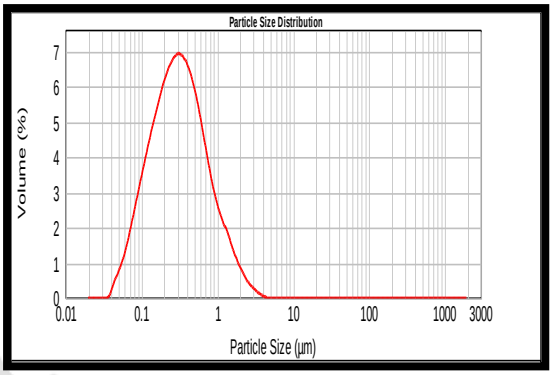


Figure 8: Droplet size of formulation M3.**Figure 9**: Droplet size of formulation M4.

3.6.4 Spreadability:

A large diameter indicates better spreadability. The developed formulations had adequate spreadability indicating ease of application [16]. 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)**
MC1	7.2 ± 0.87
MC2	6.1 ± 0.34
MD1	5.7 ± 1.65
MD2	6.8 ± 0.99

*Represents mean ± S.D. (n = 3**)**

3.6.5. Stability studies of Microemulsion gel [22]:

All microemulsion gel was stable at 30 ^{0}C in the presence of Amlodipine (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**
MC1	√	No	98.99	√	Passed
MC2	√	No	97.00	√	Passed
MD1	√	No	97.24	√	Passed
MD2	√	No	98.33	√	Passed

3.6.6. Skin irritation study

The microemulsion and microemulsion gel was applied (24hrs.) to the albino Wistarrat'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 10). This indicated that the microemulsion and microemulsion gels were nonirritant and never produced erythema after application. Thus, the formulations were safe for transdermal application.

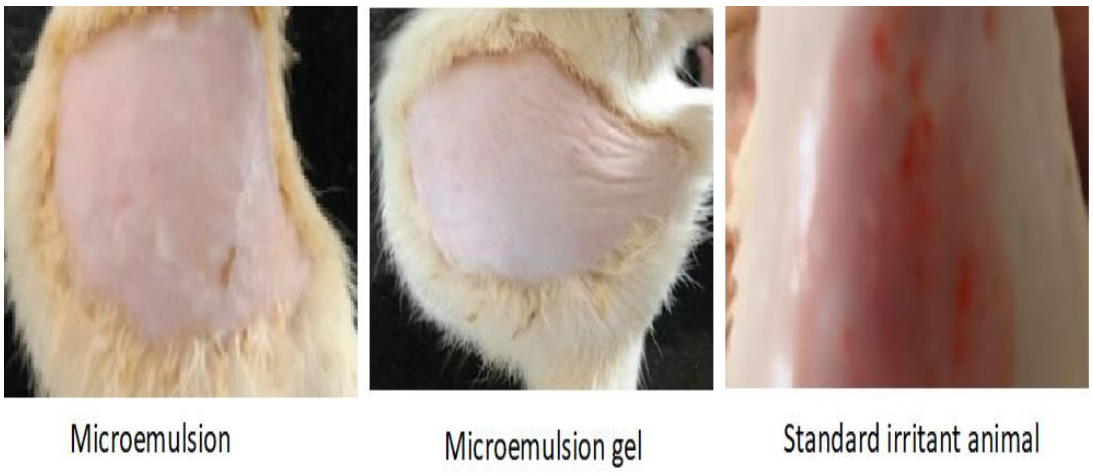


Figure 10: Skin Irritation Study

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

The concentration of Amlodipine in the receptor chambers increased steadily with time. That the Control gel shows the lowest % of drug permeation (32.11±1.39 % at 24 h) and lowest flux (0.060±0.07 mg/cm^2/h), on the other side MC1 and MD1 shows the highest % drug permeation (82.01±2.01 and 77.99±2.07 % at 24 h respectively) and highest flux (0.179±0.30 and 0.164±0.83 mg/cm^2/h respectively). As compared to microemulsion formulation to microemulsion gel % of drug permeation and flux are significantly changed. In microemulsion (C1 Formulation) highest % of drug permeation is 96.49±2.38 %. This is because adding carbopol 934 to a microemulsion reduced Amlodipine permeability, which might be due to increased viscosity and the transition from a microemulsion to a lamellar structure or a highly ordered microstructure [15].

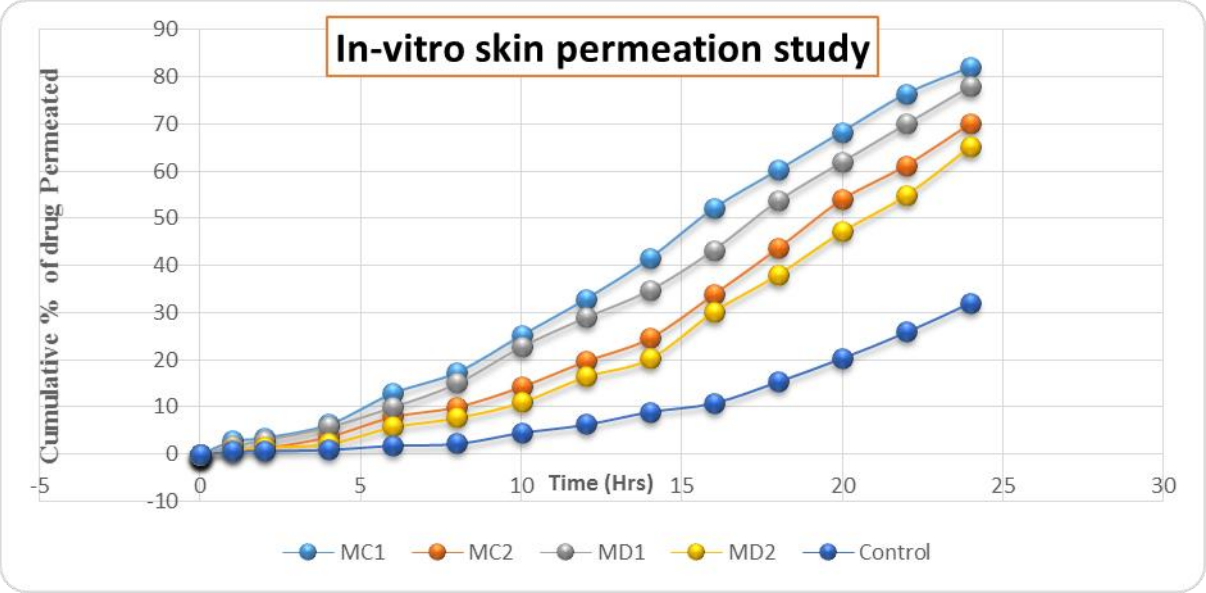


Figure 11: In vitro permeation study of microemulsion gel formulations.

Table 9: Permeation parameter of microemulsion gel.

Formulation	*Q_{24} (mg)	*Flux (Jss) (mg/cm^2 / h)	*Permeability coefficient (Kp) (cm/h)	Enhancement ratio (Er)
MC1	4.10±0.22	0.179±0.30	0.071±0.07	2.98±0.38
MC2	3.50±0.095	0.144±0.67	0.057±0.04	2.4±0.39
MD1	3.89±0.73	0.164±0.83	0.065±0.08	2.73 ±0.33
MD2	3.26±0.02	0.131±0.90	0.052±0.05	2.18±0.51
CONTROL	1.60±0.05	0.060±0.07	0.024±0.02	

*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 Amlodipine microemulsion optimized by stability study and in vitro permeation study and further processed for the formulation of Amlodipine 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 Amlodipine microemulsion formulations was found between 0.306 to 0.36 µm. Stability studies suggested that Amlodipine loaded microemulsion and microemulsion gel formulation were stable under all stability conditions. A skin irritation study suggested that Amlodipine microemulsion and microemulsion gel formulation were nonirritating and did not cause erythema. In Amlodipine loaded microemulsion gel, MC1 and MD1 show the highest % drug permeation,

5. ACKNOWLEDGEMENT: We are thankful to Abitec Corporation (US) Gattefosse India Pvt Ltd (Mumbai, India) for providing gift samplesof excipients. Also, we are very thankful to Savitribai Phule Pune University for providing research fundsfor the project.

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 HL, InHadgraft 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. Ahad A., Al-Jenoobi F. I., Al-Mohizea A. M., Aqi, M., & Kohl, K. Transdermal delivery of calcium channel blockers for hypertension. Expert Opin. Drug Deliv. 2013; 10(8): 1137–1153.
5. Williams DM, Cubeddu LX. Amlodipinepharmacokinetics in healthy volunteers. J ClinPharmacol. 1988;28:990-994
6. Chhabra G, Chuttani K, Mishra A K. Design and development ofnanoemulsion drug delivery system ofamlodipine besilate for improvement oforal bioavailability. Drug Dev Ind Pharm. 2011;37:907-16
7. S A Giannakou, P PDallas, D M Rekkas, N HChoulis. 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. FundamApplToxicol. 1992; 19(1), 1-5.
10. Kumar D., Aqil M., Rizwan, M., Sultana Y., Ali M., Investigation of a nanoemulsion as vehicle for transdermal delivery of amlodipine. Pharmazie. 2009;64(2):80-85

11. Sheikh Shafiq., FaiyazShakeel., SushmaTalegaonkar., Farhan J. Ahmad., Roop K. Khar., Mushir Ali. Development and bioavailability assessment of ramiprilnanoemulsion formulation. Eur. J. Pharm. and Biopharm. 2007; 66: 227–243.
12. Amrish Chandra., pramodkumar Sharma., RaghuveerIrchhiaya.Microemulsion-based hydrogel formulation for transdermal delivery of dexamethasone. Int. J. Pharma. 2009; 30-36.
13. 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.
14. WeiweiZhua., ChenyuGuoa., AihuaYua., Yan Gaoa., FengliangCaoa., Guang Xi Zhai. Microemulsion-based hydrogel formulation of penciclovir for topical delivery. Int. J. Pharm. 2009; 378:152–158.
15. Huabing Chen, DongshengMou, 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
16. 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.
17. Brajesh Kumar, Sanjay Kumar Jain, Sunil Kumar Prajapati, AlokMahor and Ajay Kumar.Development and Characterization of Transdermal Microemulsion Gel foran Antiviral Drug.Int. j. pharm. sci. 2010;1(6);57-74.
18. Wade A, Weller P. Handbook of pharmaceutical excipients.2nd ed.The pharmaceutical press; 1994.
19. P. Santos., A.C. Watkinson., J. Hadgraft., M.E. Lane. Application of Microemulsions in Dermal and Transdermal Drug Delivery. Skin Pharmacol Physiol. 2008;21(5):246-59.
20. Khaled Mohamed Hosny, Zainy Mohammed Banjar. The formulation of nasal nanoemulsionzaleplon in situ gel for the treatment of insomnia. Expert Opin Drug Deliv. 2013 Aug;10(8):1033-41.
21. M.J. Lawrence, G.D. Rees. Microemulsion-based media as novel drug delivery systems. Adv. Drug Deliv. Rev. 2000;45:89–121.
22. Fu-Qiang Hu, Yu Zhang, Yong-Zhong Du, Hong Yuan. Nimodipine loaded lipid nanospheres prepared by solvent diffusion method in a drug saturated aqueous system. In. J. Pharma. 2008;348:146–152.