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FORMULATION, DEVELOPMENT AND EVALUATION OF NOVEL NANOEMULSION FOR TRANSDERMAL DRUG DELIVERY OF NIMODIPINE

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ABSTRACT

The objective of the present study was to develop and optimize the nanoemulsion based transdermal drug delivery system for nimodipine, a poorly water soluble and low bioavailability drug. Characterization of the formulation was done by physicochemical studies. The pseudoternary phase diagrams were developed for various nanoemulsion formulations composed of (Triacetin+IPM), Tween 80 and PEG-400. The nanoemulsion was optimized by using *in vitro* drug permeation studies through rat skin. The pharmacokinetic evaluation and skin irritation test were carried out in healthy rats. Blood pressure reducing activity of the system was studied in hypertensive rats. The in vitro permeation rate across the rat skin varied with the varying drug: excipient ratio in the nanoemulsion formulation. The optimized formulation showed the maximum flux rate of 212.1µg/cm^2/hr was chosen for the further studies. The nanoemulsion formulation exhibited negligible skin irritation. The antihypertensive response was gradual but sustained for prolonged period of time with the transdermal system. The relative bioavailability of nanoemulsion gel was calculated to be 230% in comparison to the oral suspension. The results suggested that this novel nanoemulsion transdermal delivery system exhibited better control of hypertension than conventional oral route.

KEYWORDS: Nimodipine, nanoemulsion, pseudoternary phase diagrams, gel, transdermal delivery, anti-hypertensive.

INTRODUCTION

Transdermal drug delivery system (TDDS) allows delivery of contained drug into the systemic circulation via permeation through skin layer at a controlled rate. These system are easy to apply and remove as and when desired.[1,2] This route offers many advantages over the oral dosage form, such as improving patient compliance in long-term therapy, bypassing first-pass metabolism and sustained drug deliver, maintaining constant and prolonged drug level plasma. This approach of drug delivery is more relevant in case of chronic disorders, such as hypertension, which require long- term dosing to maintain therapeutic drug concentration.

Nimodipine is the most widely prescribed drug in the long term treatment of hypertension. Following oral administration, nimodipine is rapidly absorbed from the gastrointestinal tract (95%), but the oral bioavailability remains low (e.g. 13%) because of significant first-pass hepatic metabolism by cytochrome P450. Nimodipine also has a short plasma half-life of 1-2 hours.[3] Long- term therapy of hypertension by nimodipine oral administration may result in poor patient compliance because of low bioavailability and short plasma half-life, leading to increased frequency of administration. Nimodipine possesses ideal characteristics such as a low molecular weight (418.453g/mol), a favourable partition coefficient (octanol/water 3.5), short plasma half-life and poor oral bioavailability for formulation as a transdermal drug delivery.

Topical vehicle system can modify drug permeation through the skin but many dermal vehicles contain chemical enhancers and solvent to achieve these goals.[4] The use of these chemical enhancers may be harmful especially in chronic application, since many of them are usually irritants. It is therefore desirable to develop a topical vehicle system that does not necessitate the use of chemical enhancers to facilitate drug permeation through the skin. One of the most promising techniques for enhancement of transdermal permeation of drug is the microemulsion or nanoemulsion technique.[5,6] Nanoemulsion are thermodynamically stable transparent (translucent) dispersion of oil and water stabilized by an interfacial film of surfactant and cosurfactant molecule having the droplet size less than 100nm.[7,8] Studies have shown that nanoemulsion formulation possess improved transdermal and dermal delivery properties in vitro.[9,10,11] and in vivo[12,13] over emulsion[14] and gels.[15] This paper describes the potential of the nanoemulsion systems in transdermal delivery of nimodipine

using non-irritant, pharmaceutically acceptable ingredient without additional permeation enhancers because components of nanoemulsions themselves act as permeation enhancers.

MATERIAL AND METHOD

Components

Nimodipne was a gift sample from USV (Mumbai, India). Oleoyl macrogol-6 glycerides/ glycerides (Labrafil 1944 CS), Propylene glycol dicaprylate/dicaprate(Labrafac PG), PEG-8 caprylic/capric glycerides (Labrasol), Propylene glycol monocaprylate(Capryol PGMC), Diethylene glycol monoethylether (Transcutol P) were courtesyGattefosse SAS (France). Castor oil, olive oil and soybean oil were purchased from Genuine chemicals (Mumbai, India). Triacetin (glycerin triacetate), tween 80, tween 20 & polyethylene glycol 200 (PEG-200) were purchased from Ozone chemicals (Mumbai, India). Polyethylene glycol 400(PEG-400), propylene glycol n-butanol were purchased from E-Merck (Mumbai, India). Isopropylmyristate (IPM) was purchased from S.D. Fine chemicals (Mumbai, India). High-performance liquid chromatography (HPLC) grade methanol and acetonitrile (ACN) were purchased from Finar chemical (Ahmedabad, India). Water was obtained from Mili Q water purification system (Milipore, MA). All other chemicals and solvents procured from local market in Mumbai were of analytical grade.

Solubility of nimodipine

The solubility of nimodipine was determined by dissolving an excess amount of drug in 2 ml of oils (Oleic acid, castor oil, olive oil, labrafil 1944 CS, soybean oil, IPM, triacetin & labrafac PG), surfactants (Labrasol, Tween 80 & Tween 20), cosurfactants (PEG-200, PEG-400, propylene glycol, capryol PGMC and Transcutol P) and combination of oils in 5 ml stoppared vial and then the mixture was mixed by using vortex mixer. For equilibrium the vials were then kept at 37± 1.0ºC in an isothermal shaker (EXPO HI-TECH) for 72 hour. The equilibrated samples were transferred to centrifuged tube and rotated at 3500 rpm for 15 min. The supernatant layer obtained from centrifuge was filtered through a 0.45 µm membrane filter. The concentration of drug was determined in each component and mixture of oils by HPLC.

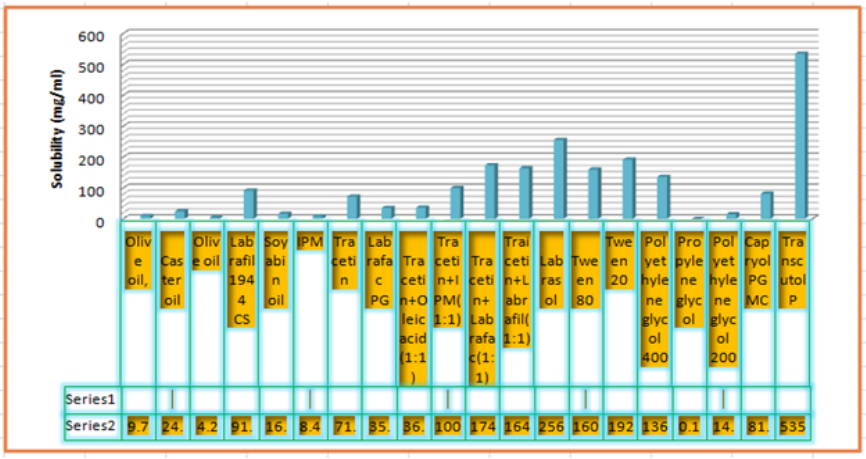


Fig. 1: Solubility of nimodipine in different components at 25ºC.

Analytical method

Quantitative analysis of Nimodipine was carried out by a validated HPLC method.[16] A HPLC (Shimadzu, Japan) equipped with LC-20AD pump, variable wavelength programmable UV/VIS detector SPD-10A, Rheodyne (Rheodyne USA) injector fitted with a 20-µl loop was used and the data were recorded and evaluated using Spinchrom software (Spinchrom, India). Chromatographic condition was C-18 column, Phenochrome® (Phenomenex USA) (5µm, 250×4.6 mm inner diameter) using a mobile phase consisting of water, methanol and ACN (25:35:40) (pH 7.2) at a flow rate of 1ml/min with UV detection at 238 nm. The mobile phase was filtered through 0.45µm filter prior to use.

Pseudo-ternary phase diagram

Water phase titration method was used for the construction of pseudoternary phase diagrams. For this method ratio of oil and Smix was taken in test tube and water was added stepwise to the mixture. The mixture was then mixed at 25ºC with the help of vortex mixer. With the help of visual observation the formulations which were showing clear and transparent regions were identified as nanoemulsion formulation. From the screening of components combination of Triacetin and IPM (1:1) was taken as the oil phase, tween 80 and PEG-400 was selected as surfactant and co-surfactant, respectively. The surfactant and cosurfactant (S_{mix}) was blended together in the weight ratio of (1:0, 1:2, 1:3, 1:1, 2:1, 3:1 and 4:1). For nanoemulsion formulation the phase diagram was studied and for this these S_{mix} ratios were chosen. The surfactant concentration was increased with respect to cosurfactant and cosurfactant concentration was increased with respect to surfactant. Sixteen different combinations in different weight ratios of oil and S_{mix} 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 were taken. For

identification of nanoemulsion region the formulation was marked on a pseudo-3- component phase diagram. Where one axis was for aqueous phase, the second axis was for oil phase and the third axis represent a blend of surfactant and co-surfactant at fixed weight ratios (Smix ratio) respectively.[17]

Selection of nanoemulsion formulation

From each phase diagram constructed, different formulas were selected from the nanoemulsion region so that the drug could be incorporated into the oil phase. For the formulation of drug loaded nanoemulsion specific quantity of drug was dissolved in oil phase and was taken in place of oil and mixed or blended with S_{mix} in test tube by using vortex mixer. In the mixture of oil and S_{mix} water was added drop by drop till a clear and transparent liquid was obtained. Selected formulations were subjected to different stability tests.

Stability studies

To overcome the problem of metastable formulation, stability studies were performed. Selected formulations were centrifuged at 3500 rpm for 30 min. The formulations that showed no phase seperations were taken for the heating and cooling cycle. Six cycles between the refrigerator temperature (4^{o}C) and 45^{o}C with storage at each temperature for not less than 48 h were done. Those formulations which were stable at these temperatures were subjected to a freeze-thaw cycle test. Three freeze-thaw cycles were done for the formulation between -21 and +25^{o}C. The formulations that survived stability test were selected for further studies and the compositions of these formulations are given in Table 1 & 2.

Table 1: Composition of nanoemulsion selected from phase diagram with Smix ratio 1:1.

Nanoemulsion	oil phase (%w/w)	Smix(%w/w)	D water(%w/w)	Nimodipine(mg)
NF1	11.11	44.44	44.44	8
NF2	10.00	40.5	50.00	8
NF3	9.09	36.36	54.55	8
NF4	8.00	32.00	60.00	8

Table 2: Composition of nanoemulsion selected from phase diagram with Smix ratio 2:1.

Nanoemulsion	oil phase (%w/w)	Smix(%w/w)	D water(%w/w)	Nimodipine(mg)
NF5	11.11	44.44	44.44	8
NF6	10.00	40.50	50.00	8
NF7	9.09	36.36	54.00	8
NF8	8.00	32.00	60.00	8

Characterization of nanoemulsion

Transmission electron microscopy (TEM)

TEM with Philips CM200 operating at 200 KV and of a 0.23 nm capable of point to point resolution and used to determine the morphology of nanoemulsion. Combination of bright field imaging at increasing magnification was used to reveal the form and size of the nanoemulsion.

A 200 mesh copper grid was used for this study and a drop of diluted nanoemulsion was applied on this and left for 2 min. A drop of phosphotungstic acid (PTA) was applied to grid and the grid was kept inverted for 1 sec. The left over PTA was washed with water and absorbed on a filter paper. The grid was kept for drying under IR lamp for half an hour and was analyzed using an instrument operated at 200 KV.

Droplet size and size distribution

Droplet size distribution of the nanoemulsion was determined by photon correlation spectroscopy that analyzes the fluctuations in light scattering due to brownian motion of the particles, using a Zetasizer (1000 HS, Malvern Instruments, U.K). The formulation (0.1 mL) was diluted in 50 mL of water in a volumetric flask, mixed thoroughly with vigorous shaking and light scattering was monitored at 25°C at a 90° angle. Droplet size distribution studies were performed at a fixed refractive index of formulation.

Viscosity determination

The viscosity of the formulations was determined by using Brookfield viscometer LV DV-E (Brookfield Engineering, USA) using spindle no. (62).

Zetapotential determination

Zeta potential was measured by photon correlation spectroscopy using Zetasizer (Nano ZS; Malvern Instruments, Worcestershire, UK) equipped with 4.0 mW He–Ne red laser (633 nm), which measures the potential range from −220 to 200 mV. Nanoemulsion formulations used for the measurement of zeta potential. All measurements were done at 25°C in triplicate.

Conductivity

The Conductivity was determined using Conductivity Meter, Testronix-15 (Microlab, Mumbai, India) in triplicate at 25 ± 0.5°C.

Refractive index

Refractive index of placebo formulations and drug-loaded formulations was determined using an Abbes type refractrometer at 25 ± 0.5°C (Erma Japan) by placing one drop of the formulation.

pH

The apparent pH of the formulation was measured by pH meter (Equip-Tronics, India) in triplicate at 25 ± 1ºC.

Table 3: Physical Characteristics of Nanoemulsion Formulations (Mean ± SD, n = 3).

Formula-tion	Droplet Size (nm)	PDI	Viscosity (cp)	Refractive Index	Conducti-vity(µs/cm)	Zetapotntial (mV)
NF-1	11.00±0.058	0.141±0.037	29.33±0.23	1.406±0.002	56.8	24
NF-2	14.67±0.048	0.301±0.029	30.00±0.81	1.407±0.026	57.2	24
NF-3	11.39±0.074	0.374±0.115	36.00±0.57	1.409±0.001	79.2	26
NF-4	14.36±0.241	0.348±0.065	31.66±0.94	1.410±0.001	69.5	30
NF-5	46.67±13.48	0.101±0.029	39.00±0.81	1.407±0.026	60.3	29
NF-6	14.36±0.241	0.328±0.065	31.66±0.94	1.410±0.001	56.2	25
NF-7	11.00±0.057	0.131±0.037	29.33±0.23	1.406±0.002	61.8	24
NF-8	10.39±0.074	0.394±0.115	36.00±0.57	1.409±0.001	55.4	24

NF: nanoemulsion formulation code.

Ex-Vivo skin permeation studies

Ex-vivo skin permeation studies were performed on a Transdermal Diffusion Cell (TDCS). The capacity of the receptor compartment of TDC was 15 ml and the size of the skin surface for permeation was 0.706 cm^2. The stratum corneum side of the skin was towards the donor cell and the drug was introduced into that, where as dermal side of the skin was in contact with the receptor phase. The receptor compartment was filled upto 15 ml with Phosphate Buffer (7.4) and Tween 80 (90:10). The permeation rate was also studied from control and neat components for this the same amount of drug was dissolved in tween 80, surfactant mixture and oil respectively. The temperature of the receptor media was maintained at 35 ± 0.5ºC and it was stirred with magnetic bar at 600 rpm. The nanoemulsion formulation was applied on the skin on donar side and 0.5 ml of aliquot was collected from the receiver compartment at time intervals (viz. 0, 1, 2, 4, 6, 8, 10, 12, 24 and 48h) period. The receiver cell was immediately replenished with the same volume of fresh media which was also maintained at 35 ± 0.5ºC. The samples were filtered with the 0.45µm membrane filter and after suitable dilution the concentration of drug in the receptor media was analyzed using HPLC.

Data analysis

The cumulative amount of nimodipine permeated through the skin (Q, µg/cm^2) was plotted as a function of time (t, h). The drug flux (permeation rate) at steady state (Js, µg/cm^2/h) was calculated from the slope of linear portion of the curve. Cumulative amount of drug permeated through the skin (mg/cm^2) was plotted as a function of time for each formulation. Drug flux (permeation rate) at steady state (*J*ss) was calculated by dividing the slope of the graph linear portion with the diffusion cell area (mg cm^{-2} h^{-1}). Permeability coefficient (*K*p) was calculated by dividing *J*ss by the initial concentration of the drug in the donor cell (cm h–1).

Formulation of optimized nanoemulsion into gel

Based on the permeation studies and characterization, NF4 was selected as the optimized formulation and converted into gel. For gel formulation carbapol 934 was selected. The placebo gel was prepared by dispersing different % concentration of carbapol 934 (1, 1.5,2 and 2.5%). The formulation which has the desired viscosity, dispersibility and better consistency was selected. 1% carbopol 934 was found better gel and used for incorporation of nanoemulsion. Carbopol 934 gel did not show any interaction and was stable for long period of time.

Characterization of nanoemulsion gel

Homogeneity

To check the consistency and homogeneity a small quantity of gel is pressed between the thumb and the index finger (whether homogeneous or not) and if there is any coarse particle appeared then the formulation was discarded.

Viscosity determination

The viscosity was determined using Brookfield viscometer LV DV-E (Brookfield Engineering, USA) using spindle No. 2 (62) in triplicate at 25 ± 0.5°C.

Measurement of gel strength and Homogeneity

Once the gel was ready after 48h it was determined for its strength by measuring the weight required to move upper plate by 3cm, when1g of each gel was placed between two 20cm×20cm plates. The gel strength was calculated by using the formula.

$$S = \frac{M \times L}{T}$$

Where S is the gel strength, M is the weight tied to the upper slide, L is the length glass slide travelled and T is time taken. Homogeneity of various gel formulations were tested by visual observations.

pH determination

The apparent pH of the formulation was measured by pH meter (Equip-Tronics, India) in triplicate at 25 ± 1ºC.

Table 4: Physical Characteristics of Transdermal Nanoemulsion Gel (TNG) Formulations (Mean ± SD, n = 3).

Transdermal Nanoemulsion Gel	Corbopol 934 (%)	pH	Viscosity (cp)	Gel strengths (gm/cm/s)
NF4	1	6.8±0.321	50.01±o.121	5.0±0.231

Skin irritancy test

Skin irritation test was performed using six rats by applying single dose of the formulation to the left ear of the rat and the right was considered as a control. For 7 days the development of erythema was monitored for using the method as reported by (Abbe *et al, 1975*).

***In vivo* studies**

Approval was taken to carry out in vivo study from Oriental College of Pharmacy, Institutional Animal Ethics Committee, Sanpada and their guidelines were followed for the studies. Adult wistar albino rats procured from Central Animal House of Oriental College of Pharmacy, India were used for in vivo experiment. In a well maintained faculty animal house the rats were kept in a rat cage. The rats were fed with standard feed and drinking water and monitored on regular basis. The rats were then divided into two groups (Group I and Group II) carrying six animals each. Group I was received TTS treatment with formulation NGF. The rat skin was shaven and the formulations were applied to the skin which was in contact with the stratum corneum. The micro porous adhesive tape was rolled over to keep the patch secured at the site of application. Group II were administered with nimodipine suspension. For collection of blood the rats were anesthetized using ether and blood samples (0.5 mL) were removed from the tail vein of the rat at time interval of 0, 2, 4, 6, 8, 10, 12, 24 and 48 h in microcentrifuge tube which was containing 8 mg of anticoagulant such as EDTA and centrifuged at 4500 rpm for 20 min. The plasma or supernatant layer was separated and stored at -21^{0}C until drug analysis was carried out by HPLC method.

Measurement of BP of rats

A blood pressure (BP) measuring instrument (Digital stoelting) with a non-invasive tail cuff and a digital BP display panel was used. The rats were trainedto stay in the rat holders in a calm and non-aggressive state during BP measurement. After recording the initial BP of rats, they were divided into 4 groups of 6 each. Group I was taken as control. Hypertension was induced in the remaining 3 groups by subcutaneous injection of methyl prednisolone acetate (MPA) (20mg/kg/wk). Two weeks later, all the rats with a minimum mean BP of 150 mm Hg were selected. After MPA treatment, group II served as toxic control and received no further treatment. Group III received nimodipine orally (8 mg in 5 ml Water). Group IV receives nanoemulsion gel (1g).BP was recorded at different time intervals (2, 4, 6, 8, 10, 12, 24 and 48 hours).

Drug excipients interaction studies

Study of drug- excipient interaction is very important criteria to be consider. Thus interactions studies were performed on nanoemulsion formulation to check the effect of excipient on drug.

The following studies were carried out on the optimized formulations.

UV Scanning

The nanoemulsion formulation which was loaded with drug dissolved in methanol and the solution was scanned for the absorbance between 200-400 nm. Pure drug was also scanned along with it.

Assay

To estimate the percent recovery of the loaded drug the nanoemulsion formulations were assayed using HPLC and the drug content was calculated.

Differential Scanning Calorimetery (DSC)

DSC of the drug-excipients mixture was taken by using differential scanning calorimeter (Perkin Elmer Pyris 6).

RESULT AND DISCUSSION

The physicochemical properties of nimodipine suggest that it has good potential for topical drug delivery. The important criteria for selection of material for the nanoemulsion

formulation development is that the components are pharmaceutically acceptable, non-irritant and nonsensitizing to the skin and fall under the GRAS (Generally Regarded as Safe) category. Non-ionic surfactants are less toxic than ionic surfactants. The higher solubility of the drug in the oil phase is important for the nanoemulsion to maintain the drug in solubilized form. Transient negative interfacial tension and fluid interfacial film is rarely achieved by the use of a single surfactant, usually necessitating addition of a cosurfactant. The presence of cosurfactant decreases the bending stress of the interface and allows the interfacial film sufficient flexibility to take up different curvatures required to form a nanoemulsion over a wide range of compositions.[18]

Therefore, the aim of the present study was to develop and evaluate thermodynamically stable o/w nanoemulsions of nimodipine for transdermal drug delivery. These formulations were prepared by using a combination of Triacetin and IPM as the oil phase, Tween 80 and PEG-400 as surfactant and cosurfactant, respectively. In the present study based on the solubilization results it was suggested that the surfactant and cosurfactants which showed the highest solubility for drug would have poor affinity for the oil phase for nanoemulsification.[19] The surfactant which gave the maximum nanoemulsion region without the use of cosurfactant was selected as surfactant for the formulation. The highest solubilization capacity for oil was observed with Tween 80 as the maximum nanoemulsion region was found with the same. PEG-400 was showing the maximum nanoemulsion region and selected as a cosurfactant.

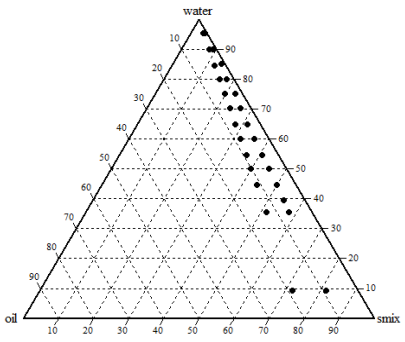
Pseudo-ternary phase diagram

The construction of phase diagrams made it easy to find out the concentration range of components for the existence range of nanoemulsion. No change was found in the phase behaviour of the pseudoternery phase diagram when nimodipine was loaded in the formulations. The ratio of Tween 80 and PEG-400 (S_{mix}) was selected at 2:1 and 1:1.[20]

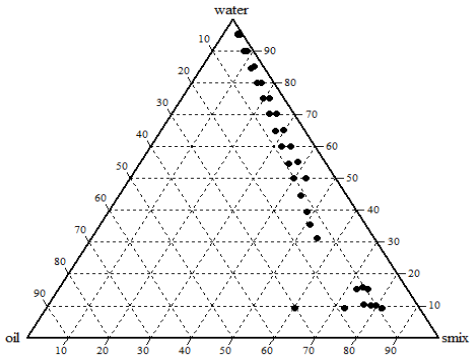
The surfactant and cosurfactant plays an important role in influencing the phase properties such as size and position of nanoemulsion region (15). Figure 2. [Pseudoternary phase diagrams showing nanoemulsion (shaded area) region of Triacetin and IPM (1:1) oil, Tween 80 (surfactant), PEG-400 (cosurfactant) at different S_{mix} ratios: A) S_{mix} 1:0, B) S_{mix} 1:1, C) S_{mix} 1:2, D) S_{mix} 1:3, E) S_{mix} 2:1, F) S_{mix} 3:1, G) S_{mix} 4:1.] shows the S_{mix} ratio 1:0 (fig.2A) has a low nanoemulsion area. Might be; at lower concentration the surfactant is not able to sufficiently reduce the o/w interfacial tension so that the surfactant concentration was

increased with respect to cosurfactant. An o/w nanoemulsion region was found towards the water-rich apex of the phase diagram. As the surfactant concentration was increased with respect to cosurfactant, a larger nanoemulsion region was observed. In S_{mix} ratio 1:0(fig. 2A) and 1:1 (fig. 2B), the maximum concentration of oil that could be visualized to be solubilized in the phase diagram was 18.18% w/w, 30.30% w/w by using 72.73% w/w and 60.61% w/w S_{mix} respectively.

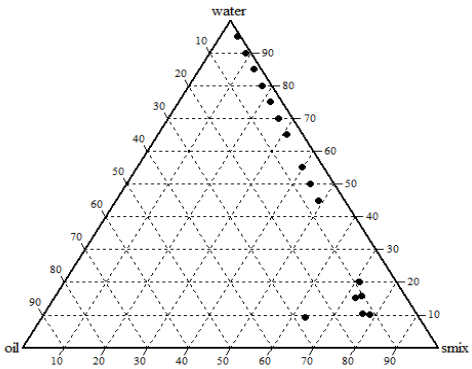
The maximum concentration of oil that could be solubilized by S_{mix} ratio 2:1 (fig. 2E) was 20.00% w/w by using 70.00% w/w of S_{mix}. In S_{mix} ratio 3:1 (fig. 2F), the maximum concentration of oil that could be solubilized in the phase diagram was 30.30% w/w using 60.61% w/w of S_{mix}. Though, large nanoemulsion region was found with S_{mix} ratio 4:1 (fig. 2G), a decreased solubilization capacity for oil was observed, which may have been due to increased concentration of the surfactant. The maximum concentration of oil that could be solubilized in the phase diagram was same as 1:0 S_{mix} and 1:1 S_{mix} ratio. Therefore, there was no need to attempt a S_{mix} ratio of 5:1. As the cosurfactant concentration was increased with respect to surfactant a limited nanoemulsion region was obtained at S_{mix} ratio 1:2 and 1:3 (fig 2C, fig2D). Hence, it was attempted up to S_{mix} 1:3.



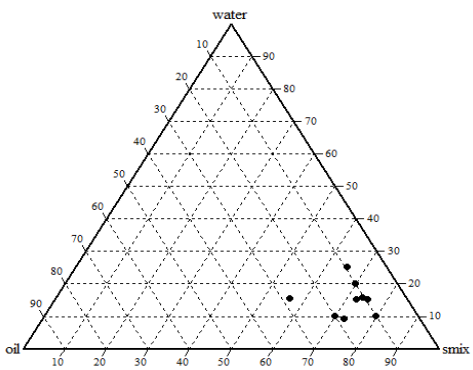
A) S_{mix} 1:0



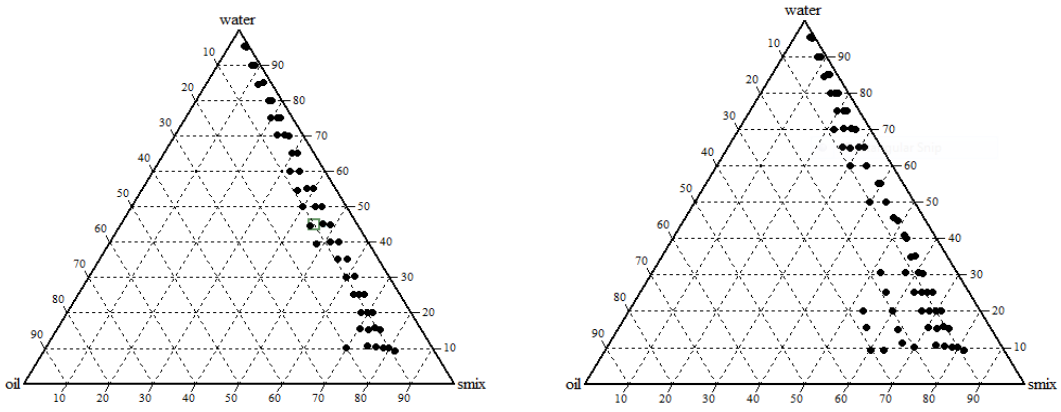
B) S_{mix} 1:1



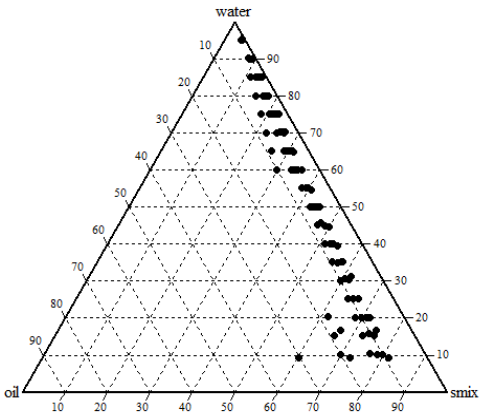
C) S_{mix} 1:2



D) S_{mix} 1:3



E) S_{mix} 2:1 F) S_{mix} 3:1



G) S_{mix}

Figure 2. Pseudoternary phase diagrams showing nanoemulsion (shaded area) region of Traicetin and IPM (1:1) oil, Tween 80 (surfactant), PEG-400 (cosurfactant) at different S_{mix} ratios: A) S_{mix} 1:0, B) S_{mix} 1:1, C) S_{mix} 1:2, D) S_{mix} 1:3, E) S_{mix} 2:1, F) S_{mix} 3:1, G) S_{mix}.

Stability Studies

To avoid the possibility of metastable formulation[21], the nanoemulsions were tested for their thermodynamic stability by using centrifugation, a heating-cooling cycle and a freeze-thaw cycle. Few selected formulations were chosen from the o/w nanoemulsion region of the phase diagram constructed at S_{mix} 1:1, 2:1, 3:1 and 4:1 respectively. The formulations which show no turbidity and phase separation were subjected for characterization of nanoemulsion formulation. Thermodynamic stability confers long shelf life to the nanoemulsion.[22]

Characterization of nanoemulsion

In present work, optimized drug loaded nanoemulsion was selected for characterization studied. The nanoemulsion was colloidal dispersions having average droplet size ranging

from 11.07 to 46.18 nm (Fig.4). Uniformity of droplet size within the formulation was also calculated. It is measured by a value of polydispersity index (PI). The nanoemulsion formulation exhibited a narrow size distribution (PI= 0.131). The results of dynamic light scattering (DLS) measurements were in agreement with the droplet size measured by TEM (Fig.3).

Viscosity of the nanoemulsion formulation was expected to be very low for the present work the viscosity measured was (29.33±0.235cp). The low viscosity may be due to presence of low amount of tween 80 (a fatty acid polyhydric alcohol ester having high intrinsic viscosity) and also the low concentration of oil.

Refractive index is the net value of the components of nanoemulsion and indicates isotropic nature of formulation. The mean value of the refractive index for the formulation was found to be 1.406±0.002.

The pH value for the optimized drug loaded nanoemulsion formulation was recorded 5.5± 0.219, which is favorable for transdermal application because the pH of the skin is in the range of 4.5 to 5.5.

Conductivity of the nanoemulsions was measured to determine the phase system whether the nanoemulsion is (o/w or w/o) type of the formulation.[23] Oil-in-water nanoemulsions shows high conductivity as water is in the form of the external phase. From the conductivity results the nanoemulsion formulations were detected as oil-in-water nanoemulsions.

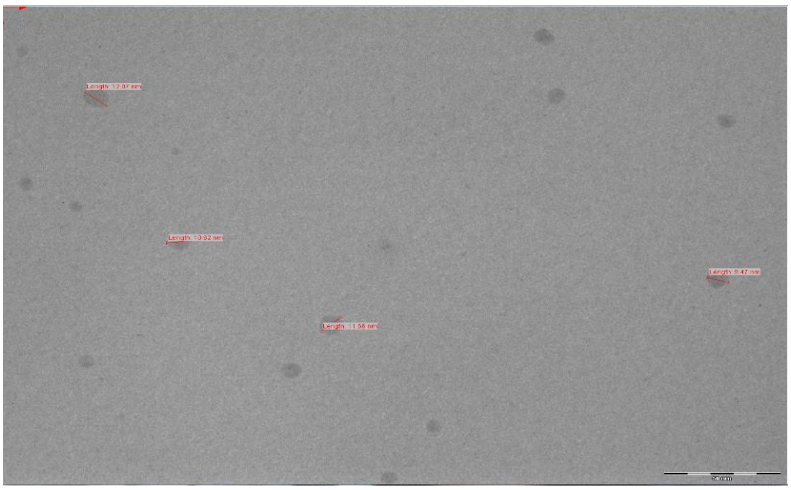


Figure 3. Transmission electron microscopic positive images of nimodipine nanoemulsion.

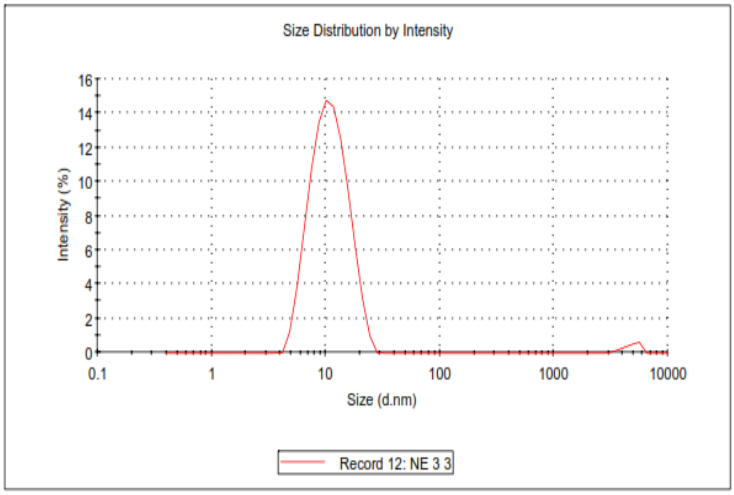


Fig. 4: Average droplet size of optimized drug loaded nanoemulsion containing oil phase (Triacetin: IPM) Tween 80 and PEG-400.

***Ex-vivo* skin permeation studies**

The *ex-vivo* permeation profile is an important tool that predicts in advance how a drug would behave *in vivo*.[24] The effect of the S_{mix} on the skin permeation of nimodipine was evaluated. Different nanoemulsions were prepared for this purpose with two different S_{mix} ratios viz 2:1 and 1:1. The composition of nanoemulsion has been shown in table 1 and 2. The permeation parameters of the tested nanoemulsion formulations are presented in table 5. The permeation profiles of nimodipine through rat skin from various vehicles are shown in fig. 5,6 and 7.

Table 5. Permeability Parameters of Nanoemulsion Formulations (Mean ± SD, n=3).

Formulation code	J_{ss} (µg/cm^2/h)	K_p(cm/hr ×$^{10-3}$)	ER
NF1	173.19±1.72	21.60±1.23	3.0±1.09
NF2	173.80±1.56	21.70±1.54	3.0±1.67
NF3	174.90±0.98	21.86±0.87	3.0±0.65
NF4	212.10±0.79	26.52 ±0.98	3.6±0.79
NF5	160.92±1.21	20.1±0.24	2.4±0.98
NF6	163.94±2.1	20.41±0.98	2.7±0.93
NF7	167.85 ±1.98	20.98±1.10	2.9±0.723
NF8	167.92±0.97	21.10±0.45	2.9±1.02
Neat oil	46.69±0.99	05.80±0.65	0.5±0.25
Neat surfactant	53.07±0.23	06.6±0.55	0.6±0.53
Control	87.18±1.78	07.21±1.51	-

J_{ss}: Flux.

K_p: Permeability coefficient.

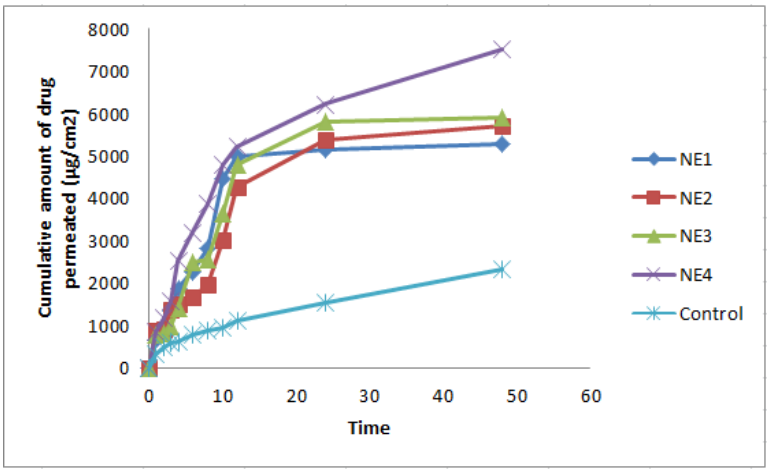


Fig. 5: Permeation profile of nimodipine through excised rat skin from nanoemulsions formulated with S_{mix} 1:1 (mean ± SD, n=3).

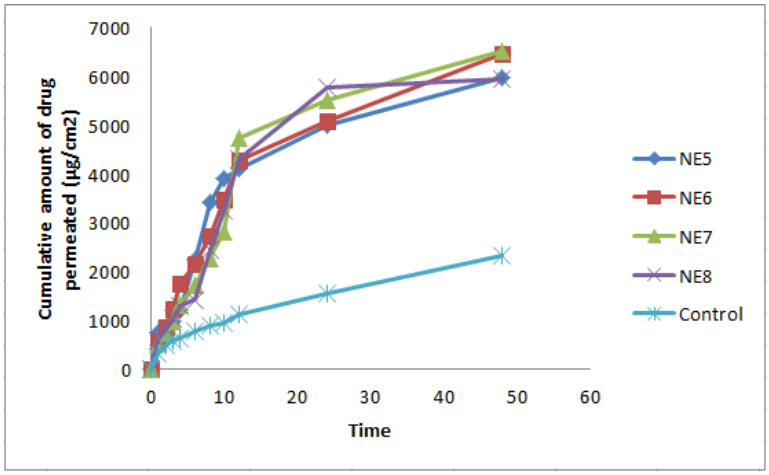


Fig. 6: Permeation profile of nimodipine through excised rat skin from nanoemulsions formulated with S_{mix}2:1 (mean ± SD, n=3).

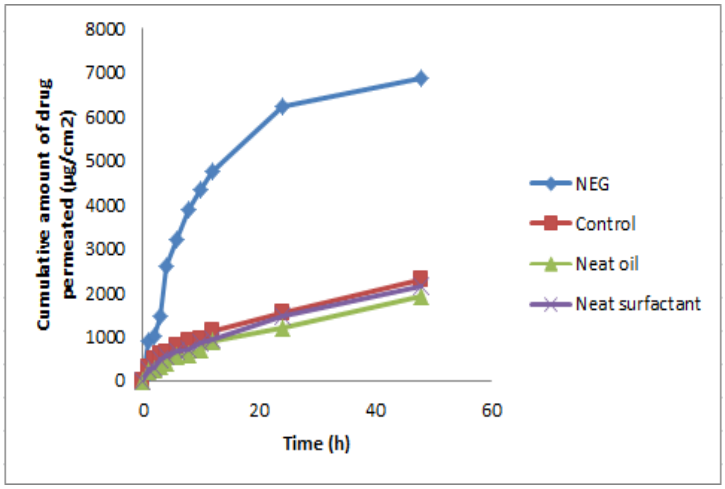


Fig. 7: Comparison of permeation profile of nimodipine through excised rat skin from optimized nanoemulsion gel (NEG) with control and drug loaded neat component.

The content of S_{mix} in the nanoemulsion formulation was found to affect the skin permeation rate of nimodipine directly. As the content of S_{mix} 2:1 increased, the skin permeation decreased. This might be due to a decreased thermodynamic activity of a drug in the nanoemulsion at the higher content of surfactant[25]. The thermodynamic activity of a drug in the formulation is a significant driving force for the release and penetration of the drug into the skin.With increased surfactant concentration, affinity to the vehicle might have become greater leading to observed slow release of the drug/or a poor transfer from the vehicle to the skin[26]. Another possible reason that could have an additive effect was the hydration effect of water[27].When the water content was increased in the formulation, the hydration of stratum corneum would have increased. It is the water in nanoemulsion that could hydrate the skin causing the corneum cells to swell, thus making the channels for drug passage wider. Therefore, with the increased amount of the water in the system, the cumulative permeation amount might have improved.

Permeation of nimodipine was also considered from the neat oil phase as oil has permeation enhancing properties. However, as fig. 3 shows, a relatively lower flux value (46.69µg/cm^2/h) was obtained as compared to nanoemulsion formulations, which might be ascribed to the greater affinity of the drug for the oil phase because of its lipophilic nature and therefore lesser partitioning of the drug from the vehicle to the skin ($p<0.01$). Permeation was also carried out from neat surfactant in order to investigate whether the nanoemulsions had any superior effect. Comparatively far lower flux (53.07µg/cm^2/h) could be attained with Tween 80 in contrast with the nanoemulsions and this was statistically significant ($p<0.01$) (Table-2). Again drug release from the vehicle might have played a crucial role.

Among the formulations tested, the formulation NF4, which was composed of 0.008% nimodipine, 8.00% oil triacetin: IPM (1:1) and 32.00% S_{mix} (1:1) and 60% of water, showed the most favourable permeation profile (Fig. 2). The skin permeation rate of nimodipine from this nanoemulsion was as high as 212.1 µg/cm^2/h.

Surfactant present in the nanoemulsions might cause increased membrane fluidity, solubilisation or extraction of lipid present in the stratum corneum leading to alterations in the tight junction properties[28] which might have become the cause of improved permeation. The nanosized droplets in nanoemulsions lead to an enormous increase in the interfacial area, which influences transport properties of drugthrough the interface.[29] It is assumed in

addition that the low interfacial tension and the continuously and spontaneously fluctuating interfaces of nanoemulsions are supposed to facilitate transfer of the drug to the skin.

Pharmacokinetic Evaluation of gel

To judge the efficacy of the developed nanoemulsion formulation the pharmacokinetic study was carried out on rats against the oral dosage form. The data so obtained was subjected to pharmacokinetic analysis. The blood plasma levels of nimodipine after transdermal and an oral administration is shown in Fig.8. The pharmacokinetic parameters were calculated from the blood plasma concentrations of the drug and summarized in table. 6. The results of oral suspension was compared with nanoemulsion transdermal gel, gel was showing better permeation and release of nimodipine. The maximum drug concentration, C_{max}, after oral administration was 41.2± 3.87ng/ml and T_{max} was 2± 0.5 hours. For the nanoemulsion gel, C_{max} and T_{max} were 48.7± 11.46 ng/ml and 12± 3.54hours respectively. The pharmacokinetic parameters obtained from the nimodipine nanoemulsion gel were significantly different from oral nimodipine suspension. The overall mean value of AUC_{0-t} by transdermal route was 2 times higher than that of oral route and the difference was found to be statistically significant ($p<0.05$) showing improved bioavailability of nimodipine from nanoemulsion gel. This could be due to avoidance of first-pass hepatic metabolism for transdermal route. Though oral route has faster onset of action, the formulated nanoemulsion transdermal gel product would lead to better therapy.

Table 6: Pharmacokinetic parameters of Nimodipine oral suspension and Transdermal gel administration.

Formulation	C_{max} (ng/ml)	T_{max} (h)	AUC_{0-t} (ng h/ml)	$AUC_{0-\infty}$ (ng h/ml)	K_e (h^{-1})	$T_{1/2}$ (h)	F (%)
Oral suspension	41.2± 3.87	2± 0.5	424 ± 15.39	472±28.21	0.027	11.55	
Transdermal gel	48.7± 11.46	12± 3.5	1272± 145.2	1521±201.1	0.022	13.86	230

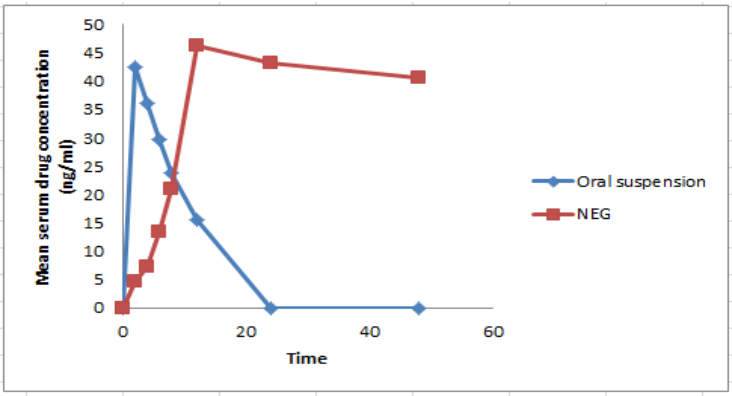


Fig. 8: Nimodipine concentrations in the rat serum after transdermal and oral treatment Pharmacodynamic evaluation in hypertensiv rats.

Hypertension was successfully induced in rats by MPA administration as highly significant difference ($p<0.001$) was observed in post treatment BP values in control and toxic control groups. Glucocorticoids like methyl prednisolone etc are known to cause systemic hypertension on subcutaneous administration.[30] The rats were found to be hypertensive (minimum mean BP 150mm Hg) up to 72 hours thus allowing the BP measurements after application of 2-day NGF.

The result in table 7-10 indicated that the significant hypertension was produced when MPA administered in the normotensive rats. The oral administration of nimodipine significantly ($p<0.05$) controlled the hypertension initially, the maximum effect shown by the oral route was at 2 hr. But after 2 hours the BP started rising and reaches to its initial value until at 48 hr. Whereas, the administration of nimodipine through transdermal gel showed a gradual decrease of BP, where the maximum effect was observed at 12 hours ($p<0.05$). Though the gel produced a peak effect at 12hr, it started exerting effect ($p<0.05$) right from the first hour and continued for 48 hours. Thus the transdermal gel release and permeate the drug in-vivo gradually over a period of time, which results in prolonged control of hypertension for 48 hours. Though the action of oral nimodipine was quick but after few hours its effect was decreased.

Table 7: Reduction in blood pressure level of rats after treatment with nanoemulsion gel without drug (Control).

Group	Treatmeent	Rats	Initial blood pressure Level (mm Hg) ±SD)	**Blood pressure at varying time points (mm Hg ± SD)							
			0	2 hrs	4 hrs	6 hrs	8 hrs	10 hrs	12 hrs	24hrs	48 hrs
I	Control*	1	96±0.9	96±0.7	97±0.7	96±0.9	96±0.9	96±0.8	96±0.9	96±0.4	96±0.6
		2	96±0.7	96±0.8	96±0.7	96±0.8	96±0.8	96±0.7	96±1.2	96±0.3	96±0.9
		3	95±0.5	95±0.7	95±0.7	95±0.9	95±0.9	95±0.9	95±0.6	95±0.5	95±0.7
		4	96±0.3	96±0.5	96±0.6	96±0.8	96±0.7	96±0.9	96±0.5	96±0.5	96±0.6
		5	96±0.7	96±0.6	96±0.8	96±0.9	96±0.6	96±0.7	96±0.5	96±0.7	96±0.6
		6	96±0.6	96±0.5	96±0.9	96±0.8	96±0.8	96±0.8	96±0.7	96±0.6	96±0.6

*No treatment was given.

**Mean of three observations ± S.D.

Table 8: Reduction in blood pressure level of rats after treatment with nanoemulsion gel without drug (Placebo Control).

Group	Treatmeent	Rats	Initial blood pressure Level (mmHg ±SD)	** Blood pressure at varying time points (mm Hg ± SD)							
			0	2 hrs	4 hrs	6 hrs	8 hrs	10 hrs	12 hrs	24 hrs	48 hrs
II	Placebo/Toxic Control*	1	157±1.2	152±0.7	155±0.2	151±0.9	156±0.7	153±0.6	152±0.9	155±0.8	152±0.7
		2	158±0.9	155±0.8	155±0.3	158±0.4	154±1.2	153±0.7	152±0.6	157±0.4	156±0.6
		3	157±0.8	154±0.8	156±0.4	157±0.4	153±0.9	152±0.5	158±0.7	156±0.7	154±0.7
		4	158±0.1	152±0.7	154±0.4	157±0.3	156±0.8	154±0.7	156±0.6	153±0.4	157±0.6
		5	157±0.2	157±0.5	154±0.9	154±0.3	153±0.3	156±0.6	157±0.7	153±0.6	153±0.5
		6	158±0.4	152±0.4	156±0.7	153±0.4	156±0.4	154±0.5	157±0.6	154±0.5	155±0.7

*After induction of hypertension no treatment was given.

**Mean of three observations ± S.D.

Table 9: Reduction in blood pressure level of rats after oral treatment with nimodipine suspension (Positive Control).

Group	Treatmment	Rats	Initial blood pressure Level (mm Hg ±SD)	** Blood pressure at varying time points (mm Hg ± SD)							
			0	2 hrs	4 hrs	6 hrs	8 hrs	10 hrs	12 hrs	24 hrs	48 hrs
III	Positive Control*	1	154±0.9	88±0.4	98±0.4	106±0.3	112±0.1	122±0.7	132±0.4	139±0.3	152±0.5
		2	152±0.8	90±0.4	97±0.3	110±0.2	120±1.2	129±0.6	135±0.3	142±0.6	155±0.6
		3	156±0.8	87±0.4	99±0.5	113±0.3	121±0.9	130±0.4	135±0.4	144±0.5	156±0.4
		4	155±0.2	87±0.6	97±0.8	108±0.5	125±0.8	134±0.4	137±0.6	142±0.4	147±0.5
		5	156±0.3	88±0,9	92±0.3	109±0.5	123±0.3	134±0.5	141±0.4	151±0.4	157±0.8
		6	156±0.5	83±0.4	98±0.8	109±0.3	126±0.4	137±0.3	139±0.7	149±0.7	153±0.9

*Formulation with nimodipine suspension.

**Mean of three observations ± S.D.

Table 10: Reduction in blood pressure level of rats after treatment with nimodipine nanoemulsion gel formulation (Formulation control).

Group	Treatmeent	Rats	Initial blood pressure Level (mmHg ±SD)	** Blood pressure at varying time points (mm Hg ± SD)							
			0	2 hrs	4 hrs	6 hrs	8 hrs	10 hrs	12 hrs	24 hrs	48 hrs
IV	Formulationni modipinenanoe mulsion gel*	1	157±0.9	132±0.3	122±1.1	112±1.12	111±1.1	107±1.6	99±1.8	102±0.3	111±0.4
		2	158±0.2	141±0.3	137±0.9	121±1.2	113±1.23	109±0.9	101±0.7	103±0.8	102±0.3
		3	157±0.4	132±0.5	129±0.8	121±1.2	126±1.7	115±1.7	103±0.2	111±0.8	114±0.5
		4	156±1.2	139±0.9	129±0.7	111±0.3	111±0.9	108±.8	98±0.3	102±0.3	112±0.3
		5	157±0.9	136±0.7	128±0.7	113±0.9	113±0.9	105±0.8	99±0.5	102±0.4	110±0.6
		6	158±0.2	138±0.6	126±0.8	112±0.9	112±0.8	106±0.5	99±0.5	101±0.3	109±0.4

*The optimized formulation containing drug.

**Mean of three observations ± S.D.

Skin Irritation Test

The test results are tabulated below.

Table 11: Skin irritation scores of NEG.

Rats	Intact skin				Abraded skin			
	24 hr		72hr		24 hr		72hr	
	A*	B**	A*	B**	A*	B**	A*	B**
1	1	1	0	0	1	1	1	0
2	0	0	1	0	1	0	2	0
3	1	1	0	1	0	2	1	1

A*= erythema formation score; B** = Oedema formation score.

Table 12: Skin irritation scores of NEG (Average).

Rats	Intact skin (i)		Abraded skin (ii)		Combined average (i + ii)
	24 hr	72hr	24 hr	72hr	
1	1*	1*	1*	1*	-
2	1	1	1	2	-
3	2	1	2	2	-
Average	1**		1.5**		1.25

*Total of A & B from Table 8; **average of all six readings of 24 and 72 hr.

The skin irritation test of the transdermal formulations showed a skin irritation score (erythema and edema) of less than 2. Acoording to Draize et al, materialsproducing score of 2 or less are considered negative (no skin irritation).[31] Hence, the developed transdermal formulations were free of skin irritation.

Drug excipients interaction studies

To determine any interference & interaction of the drug with other ingredients of the formulation the drug excipint interaction studies were performed. i.e. oil & surfactant mixture. However, in the interaction studies the drug was shown to be fairly stable and inert to the excipients used in the nanoemulsion.

The UV spectrum revealed that the formulation & the pure drug solution exhibited the same absorption maxima (λ_{max}). Fig. 9.

The assay results were found satisfactory with the percentage recovery ranging from 99.11% to 99.13% (mean 99.12) indicating that the drug was undecomposed and stable in the formulation. Table 13.

DSC thermogram of drug loaded nanoemulsion and the pure drug shows that the drug loaded nanoemulsion has same melting point (127.8ºc), area and the intensity of the peak with respect to the pure drug. So no interactions were seen between drug and excipients. Fig, 10 and 11.

From the above mentioned studies, the results reveal that the drug remained intact and no chemical interaction occur between the drug and excipients.

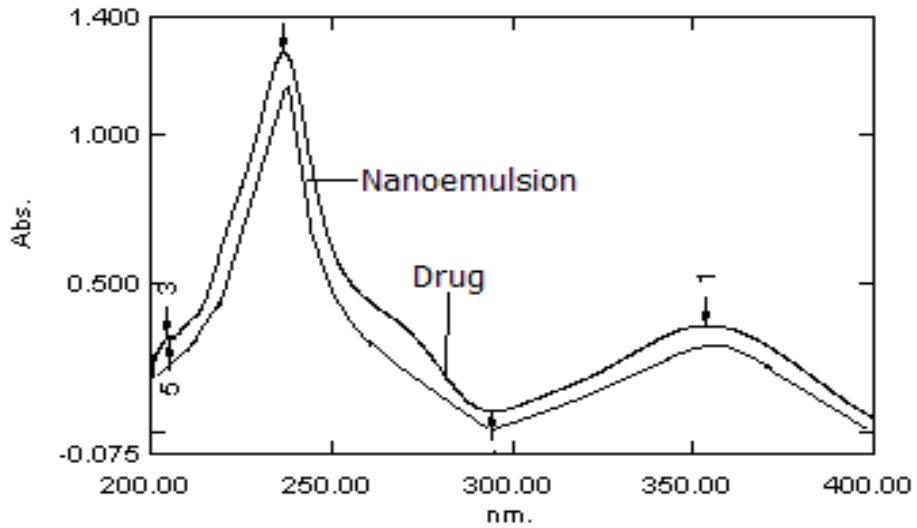


Fig. 9: UV scan of pure drug and drug loaded formulation for interaction studies.

Table 13: Drug content and drug recovery of nanoemulsion.

Formulation	Mean drug content (mg) (n=3)		Percentage Drug Recovery (±SD)
	Loaded	Assayed	
Nanoemulsion	8	7.93	99.12 (±0.03)

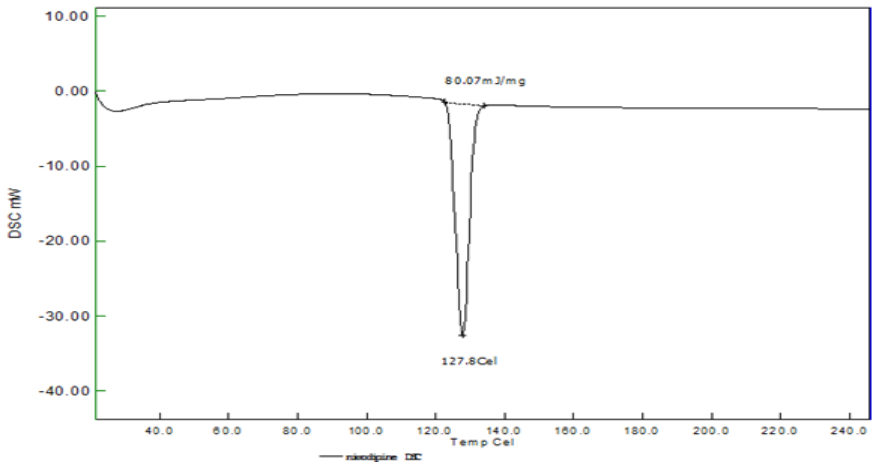


Fig.10: DSC thermogram of pure drug.

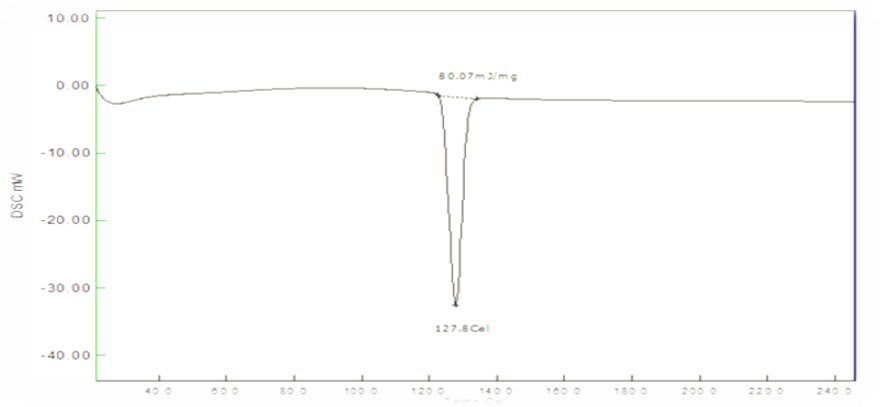


Fig.11: DSC thermogram of drug excipients mixture.

CONCLUSION

A novel nanoemulsion transdermal gel formulation of nimodipine has been successfully attempted. Results have revealed that proper management of oil, polymer and drug could give desirable outcome. From in vitro and in vivo data it can be concluded that the developed nanoemulsions have great potential for transdermal drug delivery. The option has good probability to move further via clinical & scale-up protocols. This technology can be explored for other antihypertensive molecule as well so as to achieve better control over the disease.

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