Research Article

Development of Nanoemulsion as Carrier for Transdermal Delivery of Valsartan

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ABSTRACT

The aim of the present study was to develop potential nanoemulsion for transdermal delivery of valsartan. The o/w nanoemulsion was prepared by screening the excipients from the nanoemulsion region of pseudoternary phase diagram. The prepared nanoemulsions were subjected to different thermodynamic stability tests. The composition of optimized formulation was Triacetin (13.6%), Chemophore EL (23.9%), PEG 400 (7.9%), and water (54.6%) as oil, surfactant, co-surfactant and aqueous phase, respectively, showing globule size (4.49 nm), polydispersity (0.651), and viscosity (82.55 ± 3.12 cP). The *in vitro* permeation of valsartan through rat skin was found to be significantly ($p < 0.01$) increased as compared to conventional gel formulation. The present study revealed that the nanoemulsion as suitable carrier for transdermal delivery of valsartan.

Keywords: Nanoemulsion, valsartan, transdermal delivery.

INTRODUCTION

Among the different reasons for poor bioavailability of the drug, the extensive first-pass metabolism of the drug is one of them. The transdermal delivery of drugs that are undergo extensive first-pass metabolism is an alternative way to improve the bioavailability. However, for delivery of drug through transdermal route, the drug candidate should posses some criteria like: low molecular weight, low melting point, high log partition coefficient etc.

Many efforts have been made to develop and to improve the transdermal delivery of drugs. It includes formulation of liposomes, drug nanocrystals, and lipid nanocarriers, such as solid lipid nanoparticles (SLN), nanostructured lipid carriers (NLC) or nanoemulsions. In recent years, much attention has focused on lipid-based formulations for transdermal delivery of drugs. In fact, the most popular approach is the incorporation of the active drugs into inert lipid vehicles such as oils, surfactant dispersions, self-emulsifying formulations, self-microemulsifying formulations, emulsions, microemulsions, nanoemulsions, and liposomes[1-15]. One of the promising technologies is nanoemulsion drug delivery system, owing to their small size, provide a large interfacial area for rapid drug release, thereby enhanced bioavailability, reduce dose size, more consistent temporal profiles of drug absorption, and the protection of drugs from the hostile environment of the body. In addition, nanoemulsion could be used as a reservoir for sustained release of drug for prolonged period of time, result in avoiding high concentration of drug in the blood[16,17].

Valsartan is an antihypertensive agent which selectively inhibits the type 1 angiotensin II receptor. It is undergo significant first pass metabolism, thereby low oral bioavailability of about 25%[18]. It has low molecular weight (435.5) and melting point (116-117^{o}C) with a log partition coefficient of 4.5 and a mean biological half-life of 7.5 hours, there are no reports of skin irritation attributed to valsartan.

The objectives of the present study were to develop potential nanoemulsion formulations for transdermal delivery of valsartan. In this perspective, nanoemulsion formulations were developed and converted into gel for easy application and better skin adherence. The formulations were subjected to *in vitro*

characterization, *in vitro* permeation study through rat skin, and skin irritation study.

2.0 MATERIALS AND METHODS

2.1 Materials

Valsratan was obtained as gift sample from Aurobindo Pharma (Hydrabad, India). Isopropyl myristate (IPM), oleic acid, castor oil, ethyl oleate, tween 80 and polyoxy-35-castor oil (Cremophor EL) were purchased from HiMedia (Mumbai, India). Span 80 was purchased from Loba Chemicals (Mumbai, India). Glycerol triacetate (Triacetin) was purchased from E-Merck (Mumbai, India). All other chemicals used in the study were of analytical grade.

2.2 Methods

2.2.1. Screening of oil

The oil was selected on the basis of their solubilizing capacity of valsartan. The solubility of valsartan in various oils was determined by adding an excess amount of drug in 5 mL of selected oils (castor oil, Triacetin, IPM, ethyl oleate, and oleic acid) in 15 mL capacity stoppered vials and kept under magnetic stirring at temperature of 25 ± 1.0˚C for 72 hours. The equilibrated samples were centrifuged at 3000 rpm for 15 min to separate the undissolved drug. The supernatant was taken and filtered through 0.45 µm membrane filter. 0.25 ml of the filtrate was diluted 1000 times with methanol and the absorbance of the sample was determined using UV-Visible spectrophotometer (UV-1700, Shimadzu, Tokyo, Japan) at 250 nm. The concentration of drug was determined from the regression equation obtained by plotting the standard curve of absorbance *versus* concentration of valsaratn in methanol (µg/ml).

Screening of surfactants

Surfactant was selected on the basis of its emulsification ability of oil in water. The modified method as reported earlier was used for the screening[19]. The accurate amount (300 mg) of surfactant was added in to 300 mg of the selected oily phase and the mixture was gently homogenized at 45–60˚C. The isotropic mixture of 50 mg was accurately weighed and diluted with distilled water to yield a final emulsion volume of 50 ml. The ease of formation of emulsion was monitored by noting the number of volumetric flask inversions required to give uniform emulsion. The emulsion was allowed to stand for 2 hr to note for any change in turbidity through visual observation and their transmittance was assessed at 250 nm by colorimeter (6051 Jenway, UK) using distilled water as blank.

Screening of co-surfactants

The cosurfactant was selected by mixing 100 mg of cosurfactant with 200 mg of the previously selected surfactant and the surfactant-cosurfactant (S_{mix}) was added to the selected oil phase. The mixture was gently heated at 45–60˚C for homogenizing the components. The 50 mg of isotropic mixture was accurately weighed and diluted to 50 ml with double distilled water to yield fine emulsion. The ease of formation of emulsions was monitored by noting the number of volumetric flask inversions required to give uniform emulsion. The resulting emulsions were observed visually for the relative turbidity. The emulsions were allowed to stand for 2 hr to note for any change in turbidity and their transmittance was assessed at 250 nm by colorimeter using distilled water as blank. As the ratio of co-surfactants to surfactant/s is the same, the turbidity of resulting nanoemulsions will help in assessing the relative efficacy of the co-surfactants to improve the nanoemulsification ability of the surfactant/s.

2.2.2. Pseudo-ternary phase diagram

On the basis of the solubility studies of drug Triacetin, Cremophor EL, and PEG 400 was selected as the oil phase, surfactant, and cosurfactant, respectively. Distilled water was used as an aqueous phase. Surfactant and cosurfactant (S_{mix}) were mixed in different weight ratios (1:3, 1:2, 1:1, 2:1, and 3:1). These S_{mix} ratios were chosen in increasing concentration of surfactant with respect to cosurfactant and increasing concentration of cosurfactant with respect to surfactant for detailed study of the phase diagrams. For each phase diagram, oil and S_{mix} at specific ratio was mixed thoroughly in different weight ratios from 1:9 to 9:1 in different glass vials. Sixteen different combinations of oil and Smix, 1:9, 1:8, 1:7, 1:6, 1:5, 2:8 (1:4), 1:3.5, 1:3, 3:7 (1:2.3), 1:2, 4:6 (1:1.5), 5:5 (1:1), 6:4 (1:0.7), 7:3 (1:0.43), 8:2 (1:0.25), and 9:1 (1:0.1) were made so that maximum ratios were covered for the study to delineate the boundaries of phases precisely formed in the phase diagrams. Pseudo-ternary phase diagrams were developed using aqueous titration method. Slow titration with aqueous phase was performed for each weight ratio of oil and S_{mix} and visual observation was made for transparent and easily flowable o/w nanoemulsions. The physical state of the

nanoemulsion was marked on a pseudo-three-component phase diagram with one axis representing aqueous phase, the other representing oil and the third representing a mixture of surfactant and cosurfactant at fixed weight ratios (S_{mix}).

2.2.3. Selection of nanoemulsion formulations from phase diagrams

From each phase diagram constructed, different formulas were selected from the nanoemulsion region so that the drug could be incorporated into the oil phase. The formulation was chosen with the criteria of; maximum oil being emulsified with minimum amount of S_{mix}. Exactly 1.5% (m/m) of valsartan, which was kept constant in all the selected formulations, was dissolved in the oil phase of nanoemulsion formulation. Selected formulations were subjected to different thermodynamic stability tests.

2.2.4. Formulation of nanoemulsion

From each phase diagram constructed, different formulas were selected from the nanoemulsion region so that the drug could be incorporated in to oil phase. 1.5% (m/m) of valsartan, which was kept constant in all the selected formulations, was dissolved in the oil phase of nanoemulsion formulation.

2.2.5. Thermodynamic stability studies

To overcome this problem of metastable formation, thermodynamic stability tests were performed, which were as follows[20]

1. Centrifugation: Selected formulations were centrifuged at 3500 rpm for 30 min.
2. Heating cooling cycle: Those formulations that did not show any phase separation on centrifugation were taken for heating and cooling cycle. Six cycles between refrigerator temperature of 4°C and 45°C with storage at each temperature of not less than 48 h were studied. Those formulations which were stable at these temperatures were subjected to freeze-thaw cycle test.
3. Freeze thaw cycle: Three freeze thaw cycles were done between -21˚C and +25˚C with storage at each temperature for not less than 48 h for the formulations.

Those formulations, which passed these thermodynamic stress tests, were selected for further studies.

2.2.6. Characterization of nanoemulsion

Globule size analysis

Globule size of the nanoemulsion was determined by photon correlation spectroscopy. The formulation (0.1 ml) was dispersed in 50 ml of water in a volumetric flask and gently mixed by inverting the flask. Measurement was done using a Zetasizer 1000 HS (Malvern Instrument, UK). Light scattering was monitored at 25°C at a 90° angle.

Viscosity

The viscosity of the formulations (0.5 g) was determined without dilution using Brookfield DVE viscometer (Brookfield Engineering Laboratories, Inc., Middleboro, MA) using spindle no. 63 at 25 ± 0.5˚C. The software used to calculate the viscosity was Rheocalc V2.6.

Transmission electron microscopy (TEM)

The surface topography of the nanoemulsion was studied using transmission electron microscopy (TEM). A JEMCX 100II operating at 200 kV capable of point-to-point resolution was used. A combination of bright-field imaging at increasing magnification and of diffraction modes was used to reveal the form and size of the nanoemulsion. To perform the TEM observations, the nanoemulsion was directly on the holey film grid and observed after drying.

Refractive index

The refractive index of placebo formulation and drug loaded formulations was determined using an Abbe-type refractometer (Macro Scientific Works, Delhi, India).

2.2.7. *In vitro* skin permeation studies

In vitro skin permeation studies were carried out on modified Keshary Chein-diffusion cell with an effective diffusional area of 3.14 cm^2 and 20 ml receiving chamber capacity, using rat abdominal skin. The full thickness of rat skin was excised from the abdominal region and hairs were removed with an electric clipper. The subcutaneous tissue was removed surgically and the dermis side was wiped with isopropyl alcohol to remove adhering fat. The cleaned skin was washed with distilled water and stored at -21°C until further use. The skin was bought to room temperature and mounted between the donor and receiver compartment of Chein-diffusion cell where the stratum corneum side was facing the donor compartment and the dermal side was facing the receiver compartment. The receiver chamber was filled

with ethanolic phosphate buffer saline pH 7.4 (30:70%, v/v). The receiver fluid was stirred with magnetic stirrer at a speed of 100 rpm and the temperature was maintained at 37 ± 1°C. One mililiter of nanoemulsion formulation (1.5% mg/ml valsartan) was placed into the donor compartment and sealed with paraffin film to provide occlusive conditions. Samples were withdrawn at regular intervals (0.5, 1, 2, 4, 8, 12, 18, and 24 hrs), filtered through 0.45 µm membrane filter and analyzed for drug content by UV-Visible spectrophotometer (UV-1700, Shimadzu, Tokyo, Japan) at 250 nm.

2.2.8. Permeation data analysis

The cumulative amount of valsartan permeated through rat skins were plotted as a function of time. The permeation rates of drug at a steady-state (J_{ss}, µg/cm^2/hr) through the rat skins were calculated from the slope of linear portion of the cumulative amount permeated through the rat skins per unit area versus time plot. The permeability co-efficient (K_p) of the drug through the membrane was calculated using the following equation[21].

$$Kp = \frac{J_{ss}}{C} \quad \text{..... (2)}$$

Where, C is the initial concentration of the drug in the donor compartment.

The penetration enhancing effect was calculated in terms of enhancement ratio (E_r) by using the following equation:

$$Er = \frac{J_{ss} \text{ of formulation}}{J_{ss} \text{ of control}} \quad \text{.....(3)}$$

2.2.9. Preparation of nanoemulsion gel formulation

On the basis of permeation study, nanoemulsion formulation NE31 was formulated to gel formulation as it shows higher permeability through rat skin. One gram of carbopol 934P was dispersed in sufficient quantity of distilled water. After complete dispersion, the solution was kept in dark for 24 h for complete swelling of carbopol 934P. The valsartan loaded nanoemulsion (NE31) was slowly added to the viscous solution of carbopol 934P under magnetic stirring. The pH was adjusted between 6-8 using 0.5 ml of triethanolamine and 1 ml of PEG 400 was incorporated to prevent the evaporation of moisture. The nanoemulsion gel formulation was kept at ambient condition after which *in vitro* permeation study of 1 g of nanoemulsion gel (1.5% mg/g) was performed using similar method as mentioned in Section 3.2.14.

2.2.10. Skin irritation study

Skin irritation study was performed on four Wister albino rats. Hairs from the back side were depleted with the help of depilatories and area was marked on both the sides. One side served as control while other as test. After 24 h gel formulation (500 mg/rat) of nanoemulsion gel (NG31) was applied for 7 days and observations were made for any sensitivity and the reaction if any was graded as: A: no reaction; B: slight, patchy erythema; C: moderate but patchy erythema; D: moderate erythema; and E: severe erythema with or without edema[22].

3.0 RESULT AND DISCUSSION

3.1 Selection of excipients

In this study, oil was selected on the basis of drug's solubility in it while the surfactant and co-surfactant were selected on the basis of their oil solubilizing and nanoemulsification efficiency.

Selection of oil

The capability of nanoemulsion to upload the drug in dissolved state in highly influenced by the solubility of the drug in the oil phase. Furthermore, oil of low drug solubility would require higher amount of oil to incorporate the desired dose of drug. Consequently, higher amount of S_{mix} would be required to maintain the miscibility of oils which might increase the side effects, toxicity and skin irritation of the system. Therefore, consideration was given to the solubility of the drug in the oil phase for the selection of oil. In the present study, solubility of valsartan in different oils was determined and presented in Fig. 1. The solubility of valsartan was found to be highest in Triacetin (49.894 ± 0.184 mg/ml) and castor oil (45.117 ± 0.973 mg/ml) as compared to the other oils. Thus, Triacetin was selected as the oil phase for the development of nanoemulsion formulation.

Screening of surfactants

The most critical problem related in the development of nanoemulsion based drug delivery systems is the toxicity of the surfactants. Large amounts of surfactants may cause skin irritation when administered transdermally. It is therefore important to determine the surfactant concentration properly and use the minimum concentration in the development of nanoemulsion formulation. In the present study, screening of surfactants was

made on the basis of their solubilization capacity for oil in water. It was reported that surfactant's solubilization capacity for oils is very important criteria, because it is not necessary that the surfactant which has the good solubilization capacity for drug definitely would have good affinity for the oil phase. Therefore, in the present study emulsification ability of different surfactants for Triacetin was determined. Lower the inversion requires of surfactant for oils means greater the nanoemulsification capacity of surfactant consequently higher the nanoemulsification area. These studies indicated that for Cremophor EL requires minimum number of inversion to emulsify the maximum amount of Triacetin followed by PEG 400, Tween 80 and Span 80 (Table 1). It is also important criterion for the selection of the surfactants is that the HLB value of the surfactant, should be greater than 10 to form o/w nanoemulsion. Hence, in the present study Cremophore EL (HLB value 12) was chosen as the surfactant for the nanoemulsion development.

Screening of co-surfactants

An important criterion for selection of the surfactant and co-surfactant is that HLB achieved by the combination should be greater than 10 to form o/w nanoemulsion. Experimentally it was found that an appropriate blend of low and high HLB surfactants leads to the formation of a stable nanoemulsion upon dilution with water. In most cases, single-chain surfactants alone are incapable to reduce the o/w interfacial tension to produce stable nanoemulsion. Co-surfactants provide sufficient flexibility to interfacial film required to take up different curvatures that is essential for formation of nanoemulsion over a broad range of composition. Co-surfactants are also added to achieve nanoemulsion systems at low surfactant concentration. Amphiphilic nature, hydrophobic chain and terminal hydroxyl groups of co-surfactants make them enable to intermingle with surfactant monolayer at the interface resulting into changes in their packing arrangement, which in turn can affect the curvature of the interface and interfacial energy. Co-surfactant was selected by evaluating its emulsification efficiency i.e. capability for oil. Like the method used for the selection of suitable surfactant, suitable surfactant co-surfactant was selected by inversion technique. Among the different co-surfactants (PEG 400, Tween 80 and Span 80), PEG 400 required to minimum inversion for emulsification of Triacetin and the transmittance was found to be maximum (Table 1). Hence, PEG 400 was selected as the co-surfactant for the nanoemulsion formulation development.

3.2. Construction of pseudoternary phase diagram

The zone of nanoemulsion formulation can be explained with the help of the pseudo-ternary phase diagrams. Phase diagrams were constructed using Triacetin as oil phase and Cremophor EL and PEG 400 as the surfactant and co-surfactant, respectively. Effect of surfactant and co-surfactant mass ratio on nanoemulsion formation was assessed for the further optimization of the system. Low o/w nanoemulsion area was observed toward the water rich apex of the phase diagram when Triacetin was used without co-surfactant i.e. at the S_{mix} ratio 1:0. It was observed that the surfactant alone was ineffective to reduce the o/w interfacial tension and failed to provide desirable nanoemulsion formulation. A large nanoemulsion gel area was obtained toward the surfactant rich apex which upon dilution with water broken down before converting into coarse emulsion. Upon increasing the amount of co-surfactant with respect to surfactant i.e. S_{mix} ratio 1:1, the maximum amount of oil that could be solubilized was 6.8% (w/w) with 16.0% (w/w) of S_{mix} (1:1) at the maximum content of water and gel area turn into easily flow able o/w nanoemulsion area in the presence of PEG 400 as a cosurfactant (Fig. 2a). This might be due to the fact that the incorporation of cosurfactant could have enhanced the penetration of the oil phase in the hydrophobic zone of the surfactant monomers, which in turn reduced the interfacial tension and increased the flexibility and fluidity of the interface, ultimately leading to increased entropy of the system. When co-surfactant concentration was doubled i.e. S_{mix} ratio 1:2 (Fig. 2b) the total area of nanoemulsion decreased as compared to S_{mix} ratio 1:1. Further increment of co-surfactant concentration i.e. S_{mix} ratio 1:3 (Fig. 2c) led to considerable reduction in microemulsion area. The maximum amount of oil i.e. 7.2% (w/w) and 8.4% (w/w) could be solubilized by using 25.6% (w/w) and 22.9% (w/w) of S_{mix} at the ratio of 1:2 and 1:3 respectively at the maximum content of water. Higher concentration of co-surfactant appeared to have a destabilizing effect on the formation of nanoemulsion resulting into substantial reduction of nanoemulsion area. On the other

hand, when the surfactant concentration of S_{mix} was increased from 1:1 to 2:1 (Fig. 2d) and 1:3 (Fig. 2e) increased in nanoemulsion region was observed in comparison to 1:1. It was observed that the oil solubility of 12.2% (w/w) and 14.2% (w/w) of S_{mix} ratio of 2:1 and 3:1 respectively at the maximum content of water.

The degree of reduction in the surface tension at the oil-water interface brought about by the surfactant and alteration in dispersion entropy were found to affect the free energy of nanoemulsion formulation. Thus, the system in which the surfactant or the S_{mix} concentration employed is able to enhance the dispersion entropy, reduce the interfacial tension, augment the interfacial area, lower the free energy system to a very small value would lead to the prospective nanoemulsion for transdermal delivery.

3.3. Selection of nanoemulsion formulations

Nanoemulsion containing 1.5% valsartan were prepared using Triacetin as the oil phase, Cremophore EL as the surfactant, and PEG 400 as the cosurfactant using phase titration (spontaneous emulsification) method. The composition of selected formulations is presented in Table 2. S_{mix} (Cremophore EL and PEG 400) was used at different ratio (1:1, 1:2, 1:3, 2:1, and 3:1). No change was observed in the phase behavior of the pseudoternary phase diagram when valsartan was incorporated in the formulations, showing desirable stability of nanoemulsions. Formulations were subjected to thermodynamic stability study to exclude the metastable formulations.

3.4. Thermodynamic stability study

The stability of nanoemulsion was evaluated after subjecting to different stress condition like centrifugation, heating-cooling cycle and freeze-thaw cycle. After performing thermodynamic studies it was observed that formulations which were developed from Smix ratio 1:1, 2:1, and 3:1 had shown good stability (Table 3). No phase separation, creaming, cracking or turbidity was observed. Very low interfacial tension between oil and water and small droplet size could be a possible reason for thermodynamic stability of these formulations.

3.5. Characterization of nanoemulsion

3.5.1. Globule size analysis

The globule size analysis of the optimized formulations was done using Zetasizer. The globule size was decreased with increase in surfactant in the S_{mix} mixture of formulations (Table 4). This result is in accordance with the report that the addition of surfactant to nanoemulsion systems causes the interfacial film to condense and stabilize, while the cosurfactant causes the film to expand. The mean globule size of the formulation, NE31, containing 13.6% of oil was 4.49 nm while as formulation NE21, containing 11.1% of oil was 57.45 nm. The mean globule size of formulation NE11, containing 11.1% of oil was 58.18 nm. The difference in the droplet size between the formulations is not statistically significant ($p > 0.05$). There is only marginal difference in the mean globule size between the formulations NE21 and NE11. The polydispersity values of all the formulations were found to be within the range of 0.65 – 0.87. The polydispersity values was found minimum in the case of formulation NE31, suggesting the uniformity in the globule size of the formulation.

3.5.2. Viscosity determination

The viscosity of the selected formulations was determined. The values are shown in Table 4. It was observed that the viscosity of all the formulations is less that 95 cP. There marginal difference in the viscosity between the formulations. The difference in the viscosity between the formulations is not statistically significant ($p > 0.05$). Formulation, NE31, has the minimum viscosity (82.55 ± 3.12 cP). Instead a hypothesis can be put forward that due to the higher viscosity of the nanoemulsions the droplets were not able to coalesce and thus retain their nanosize.

3.5.3. Transmission electron microscopy

The nanoemulsion appeared dark and with bright surroundings (Fig. 3). Some droplet sizes are measured using TEM, as it is capable of point-to-point resolution. The droplet size is in agreement with the results obtained from globule size analysis using zetasizer.

3.5.4. Refractive index

The refractive index of placebo formulations and drug loaded formulations was determined using an Abbe refractometer. The values of the refractive index of drug-loaded formulations and placebo formulations are given in Table 5. When the refractive index values for formulations were compared with those of the placebo, it was found that there were no significant differences ($p > 0.05$) between the values. Therefore, it can be concluded that the nanoemulsion

formulations were not only thermodynamically stable but also chemically stable and remained isotropic; thus, there were no interactions between nanoemulsion excipients and drug.

3.6. Formulation of nanoemulsion gel

The optimized nanoemulsion formulation was selected based on the globule size, polydispersity value, viscosity and stability of nanoemulsion and drug. From the above result it has been shown that formulation NE31, having optimum globule size (4.49 nm), minimum polydispersity value (0.651), lower viscosity (82.55±3.12 cP) and stability of nanoemulsion and drug. Therefore, formulation NE31 was selected for the formulation of nanoemulsion gel. Carbopol 934P was used as gelling agent for the formulation of nanoemulsion gel of nanoemulsion formulation NE31. The nanoemulsion gel (NG31) of nanoemulsion formulation (NE31) was subjected to evaluation of further *in vitro* permeation study.

3.7. *In vitro* skin permeation studies

The permeation ability of valsartan from various nanoemulsion formulations (NE 31, NE21, and NE11), nanoemulsion gel (NG31) and conventional gel were evaluated using *in vitro* permeation experiments through rat skin. The permeation profiles of NE31, NE21, NE11, NG31, and conventional gel through rat skins are shown in Fig. 4. *In vitro* skin permeation was highest in formulation NE31 and lowest for conventional gel. The formulation NG31 showed an intermediate skin permeation profile. The skin permeation profile of NE31 was significantly ($p < 0.05$) different when compared with that of conventional gel and NG31. The cumulative amount of valsartan from nanoemulsion formulation (NE31) through the excised rat skin was 28.5 mg/cm^2 at 24 h after application. After nanoemulsion was mixed with carbopol 934P, the cumulative amount of valsartan became 18.9 mg/cm^2. The result showed that addition of carbopol 934P into nanoemulsion decreased markedly the permeability of valsartan. It might attribute to the increased viscosity and transform from nanoemulsion to lamellar structure or a highly ordered nanostructure. The significant difference in valsartan permeation between nanoemulsion formulations, NG31, and conventional gel was probably due to the mean size of internal phase droplets, which were significantly smaller in nanoemulsions. The maximum release in NE31 could be due to having the lowest droplet size and lowest viscosity of all the nanoemulsions.

3.8. Permeation data analysis

Permeability parameters like steady-state flux (J_{ss}), permeability coefficient (K_p), and enhancement ratio (E_r) were significantly increased in nanoemulsions and the NG31 formulation as compared with conventional gel ($p < 0.05$). This is because nanoemulsions and NG31 excipients contain permeation enhancers like Triacetin. The permeability parameters of different formulations are given in Table 6. The J_{ss} and K_p of NE31 and NG31 were found to be 283.119 ± 4.164 µg/cm^2/h, 1.883 × 10^{-2} ± 0.037 cm/h and 256.610 ± 4.128 µg/cm^2/h, 1.710 × 10^{-2} ± 0.031 cm/h. The enhancement of the flux of valsartan was increased 7.219 and 6.543 times for NE31 and NG31, respectively as compared with conventional gel formulation of valsartan.

3.9. Skin Irritation study

The skin irritation study of nanoemulsion (NE31) and nanoemulsion gel (NG31) were performed to evaluate the safety of the optimized nanoemulsion formulation. The mean values of skin irritancy score following 7 days application for formulation NE31 and NG31 were found to be 1.76 ± 0.51 and 1.83 ± 0.34, respectively. Van-Abbe *et al*. reported that a value between 0 and 9 indicates that the applied formulation is generally not an irritant to human skin [22]. This value concluded that the formulations NE31 and NG31 were safe for transdermal delivery of valsartan.

Table 1: Emulsification capability of surfactants and cosurfactants

Surfactants	Maximum number of inversions*	% transmittance*	Cosurfactants + Cremophor EL	Maximum number of inversions*	% transmittance*
Cremophor EL	24 ± 3	94 ± 5	PEG 400	18 ± 3	92 ± 4
Span 80	39 ± 2	31 ± 3	Span 80	41 ± 2	37 ± 3
Tween 80	36 ± 4	54 ± 4	Tween 80	32 ± 4	59 ± 4
PEG 400	29 ± 3	71 ± 6			

*Mean ± SD, n = 3

Table 2: Composition of selected nanoemulsion formulations

Code	Oil:S_{mix} Ratio	S_{mix} Ratio	Components (% m/m)			
			Oil (%)	Surfactant (%)	Cosurfactant (%)	Water (%)
NE13	1:2.34	1:3	6.8	4.2	11.8	77.2
NE12	1:3.50	1:2	7.2	8.9	16.7	67.2
NE11	1:3.03	1:1	8.4	10.8	12.1	68.7
NE21	1:4.95	2:1	12.2	35.1	17.3	35.4
NE31	1:2.33	3:1	14.2	21.8	8.5	55.5

Table 3: Thermodynamic stability studies (centrifugation, heating-cooling cycle, and freeze-thaw cycle) of different nanoemulsion formulations

Code	Thermodynamic stability test results		
	Centrifugation	Heating cooling cycle	Freeze thaw cycle
NE13	✓	✓	---
NE12	✓	✓	---
NE11	✓	✓	✓
NE21	✓	✓	✓
NE31	✓	✓	✓

√: Test passed; --- Test failed

Table 4: Globule size, polydispersity index, viscosity of different nanoemulsion formulations

Formulation	Component (% m/m)				Globule size (nm)	PDI*	Viscosity (cP)**
	Oil	Surfactant	Co-surfactant	Water			
NE31	13.6	23.9	7.9	54.6	4.49	0.651	82.55±3.12
NE21	11.1	36.7	18.3	33.9	57.45	0.673	88.41±3.09
NE11	7.7	11.7	11.7	68.9	58.18	0.872	94.82±2.56

*PDI: polydispersity index; **Mean ± standard deviation (n = 6)

Table 5: Refractive index of drug loaded and placebo nanoemulsion formulations determined by refractometer

Formulation	Refractive index (Mean ± S.D.; n = 6)	
	Drug loaded	Placebo
NE31	1.355 ± 0.013	1.354 ± 0.010
NE21	1.381 ± 0.023	1.378 ± 0.019
NE11	1.357 ± 0.016	1.354 ± 0.002

Table 6: Permeability parameters of different nanoemulsions and nanoemulsion gel formulation (Mean ± S.D.; n = 3)*

Formulation	J_{ss} (µg/cm^2/h)	$K_p \times 10^{-2}$ (cm/h)	E_r
Conventional gel	39.221 ± 2.208	0.261 ± 0.026	-
NE31	283.119 ± 4.164	1.883 ± 0.037	7.219
NE21	238.009 ± 3.561	1.586 ± 0.009	6.068
NE11	182.675 ± 4.002	1.217 ± 0.041	4.658
NG31	256.610 ± 4.128	1.710 ± 0.031	6.543

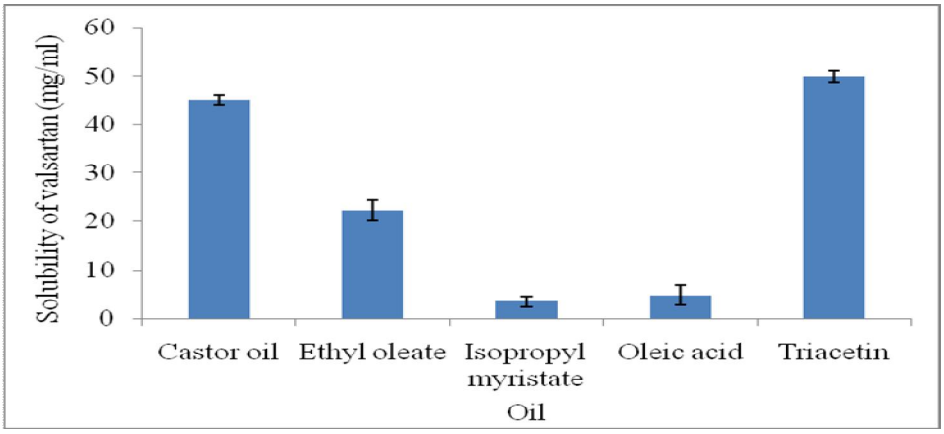

Fig. 1: Solubility of valsartan in different oils

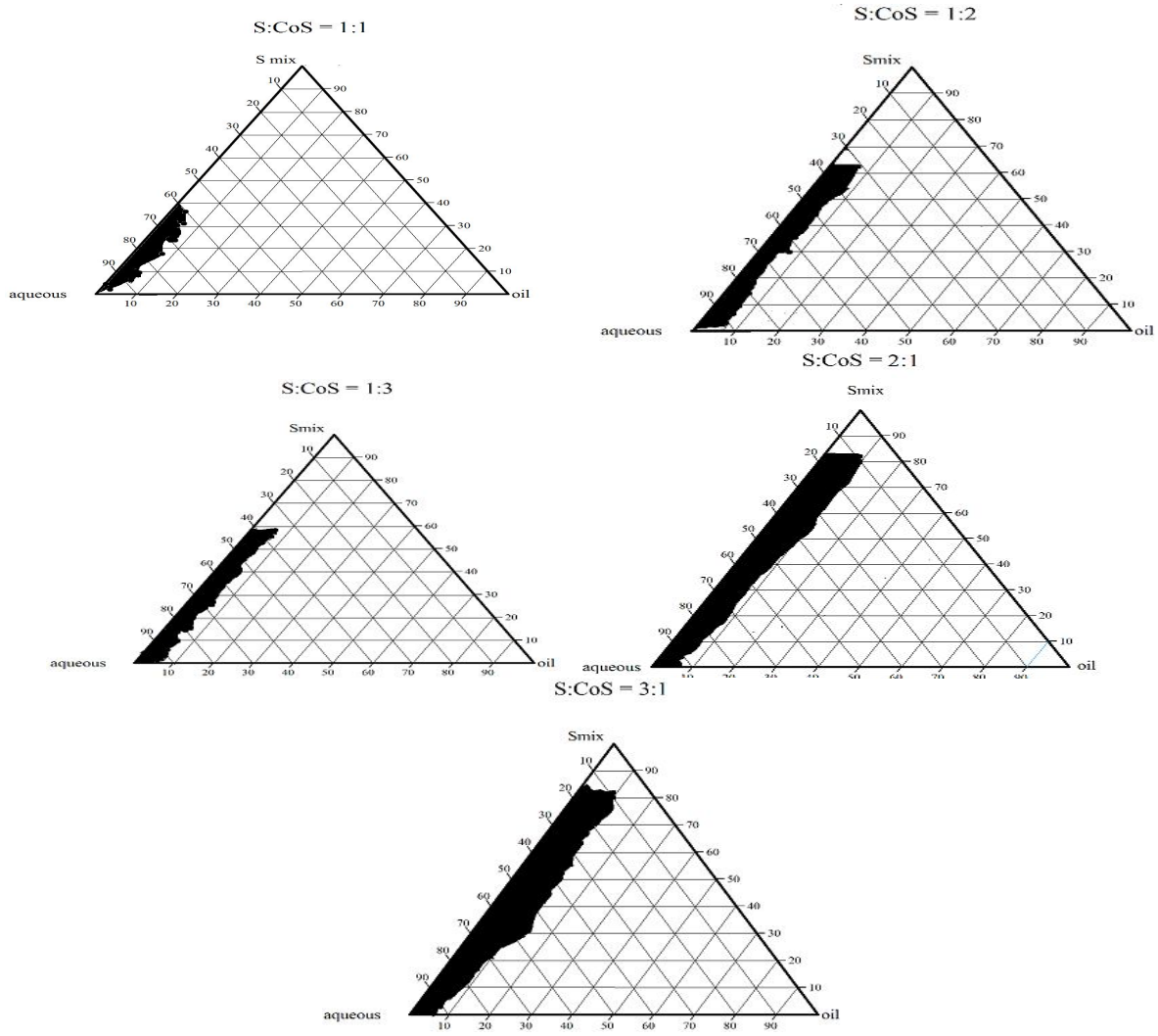

Fig. 2: Pseudoternary phase diagrams showing the o/w nanoemulsion (shaded area) regions of Triacetin (oil), Cremophore EL (surfactant), PEG 400 (cosurfactant) at different Smix ratios a) Smix 1:1 b) Smix 2:1 c) Smix 3:1 d) Smix 2:1 and e) Smix 3:1

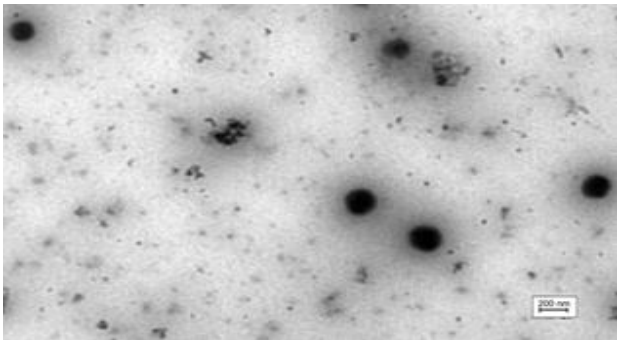

Fig. 3: Transmission electron microscopy images of globule of NE31 nanoemulsion formulation

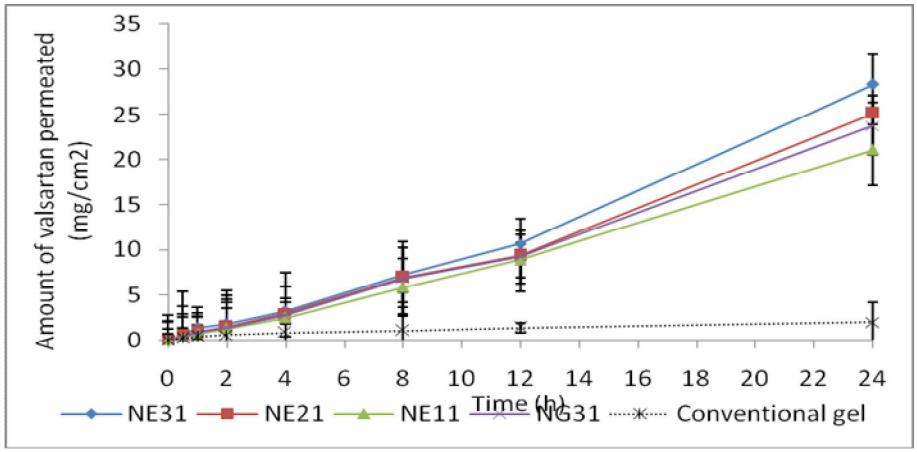

Fig. 4. In vitro permeation profile of valsartan from different nanoemulsion formulation (NE31, NE21, and NE11), nanoemulsion gel (NG31), and conventional gel through excised rat skin (n = 6)

4. CONCLUSION

The nanoemulsion formulation NE31, which contained Triacetin (13.6%), Cremophore EL (23.9%), PEG 400 (7.9%), and distilled water (54.6%) was selected as optimized formulation on the basis lowest globule size (4.49 nm), optimum polydispersity (0.651), suitable viscosity (82.55 ± 3.12 cP), low surfactant (23.9%) and cosurfactant (7.9%) concentration, and highest steady-state flux through rat skin (283.119 ± 4.164 µg/cm^2/h), and 7.219 fold enhancement of permeation valsartan as compared to conventional gel. The nanoemulsion gel (NG31) showed the desired flux as require for valsartan therapeutic activity. Therefore, it can be concluded that the nanoemulsion gel formulation (NG31) of valsartan has potential for transdermal delivery of valsartan.

5. REFERENCES

1. Stella VJ, Haslam J, Yata N, Okada H, Lindenbaum S and Higuchi T. Enhancement of bioavailability of a hydrophobic amine antimalarial by formulation with oleic acid in a soft gelatin capsule. J. Pharm. Sci. 1978; 67: 1375–1377.
2. Palin KJ, Phillips AJ and Ning A. The oral absorption of cefoxitin from oil and emulsion vehicles in rats. Int. J. Pharm. 1986; 33: 99–104.
3. Serajuddin ATM, Shee PC, Mufson D, Bernstein DF and Augustine MA. Effect of vehicle amphiphilicity on the dissolution and bioavailability of a poorly water soluble drug from solid dispersion. J. Pharm. Sci. 1988; 77: 414–417.
4. Wakerly MG, Pouton CW, Meakin BJ and Morton FS. Self emulsification of vegetable oil-non-ionic surfactant mixtures. ACS Symp. Series. 1986; 311: 242–255.
5. Toguchi H, Ogawa Y, Iga K, Yashiki T and Shimamoto T. Gastrointestinal absorption of ethyl 2-chloro-3-(4-(2-

methyl-2-phenylpropyloxy) phenyl) propionate from different dosage forms in rats and dogs. Chem. Pharm. Bull. 1990; 38: 2792–2796.
6. Charman SA, Charman WN, Rogge MC, Wilson TD, Dutko FJ and Pouton CW. Self-emulsifying drug delivery systems: formulation and biopharmaceutic evaluation of an investigational lipophilic compound. Pharm. Res. 1992; 9(1): 87–93.
7. Kararli TT, Nedham TE, Griffin M, Schoenhard G, Ferro LJ and Alcorn L. Oral delivery of a renin inhibitor compound using emulsion formulations. Pharm. Res. 1992; 9: 888–893.
8. Myers RA and Stella VJ. Systemic bioavailability of penclomedine (NSC-338720) from oil-in-water emulsions administered intraduodenally to rats. Int. J. Pharm. 1992; 78: 217–226.
9. Craig DQM, Lievens HSR, Pitt KG and Storey DE. An investigation into the physicochemical properties of self-emulsifying systems using low frequency dielectric spectroscopy, surface tension measurements and particle size analysis. Int. J. Pharm. 1993; 96: 147–155.
10. Aungst BJ, Nguyen N, Rogers NJ, Rowe S, Hussain M, Shum L and White S. Improved oral bioavailability of an HIV protease inhibitor using Gelucire 44/14 and Labrasol vehicles. B.T. Gattefosse. 1994; 87: 49–54.
11. Shah NH, Carvajal MT, Patel CI, Infeld MH and Malick AW. Self-emulsifying drug delivery systems (SEDDS) with polyglycolized glycerides for improving *in vitro* dissolution and oral absorption of lipophilic drugs. Int. J. Pharm. 1994; 106: 15–23.
12. Schwendener RA and Schott H. Lipophilic 1-beta-D-arabinofuranosyl cytosine derivatives in liposomal formulations for oral and parenteral antileukemic therapy in the murine L1210 leukemia model. J. Cancer. Res. Clin. Oncol. 1996; 122: 723–726.
13. Burcham DL, Maurin MB, Hausner EA and Huang SM. Improved oral bioavailability of the hypocholesterolemic DMP 565 in dogs following oral dosing in oil and glycol solutions, Biopharm. Drug Dispos. 1997; 18: 737–742.
14. Kang BK, Lee JS, Chon SK et al. Development of self-microemulsifying drug delivery systems (SMEDDS) for oral bioavailability enhancement of simvastatin in beagle dogs, Int. J. Pharm. 2004; 274: 65–73.
15. Bouchemal K, Briancon S, Perrier E and Fessi H. Nano-emulsion formulation using spontaneous emulsification: solvent, oil and surfactant optimization, Int. J. Pharm. 2004; 280: 241–251.
16. Osborne DW, Ward AJ and Neil KJ. Microemulsions as topical delivery vehicles: *in-vitro* transdermal studies of a model hydrophilic drug. J Pharm Pharmacol. 1991; 43: 450-454.
17. Trotta M, Pattarino F and Gasco MR. Influence of counter ions on the skin permeation of methotrexate from water-oil microemulsions. Pharm Acta Helv. 1996; 71: 135-140.
18. Ahad A, Aqil M, Kohli K, Sultana Y, Mujeeb M and Ali A. Role of novel terpenes in transcutaneous permeation of valsartan: effectiveness and mechanism of action. Drug Dev Ind Pharm. 2011; 37: 583–596.
19. Choudhury S, Dasgupta S, Dey S and Mazumder B. Topical delivery of aceclofenac as nanoemulsion comprising excipients having optimum emulsification capabilities: Preparation, characterization and *in vivo* evaluation. Expert Opin. Drug. Deliv. 2013; 10(4): 411-420.
20. Shafiq-un-Nabi S, Shakeel F, Talegaonkar S, et al. Formulation development and optimization using nanoemulsion technique: a technical note. AAPS PharmSciTech. 2007; 8: E28-E35.
21. Williams AC and Barry BW. Terpenes and lipid protein partitioning theory of skin permeation enhancement. Pharmaceutical Research. 1991; 8: 17-24.
22. Van Abbe NJ, Nicholas P and Boon E. Exaggerated exposure in topical irritancy and sensitization testing, J. Soc. Cosmet. Chem. 1975; 26: 173–187.