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RESEARCH ARTICLE

DESIGN AND DEVELOPMENT OF *O/W* NANOEMULSION FOR THE TRANSDERMAL DELIVERY OF ONDANSETRON

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Oil-in-water nanoemulsion was developed as a tool for the transdermal delivery of ondansetron, a $5HT_3$ antagonist. With an objective to select appropriate components for the formulation development, screening of oils and surfactants, co-surfactants was performed on the basis of solubility of ondansetron in oils, solibilization capacity of surfactant for different oils and nanoemulsion area of S_{mix}, respectively. Pseudo ternary phase diagrams were constructed by aqueous titration technique and various nanoemulsion formulations were developed. The developed formulations were subjected to thermodynamic stability tests. In order to evaluate the effect of nanoemulsion on skin permeation, *ex vivo* permeation of drug was performed and compared with drug solution in oil, S_{mix} and aqueous suspension. The flux of nanoemulsion formulations were in the range from 109.8-178.9 $\mu g/cm^2/h$, significantly higher ($p<0.01$) than the oil solution (control, 31.08 $\mu g/cm^2/h$), S_{mix} (14.78 $\mu g/cm^2/h$) and aqueous suspension (11.75 $\mu g/cm^2/h$). The optimized formulation was subjected to various in vitro attributes. The mean droplet size, polydispersity index, zeta potential electrical conductivity, refractive index and pH were found to be 23.70 nm, 0.27, -8.7mV, 460.17 S/cm, 1.412 and 6.2±0.219 respectively. The results of *ex vivo* permeation studies of developed nanoemulsion showed a great potential to replace oral conventional formulation and could be used for chemotherapy induced nausea and vomiting.

Key words: Nanoemulsion, Ondansetron, Ternary phase diagram, Flux, Permeability coefficient.

INTRODUCTION

Ondansetron, {1,2,3,9-tetrahydro-9-methyl-3-[(2-methyl-1*H*-imidazol-1-yl)-methyl]-4*H*-carbazol-4-one} is potent and highly selective $5HT_3$ receptor-antagonist available as an antiemetic (Currow *et al* 1997) and also indicated for the treatment and/or prophylaxis of chemotherapy- or radiotherapy- or postoperative induced emesis (Johnson *et al* 2000). The 5-HT_3 receptor antagonists are the primary drugs used to treat and prevent chemotherapy-induced nausea and vomiting (CINV) (Patel *et al* 2009). Standard regimens of ondansetron in CINV are a single dose of 8 mg by slow intravenous or intramuscular injection immediately before treatment either followed by a continuous intravenous infusion of 1 mg/h for up to 24 h, or by a further two doses of 8 mg two to four hours apart or a single dose of 32 mg given by intravenous infusion over at least 15 min. immediately before treatment. To protect against delayed emesis, these regimens are followed by oral ondansetron 8 mg twice daily or 16 mg rectally once daily, up to 5 days after the end of a course of chemotherapy.

Dosing frequency and invasive therapy of ondansetron causes great inconvenience to the cancer patients, which in turn necessitates a development a system that could maintain the therapeutic concentration of antiemetic agents safely and effectively during chemotherapy. Transdermal delivery has received increased attention in the face of growing awareness that drugs that are administered by the conventional means (tablets, capsules, parenterals) exhibit unfavorable patterns of efficacy and sometimes toxicity. Transdermal delivery could be an

effective approach to deliver the drugs by maintaining a therapeutic concentration of drug in systemic circulation for an intended period of time.

It was envisaged that the limitations of the conventional oral ondansetron therapy might be addressed by transdermal administration. Oral administration of ondansetron would be difficult at the time of frequent emesis as a chance of drug to get vomited out. Transdermal delivery could provide an alternative noninvasive route. Transdermal delivery might help in dosage reduction, and prolongation of drug release which could help in overcoming dose related side effects and increase in patient compliance, respectively. Furthermore, ondansetron suffers from low oral bioavailability about 60% due to its extensive first-pass metabolism (Simpson and Hicks, 1996). Partition coefficient (log p), molecular weight and half life of ondansetron are 2.07, 293 dalton and 3 to 5 h and depends on subjects (Sweetman, 2009) which makes it a suitable candidate for transdermal delivery. Therefore, transdermal route would be ideal for the delivery of ondansetron for the treatment of CINV.

Inspite of being a well accepted route for delivery of drugs to systemic circulation, only a few drugs could be delivered effectively through trandermal route that shows some limitation of this route. Stratum corneum of skin, the most resistant barrier to permeation of drugs is responsible for limitation of transdermal route. To overcome the limitation of this route, new delivery systems that could modify drug penetration into and through the skin are being searched. Many of them contain chemicals and non-friendly solvents to attain improved permeability**.** Such systems generally result in skin irritation, especially when chronic treatments are required. Recently, some novel carrier system like nanoemulsions are being introduced which do not require chemical enhancers to facilitate drug transportation into and through the skin. Recently, nanoemulsion is being seen as a tool for transdermal delivery of drugs as it is more stable than other vesicular systems likes liposomes, ethosomes, niosomes, etc. They are translucent mixtures of oil, surfactant, cosurfactant, and water, in which either the oil globules are dispersed in water (*o/w*) or water globule are dispersed in oils (*w/o*). These systems are single, optically isotropic and thermodynamically stable with a droplet size typically in the range of 10-100 nm (Shafiq *et al* 2007). Various studies have revealed the significance of nanoemulsions for dermal and transdermal delivery both *in vitro* and *in vivo* (Yuan *et al* 2008; Chouksey *et al* 2011). There are several facts that have been proposed to elucidate the advantages of nanoemulsions for the transdermal delivery of drugs. A large quantity of drug can be incorporated in the formulation due to the high solubilization capacity. High permeation rate of the drug may be achieved by modifying the partitioning of drugs in favor of stratum corneum. Since, it is easy to change the affinity of a drug to internal phase of nanoemulsion by varying internal phase or its composition, the components of nanoemulsions can interact with the lipid layers of stratum corneum and changes its structural organization and consequently, increases transdermal permeation of drug (Azeem *et al* 2009).

The present study is intended to investigate the potential of novel nanoemulsion formulations for the transdermal delivery of ondansetron for the prophylaxis and/or treatment of chemotherapy induced nausea and vomiting. In this perspective, nanoemulsion formulations were developed, optimized and were subjected to *in vitro* characterization and *ex vivo* permeation study.

MATERIALS AND METHODS

Ondansetron was a gift sample obtained from Cipla Ltd, India. Labrasol, transcutol, peceol were gifted by Gattefosse, Gennevilliers, France, while jojoba and olive oil were a courtesy from Nikko Chemicals (Tokyo, Japan). Acconan CC6 was obtained as a gift sample from Abitec Corporation Limited, Janesville, USA. HPLC grade acetonitrile, ethyl oleate, oleic acid, span 20, span 80, tween 80, tween 20, tween 60, PEG 400, isopropyl palmitate, isopropyl myristate were purchased from SD fine Chemicals (Mumbai, India). Water was obtained from Milli Q-water purification system (Millipore, MA). All other chemicals and solvents were of analytical grade.

Screening of nanoemulsion components

Screening of oil

The solubility of ondansetron in different oils was determined by taking 2 ml of different oils - isopropyl myristate (IPM), triacetin, labrafac, isopropyl palmitate, oleic acid, clove oil, jojoba oil, turpentine oil, castor oil, ethyl oleate, olive oil, almond oil and maisine 35 in small vial and excess amount of drug was added. The vials were tightly closed and were kept for stirring at

100 rpm and 25±1.0°C for 72 h in a water bath shaker (Nirmal International, Delhi, India). Samples were centrifuged at 3000 rpm for 15 min. The supernatant was separated, filtered through 0.22 μm membrane filter. Solubility was determined using HPLC with slight modification (Varvara *et al* 2009).

Screening of Surfactants

Five surfactants namely, tween 80, tween 60, tween 20, labrasol, acconan CC6 were used in screening of surfactant. Separately, 2.0 ml of 15 wt % solution of each surfactant were prepared in distilled water and 5 μl of oil was added with vigorous vortexing, addition of the oil was repeated until the solution became cloudy or turbid (Azeem *et al* 2009a).

Screening of co-surfactants

Selection of co-surfactant was done on the basis of nanoemulsion region. Separately, tween 20 was mixed with different types of co-surfactants namely span 80, span 20, transcutol, peceol, polyethyleneglycol 400. S_{mix} ratio 1:1 was kept constant and pseudoternary phase diagrams were constructed. Different combinations in different weight ratios of oil and S_{mix}; 1:9, 1:8, 1:7, 1:6, 1:5, 1:4, 1:3, 1:2, 1:1, 6:4 (1:0.7), 7:3 (1:0.43), 9:1; were taken so that maximum ratios can be covered to explain the boundaries of phases exactly formed in the phase diagrams (Azeem *et al* 2009a).

Effect of surfactant and co-surfactant mass ratio on nanoemulsion formation

Surfactant was mixed with co-surfactant in the weight S_{mix} ratio 1:0, 1:1, 1:2, 1:3, 1:4, 2:1, 3:1. For detail study of the phase diagrams, S_{mix} ratios were chosen in decreasing concentration of surfactant with respect to co-surfactant and increasing concentration of co-surfactant with respect to surfactant. Different combinations in different weight ratio of oil and Smix; 1:9, 1:8, 1:7, 1:6, 1:5, 02:9, 1:4, 02:7, 1:3, 3:7, 1:2, 1:1.5, 1:1, 6:4, 7:3, 8:2 and 9:1 were also taken. Aqueous titration method was used for the construction of the pseudo ternary phase diagrams. It involves stepwise addition of water to the each weight ratio of oil and Smix and mixing of the components using vortex mixer at 25°C (Chen *et al* 2004). The mixtures were evaluated visually and nanoemulsion phase was recognized as the region in the phase diagram where translucent, easily flowable and clear formulations are obtained. One axis of the pseudo-three-component phase diagram represents the aqueous phase, second one corresponds to the oil phase and the third one represents S_{mix} *i.e.* combination of surfactants and co-surfactants at a fixed weight ratio.

Development of drug containing nano-emulsion formulations

Nanoemulsion formulation of ondansetron was prepared by dissolving 0.5% of ondansetron in oleic acid. Then, required quantity of S_{mix} (Tween 80 and PEG 400) at different ratios was added to the oil phase and mixed up with the help of vortex. The final preparation was made up to 100% *w/w* by a slow addition of distilled water with continuous vortexing.

Thermodynamic stability studies of drug loaded nanoemulsions

In order to find out the stable nanoemulsion and to discard the unstable or metastable nanoemulsions, formulated nanoemulsions were subjected to thermodynamic stability testing, which comprises of various parameters. Physical stability of nanoemulsions was continuously monitored over a period of time whereas phase separation, turbidity etc. were observed at room temperature (Azeem *et al* 2009b).

Heating-cooling cycle

Formulations were subjected to different temperature 4°C and 45°C and storage of formulations at each temperature was not less than 48 h. Formulations were exposed for six cycles and then examined for stability at these temperatures.

Centrifugation test

Formulations were subjected to centrifugation at 3500 rpm for 30 min and observed for phase separation.

Freeze-thaw cycle

Three freeze–thaw cycles between -21°C and +25°C with formulation were performed and formulations were stored at each temperature for not less than 48 h.

Ex vivo skin permeation studies

Preparation of skin for permeation studies

The animal protocol to conduct skin permeation study was reviewed and approved by the Institutional Animal Ethics Committee (Approval No. 698, 2011). Abdominal skin was obtained from female albino Wistar rats weighing 200±30 g after killing by diethyl ether aspiration. The skin was cautiously removed, and the

subcutaneous tissue was excised surgically, and the dermis side was cleaned with isopropyl alcohol to eliminate adhered fat. The hairs on the skin were trimmed using electrical clipper and washed with water and examined for its integrity before further use.

Permeation studies

Ex vivo skin permeation studies across rat skin were performed using a vertical type Keshary-Chien diffusion cell. The area of diffusion between the two halves was around 0.785 cm^2. The receptor cell was filled with freshly prepared saline phosphate buffer (pH 7.4) with 0.4% of SLS. Nanoemulsion containing 0.5% mg of ondansetron was applied on the skin surface and mounted between the two halves of cells. Stratum corneum faces the donor compartment and sealed with paraffin film to provide occlusive conditions in order to prevent the evaporation of water from the formulations. In case of control, ondansetron was dissolved in aqueous saline phosphate buffer to obtain solution of 1 mg/ml. To study the permeation through neat components, same amount of drug was dissolved in Smix and oil separately and same procedure was followed. The receptor media was maintained at 37±0.5°C and magnetically stirred at 600 rpm. Samples were collected from the receiver cell at regular time interval (0, 1, 2, 3, 4, 6, 8, 12, 16 and 24 h) and replaced with the same volume of fresh media. Samples were filtered through 0.45 *μ*m membrane filter and analyzed for drug content by HPLC with slight modification (Varvara *et al* 2009).

Data analysis

The cumulative amount of permeated drug through unit surface area of skin (Q_t) was calculated using equation 1, plotted as a function of time (t, h). The skin permeation rate at steady state *i.e.* flux (J_{ss}, *μ*g/cm^2/h) was obtained from the plot. Slope of the steady state portion of the plot represents the flux whereas the x-intercept represents the lag time. The permeability coefficient (K_p, cm/h) was calculated according to the equation 2:

$$Q_t = \frac{V_r C_t + \sum_{i=0}^{t-1} V_s C_i}{A} \qquad \text{(Eq. 1)}$$

$$K_p = J_{ss} / C_d \qquad \text{(Eq. 2)}$$

where C_t is the drug concentration of the receiver solution at each sampling time, C_i the drug concentration of the *i*th sample, and V_r and V_s the volumes of the receiver solution and the sample, respectively, A represents the skin surface area and C_d is the concentration of the drug in the donor compartment.

In vitro characterization of nanoemulsion

The nanoemulsions were characterized for following *in vitro* attributes:

Droplet size and size distribution

Nanoemulsion droplet size was determined using a photon correlation spectrometer, Zetasizer 1000 HAS, Malvern Instruments, Worcestershire, UK, based on the laser light scattering phenomenon, which analyzes the fluctuations in light scattering. Nanoemulsion (0.1 ml) was diluted with 50 ml of millipore water. Light scattering was monitored at 25°C at a 90° angle. Average droplet size and size distribution of nanoemulsion were determined.

Transmission electron microscopy (TEM)

Morphology and structure of nanoemulsion were studied with the help of transmission electron microscope (TEM) (TOPCON 002B) operating at 200 KV, capable of point to point resolution. A drop of diluted (1/100 in water) nanoemulsion was allowed to deposit on the circular copper film grid of 300 mesh and observed after drying. Combination of bright field imaging at increasing magnification and of diffraction modes were used to determine the form shape and size of nanoemulsion globules.

Electrical conductivity

Electrical conductivity measurement was used to determine the nature of prepared nanoemulsion. Electrical conductivity (σ) of the nanoemulsion was assessed using a conductivity meter CDM 230 (Radiometer, Copenhagen, Denmark), having a cell constant of 0.11 cm^{-1} at the frequency of 94 Hz. The measurements were performed at 25±1°C.

Refractive index

The refractive index was determined using Abbe´s refractometer at 25±0.5°C. Standardization was performed using castor oil.

pH

The apparent pH of the formulation was determined using Decibel digital pH meter (Decibel digital technology, India) at 25±1°C.

RESULTS AND DISCUSSION

Screening of nanoemulsion components

The best criterion for screening of components of nanoemulsion is that all the excipients should be pharmaceutically acceptable for topical application and fall under GRAS (Generally regarded as safe) category. In other words, the materials should be biocompatible, non-toxic and clinically acceptable. In the present study all excipients from GRAS category were used.

Screening of oils

The capability of nanoemulsion to uphold the drug in dissolved state is highly influenced by the solubility of the drug in the oil phase. Furthermore, oil of low drug solubility would require higher amount of oils to incorporate the desired dose of drugs. 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 oils. In the present work, solubility of ondansetron in different oils of both natural as well as semi-synthetic origin was determined (**Table 1**) and was found to be highest in oleic acid (170±2.46 mg/ml) and clove oil (167±2.36) as compared to the other oils. Oleic acid has also been reported as a powerful permeation enhancer for transdermal delivery therefore it could be another advantage to use oleic acid as oily phase. Oleic acid increases the fluidity of the intercellular lipid barriers in the stratum corneum by forming separate domains which disturb the continuity of the multi-lamellar stratum corneum and creates highly permeable pathways in the stratum corneum (Puranojoti *et al* 2002; Guy and Hadgraft, 1994). Thus, oleic acid was selected as the oil phase for the development of nanoemulsion formulation.

Table 1. Solubility of ondansetron in different oils at 25°C

S. No.	Oils	Solubility (mg/ml)*
1.	Isopropyl myristate (IPM)	1.5±0.32
2.	Triacetin	1.67±0.42
3.	Labrafac	2.83±0.29
4.	Isopropyl palmitate	0.028±0.008
5.	Oleic acid	170±2.46
6.	Clove oil	167±2.36
7.	Jojoba oil	0.224±0.076
8.	Castor oil	1.93±0.091
9.	Ethyl oleate	0.64±0.12
10.	Olive oil	0.22±0.016
11.	Almond oil	0.25±0.0123
12.	Maisine 35	4.63±0.33

*Values are given as mean±SD, n=3

Screening of surfactants

In the present study, different non-ionic surfactants namely labrasol, tween 20, tween 60, tween 80 and acconan CC6 were chosen for screening. Non-ionic surfactants were included in the screening of surfactants since they are well-known for their nonirritant nature. They are less affected by changes in pH and ionic strength and are generally regarded as safe and biocompatible while the ionic surfactants were excluded from the study due to their irritant and other toxicological concern. Screening of surfactants was made on the basis of their solubilization capacity for oil in water. Although, the solubility of drugs in surfactants is also one of the criteria for the selection of surfactants, it was suggested that surfactant's solubilization capacity for oils is very important criteria (Azeem *et al* 2009a). It is not essential that the surfactant which has the good solubilizing capacity for drug definitely would have good affinity for the oil phase. In this respect, solubilization capacity of different surfactants for oleic acid was determined. Surfactant solution of 15 wt % was chosen because at this concentration surfactants were found to be more discriminatory in solubilizing the oil. At higher concentration, the effect might be lessen between the surfactants. Higher the solubilization capacity for oils means higher the nanoemulsion area consequently greater the nanoemulsification capacity of the surfactant, therefore the surfactant which had given the maximum solubilization of oils *i.e.* nanoemulsion

area was chosen for the formulation development. Tween 20 solubilized the maximum amount of oleic acid (1.52 wt %) and it was chosen as the surfactant for the nanoemulsion development. Surfactant-oil miscibility is an indicator of the prospect of nanoemulsion formation (**Figure 1**).

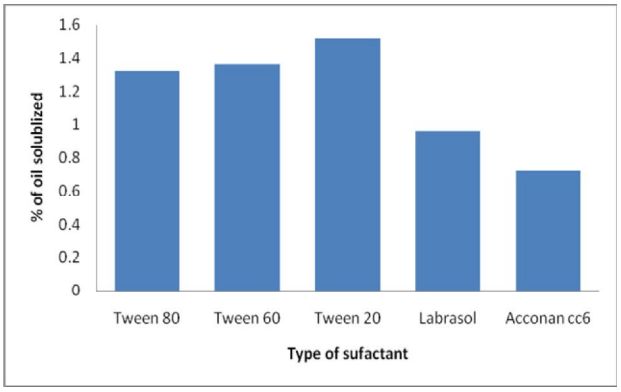

Fig. 1. Solubiliztion capacity of the different surfactants

Screening of co-surfactants

An important criterion for selection of the surfactants and cosurfactant 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 led to the formation of a stable nanoemulsion upon dilution with water (Kommuru *et al* 2001). In most cases, single-chain surfactants alone are incapable to reduce the *o/w* interfacial tension to produce stable nanoemulsion (Lawrence, 1994; Tanjarla, 1999). Co-surfactants provides sufficient flexibility to interfacial film required to take up different curvatures that is essential for formation of nanoemulsion over a broad range of composition (Lawrence and Rees, 2000; Ghosh and Murthy, 2006; Aboofazeli *et al* 1994). Co-surfactants are added to achieve nanoemulsion systems at low surfactant concentration (Kreilgaard, 2002). Amphiphilic nature, hydrophobic chain and terminal hydroxyl groups of 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 (Cavalli *et al* 1996). Co-surfactant was selected by evaluating its emulsification efficiency *i.e.* capability to provide nanoemulsion area. Tween 20 was chosen on the basis of maximum oil solubilization capacity among the tested surfactants. To select the co-surfactant, the sizes of the nanoemulsion region in the phase diagrams were compared at a fixed S_{mix} ratio (1:1) by keeping tween 20 as a surfactant and by changing the co-surfactants (**Figure 2a-e**). The presence of the co-surfactant/secondary surfactant and its type can thus affect the phase behavior of the nanoemulsion. Larger the size of nanoemulsion field, greater the nano-emulsification efficiency of the system. Amongst co-surfactants (Peceol, transcutol, span 80, span 20 and PEG 400), PEG 400 provided maximum nanoemulsion area (**Figure 2e**). Hence, it was selected as the co-surfactant for nanoemulsion formulation development.

Effect of surfactant and co-surfactant mass ratio on nanoemulsion formation

The zone of nanoemulsion formation can be explained with the help of the pseudo-ternary phase diagrams. Phase diagrams were constructed using oleic acid as the oil phase and tween 20 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. (**Figure 3a-e**). Low *o/w* nanoemulsion area was observed towards the water rich apex of the phase diagram when oleic acid was used without co-surfactant *i.e.* at the S_{mix} ratio 1:0 (**Figure 3a**). It was observed that when co-surfactant was absent or was present at very low concentration, the surfactant alone was found to be ineffective to reduce the *o/w* interfacial tension or failed to give desirable nanoemulsion formulation. Maximum concentration of oil that could be solubilized was 5.2% *w/w* at 33% *w/w* of Smix at 1:0 and a large nanoemulsion gel area was obtained towards the surfactant rich apex which upon dilution with water was 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 9.52% *w/w* with 22.22% *w/w* of Smix and whole nanoemulsion gel area turn into easily flowable *o/w* nanoemulsion area (**Figure 3b**). The presence of the PEG 400 eliminated the region of the gel from the phase diagram compared to the cosurfactant free system. This might be due to the fact that the incorporation of cosurfactant may be enhanced the penetration of the oil phase in the hydrophobic zone of the surfactant monomers consequently resulted in reduction in interfacial tension, which will directly increase the flexibility and fluidity of the interface thus increasing the entropy of the system

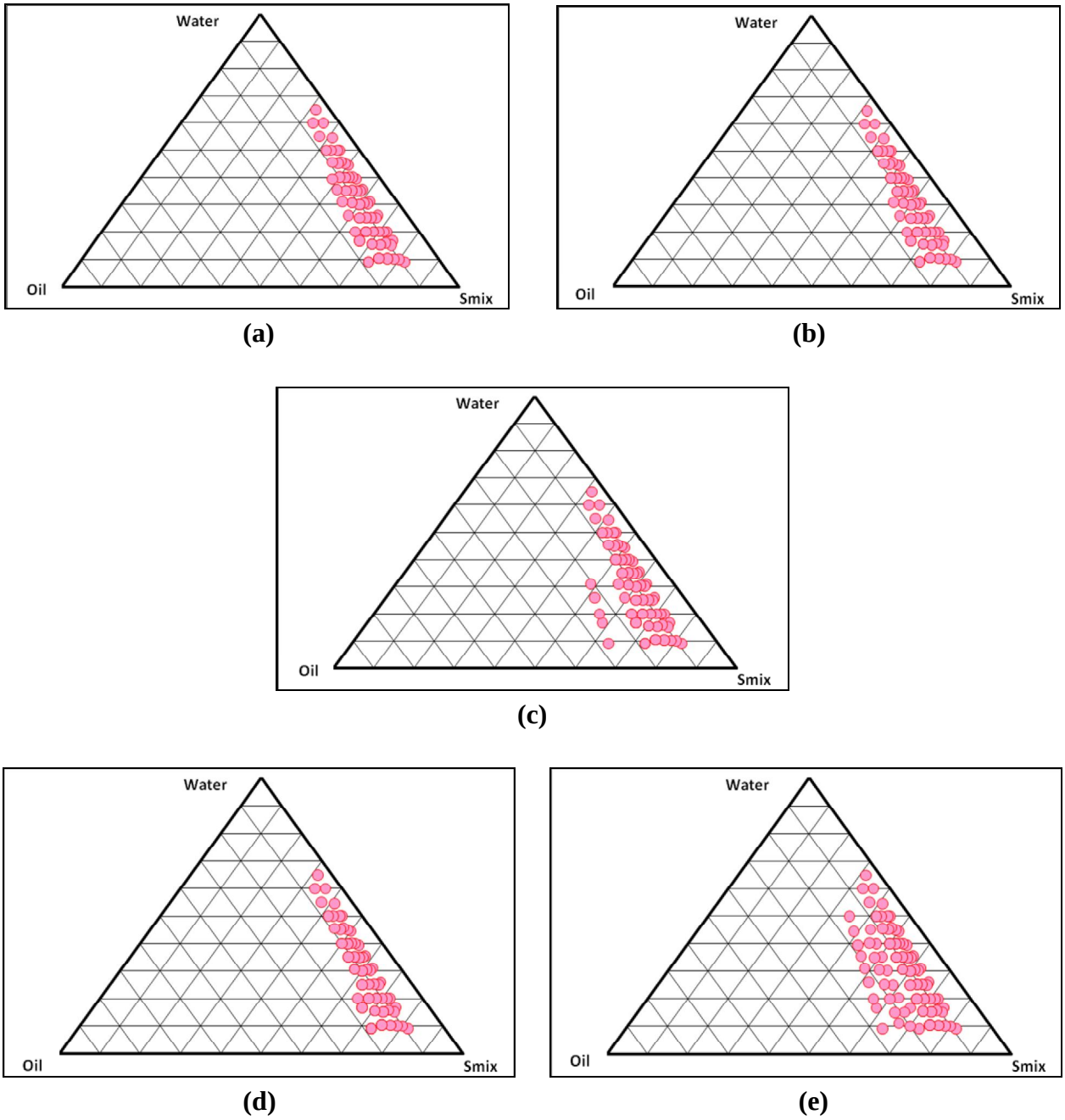

Fig. 2. Pseudoternary phase diagrams of nanoemulsion composed of oleic acid, tween 20, water and different co-surfactants (a) Peceol; (b) Transcutol; (c) Span 80; (d) Span 20; (e) PEG 400 at S_{mix} 1:1

(Warisnoicharoen *et al* 2000). When co-surfactant concentration was increased to double *i.e.* S_{mix} ratio 1:2 (**Figure 3c**) the total area of nanoemulsion decreased as compared to S_{mix} 1:1. Further, increment of co-surfactant concentration S_{mix} 1:3 (**Figure 3d**) led to the depletion of nanoemulsion area, considerably. Maximum amount of oil *i.e.* 6.3% *w/w* could be solubilized by using 21.5% *w/w* and 31% *w/w* of S_{mix} at the ratio of 1:2 and 1:3, respectively. High concentration of co-surfactant appeared to have a destabilizing effect that could be a probable factor for the substantial reduction of nanoemulsion area. On the other hand, increasing the surfactant concentration of S_{mix} from 1:1 to 2:1, depletion in nanoemulsion region was observed (**Figure 3e**) but it was higher in comparison to S_{mix} ratio, 1:0. The nanoemulsion area was further decreased at S_{mix} 3:1 and 4:1 (**Figure 3f**). It could be seen in the phase diagrams that extensive dilution of the formulations is possible in 1:1, 2:1 ratios of S_{mix} in comparison to other ratios. The surfactant and co-surfactant mass ratio was found to have pronounced effect on phase properties *i.e.* size and position of nanoemulsion zone (Hua *et al*

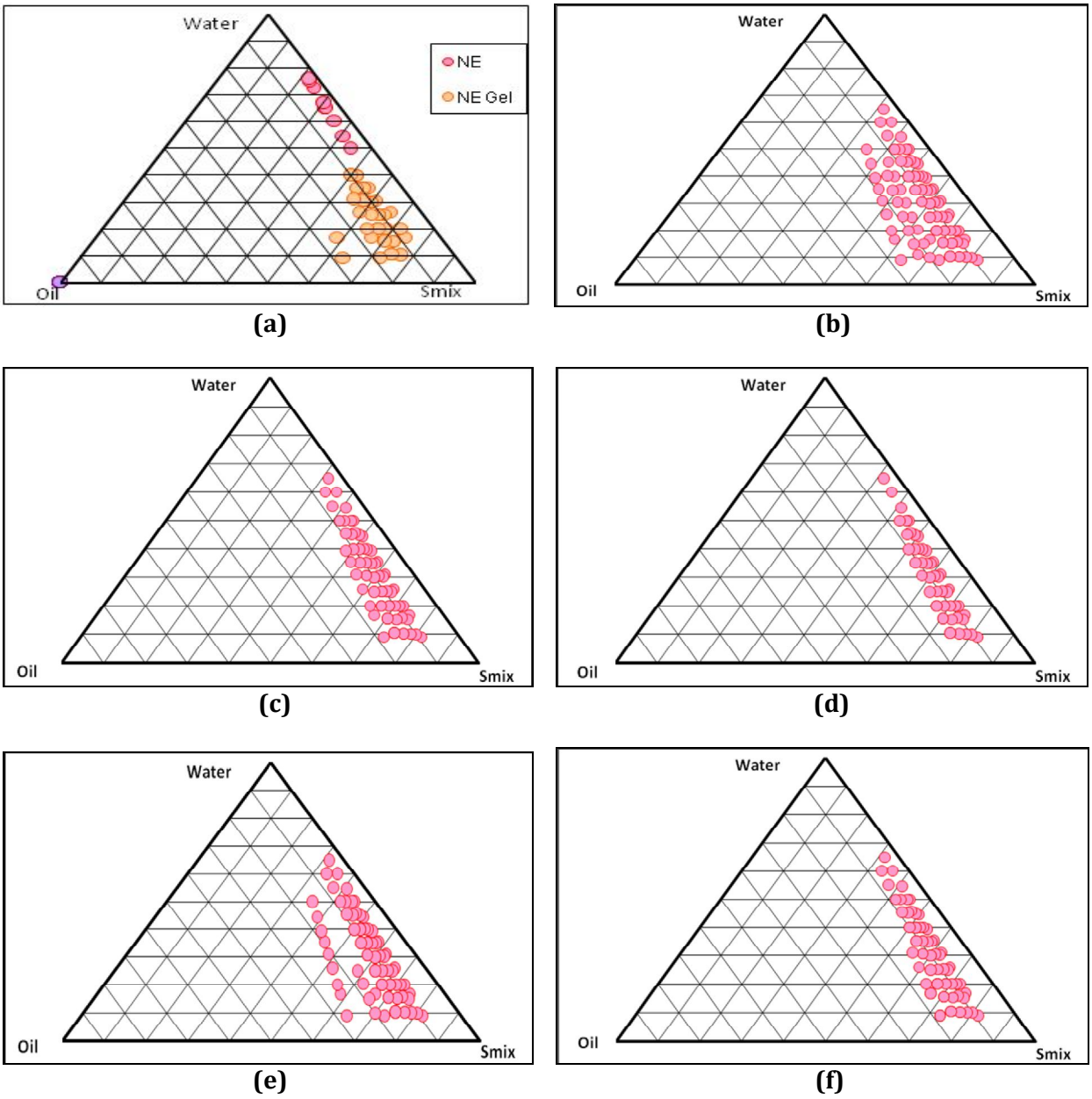

Fig. 3. Pseudoternary phase diagrams indicating *o/w* nanoemulsion region of oleic acid (oil), water tween 80 (surfactant) and PEG 400 (cosurfactant) at different S_{mix} ratios indicated in parts (**a**) (S_{mix} 1 :0), (**b**) (S_{mix} 1 :1), (**c**) (S_{mix} 1 :2), (**d**) (S_{mix} 1:3), (**e**) (S_{mix} 2 :1) and (**f**) (S_{mix} 3 :1)

2004). The concentration of oil employed also plays a role (Malcolmson *et al* 1998). S_{mix} 1:1 showed the maximum area as compared to the other ratios. Such effect is attributed to differences in the packing of surfactant and cosurfactant at the *o/w* interface. Similar findings were also reported upon varying the S_{mix} ratio of polysorbate 40/sorbitol from 1:1 to 1:3.5 (Attwood *et al* 1992).

Preparation of nanoemulsion

Nanoemulsions containing 0.5% ondansetron were prepared using oleic acid as oil phase, tween 20 as the surfactant, and PEG 400 as co-surfactant using phase titration (spontaneous emulsification) method. S_{mix} (Tween 20 and PEG 400) was at different ratio (1:1, 2:1; 3:1, 4:1, 1:2, 1:3, 1:4 etc). No change was observed in phase behavior of pseudoternary phase diagram when ondansetron was incorporated in the formulations, showing desirable stability of nano emulsions consisting of non-ionic surfactants, which were not affected by change in the pH or ionic strength (Lawrence and Rees, 2000). Formulations were subjected to thermo-dynamics to exclude metastable formulations.

Thermodynamic stability studies

The nanoemulsion formulations were subjected to various thermodynamic stability tests, which

included heating-cooling cycle, centrifugation, and freeze-thaw cycle tests. No phase separation, creaming, cracking or turbidity was observed in those formulations which were developed from S_{mix} ratio 1:1 and 1:2 as these were having high nanoemulsion area. The results showed that survived formulations had a good physical stability. Very low interfacial tension between oil and water and small droplet size could be a possible reason for thermodynamic stability of these formulations (Lawrence and Rees, 2000). Composition of formulations along with their codes is given in the **Table 2**. Large nanoemulsion region would provide the flexibility to manipulate concentration range of the ingredients to obtain maximum drug permeation and at the same time would also facilitate the selection of formulation with the low surfactant and co-surfactant concentration, desirable for preparing the non-irritating formulations. These selected formulations were subjected to *ex vivo* permeation study.

Table 2. Composition of nanoemulsion formulations

Code	% Drug	% Oleic acid	% S_{mix}	% Water
A1	0.5	5.0	15	79.5
A2	0.5	5.0	20	74.5
A3	0.5	5.0	25	69.5
A4	0.5	5.0	30	64.5
A5	0.5	5.0	35	59.5
A6	0.5	5.0	40	54.5
B1	0.5	5.0	15	79.5
B2	0.5	5.0	20	74.5
B3	0.5	5.0	25	69.5
B4	0.5	5.0	30	64.5
B5	0.5	5.0	35	59.5
B6	0.5	5.0	40	54.5

Ex vivo permeation study

In order to select the optimized formulation for *in vivo* study, *ex vivo* permeation study was performed on various nanoemulsion formulations. The flux of all the nanoemulsion formulations were found in the range from 109.8-178.9 $\mu g/cm^2/h$ (**Table 3**). These values were significantly higher ($P<0.01$) than the oil solution (31.08 $\mu g/cm^2/h$), S_{mix} (14.78 $\mu g/cm^2/h$) and aqueous suspension (11.75 $\mu g/cm^2/h$), indicating that nanoemulsion had improved the permeation of ondansetron significantly. The permeation results also indicated that composition of the formulations played an important role in permeation of ondansetron. Since, all the nanoemulsions upheld equal quantity of drug, concentration gradient is not only the factor governing permeation but other possible mechanisms were also involved in drug permeation *i.e.* enhanced membrane fluidity due to the surfactants present in the nanoemulsions, solubilization or extraction of lipids present in the stratum corneum i.e. distortion of structure of stratum corneum or alterations in the tight junction (Rege *et al* 2002; Azeem *et al* 2009b). Nanosized droplets of nanoemulsions lead to an enormous increase in the interfacial area which influences transport properties of the drug (Eccleston, 1994). It is also assumed that the low interfacial tensions, continuous and spontaneous fluctuating interfaces of nanoemulsions are supposed to be responsible for smoother permeation of drug through the skin. Another possible mechanism could be attributed to the permeation of loaded drug directly from the nanoemulsion droplets to the stratum corneum without nanoemulsion fusion at the stratum corneum, which indicated that enhancement effect of nanoemulsions was caused by the nano sized droplets, which moved easily into the stratum corneum and carried the drug through the skin barrier. Another possible reason, that could be responsible for improved permeation of drugs, was hydration of stratum corneum *i.e.* as the water content was increased from 54.5% to 79.5% in the formulations, the hydration of stratum corneum increased which might in turn led widening of transport channels due to hydration of protein of corneocytes and distortion of lipid bilayers, consequently enhancement of drug permeation.

The content of S_{mix} in the nanoemulsion formulation also affected the skin permeation

Table 3. Permeability parameters of nanoemulsion formulations

S. No.	Code	Flux (J_{ss}) ($\mu g/cm^2/h$)	Permeability coefficient (K_b)
1.	A1	109.8	0.0233
2.	A2	139.5	0.0296
3.	A3	154	0.0326
4.	A4	160.3	0.0339
5.	A5	134.3	0.0285
6.	A6	111.6	0.0236
7.	B1	166.4	0.353
8.	B2	177.4	0.367
9.	B3	178.2	0.376
10.	B4	178.9	0.379
11.	B5	172.2	0.365
12.	B6	169.4	0.359
13.	Oil solution	31.08	0.0066
14.	S_{mix}	14.87	0.0032
15.	Control (aqueous suspension)	11.75	0.0025

*Values are given as mean±SD, n=3

rate of ondensetron. As the percentage of S_{mix} (2:1) was increased from 15.0 to 30.0% the rate of permeation also increased. However, further increment of percentage of S_{mix} led to the depletion of drug permeation**.** The percutaneous permeation of ondansetron from formulations, prepared from S_{mix} ratio (2:1) was found to be in the following order B1<B6<B5<B2<B3<B4. This might be attributed to decreased thermodynamic activity of the drug in the nanoemulsion at the higher concentration of surfactants (Shah *et al* 1994). The driving force for the release and penetration of the drug into the skin is the thermodynamic activity of a drug in the formulation, at higher surfactant concentration, affinity of drug would be higher to the vehicle and thermodynamic activity would be lower that will be responsible for slow release of the drug and/or a poor transfer of drug from the vehicle to the skin. Similar pattern was seen when the nanoemulsion were prepared from S_{mix} ration 1:1 A1<A6<A5<A2<A3<A4 (**Figure 4a, 4b**). Thus, from above results it can be deduced that the composition of nanoemulsions plays an important role in permeation enhancement of drug.

Throughout the permeation study a trend was observed, *i.e.* reduction in flux and increment of lag time by increasing the amount of S_{mix} after an optimum level which might be due to reduction in thermodynamic activity of drug in the nanoemulsion at higher concentration of S_{mix}. Fortunately, this is a favorable trend in concern to safety issues as higher content of surfactant might cause skin irritation. Hence, the use of high content of surfactants is not desirable.

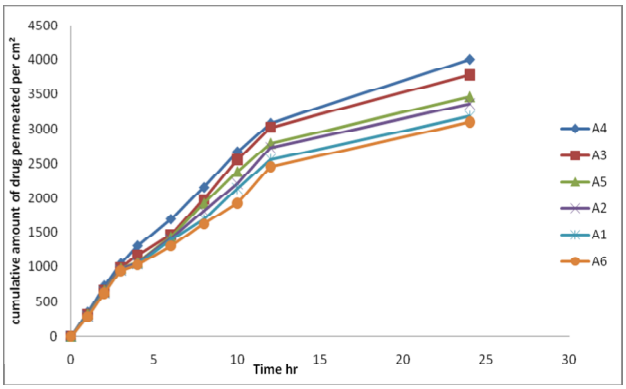

Fig. 4a. *In vitro* skin permeation of ondansetron from nanoemulsion of S_{mix} (Tween 20/ PEG 400) with S/CoS ratio 1:1

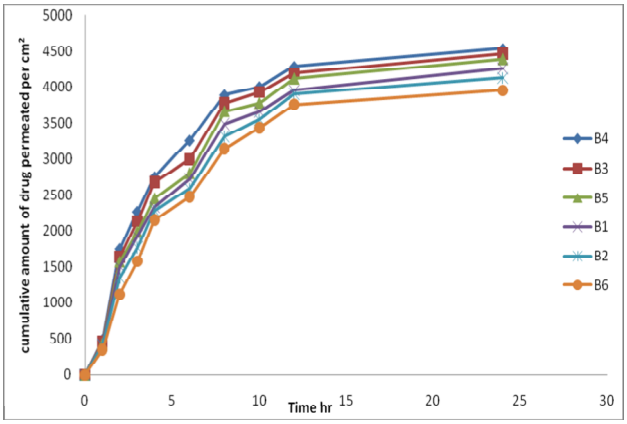

Fig. 4b. *In vitro* skin permeation of ondansetron from nanoemulsion of S_{mix} (Tween 20/ PEG 400) with S/CoS ratio 2:1

Hence, the use of high content of surfactants is not desirable.Skin irritation has been a challenging issue in the development of safe and nonirritant transdermal delivery vehicle.

Characterization of nanoemulsion

Among the formulations tested, formulation B4 which was composed of antiemetic - 0.5% ondansetron, 5.0% oleic acid, 30.0% S_{mix} and 64.5% distilled water, showed the highest permeation flux *i.e.* 178.8 $\mu g/cm^2/h$**.** Therefore, it was selected for *in vitro* characterization and preparation of nano-emulsion gel (**Table 4**).

Droplet size and size distribution

The main feature of nanoemulsions is the strict droplet size, which must be in nanometer range. Therefore, size analysis was performed to confirm whether the resultant emulsions were indeed nanoemulsions. Droplet size of the nanoemulsion was determined by photon correlation spectroscopy. The average droplet size of nanoemulsion was found to be 23.70 nm. Polydispersity index (PDI) indicates uniformity of droplet size within the formulation and its stability. The value of PDI was found to be 0.27. Low value of PDI indicated uniform distribution of nano droplets within the formulation.

Table 4. Characteristic of optimized nanoemulsion formulation (B4)

S. No.	Parameter	Value
1.	Droplet Size nm	23.70
2.	Polydispersity index	0.27
3.	Droplet Shape	Spherical
4.	Electrical conductivity µS/cm	461.37±23.26
5.	Refractive index	1.412±0.041
6.	pH	6.2± 0.22

*Values are given as mean±SD, n=3

Transmission electron microscopy

The shape of droplets was determined by TEM. TEM image revealed that the lipid emulsion droplets were almost spherical in shape, discrete, appearing dark and has the amorphous core (**Figure 5**). Some droplet sizes were measured and the droplets were in nanometer range varying from 30-60 nm. The results of TEM analysis were in agreement with the droplet size measured by dynamic light scattering.

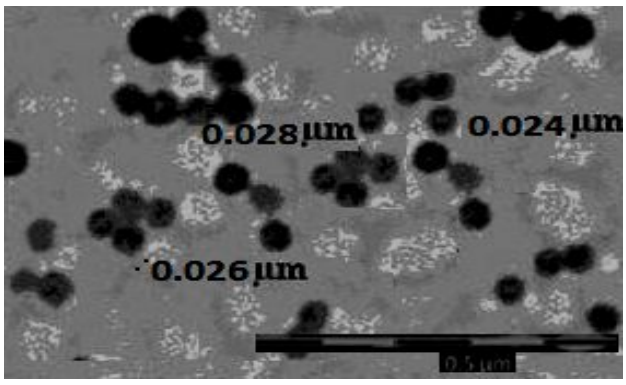

Fig. 5. TEM image of nanoemulsion formulation (B4)

Electrical conductivity

Electrical conductivity of the formulations was determined to check the stability and assert the nature of the formulation. It was found to be 460.17μS/cm. The higher conductivity of nanoemulsion is attributed to a larger percentage of water which allows more freedom for mobility of ions. There was no significant change in conductivity of formulations even after storage of one month at room temperature, indicating stability of formulations with no sign of phase inversion.

Refractive index

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 (B4) was found to be 1.412.

pH

The pH value for the optimized drug loaded nanoemulsion (B4) was recorded to be 6.2±0.219, which is favorable for topical application since the pH of the skin range from 5.5 to 7.0.

CONCLUSION

Selection of appropriate oil, surfactant/co-surfactant is vital factor to develop an efficient nanoemulsion formulation with optimum drug loading. Attention should be given to the tolerability of excipients and all the excipient must be of GRAS category. The ability of

various co-surfactants to influence the nanoemulsification of the selected surfactant and impact of surfactant: co-surfactant weight ratio in the formulation of the nanoemulsion systems was studied. Oleic acid was found to be suitable oil for ondansetron. The highest nanoemulsion region was obtained at tween 20 : PEG 400 in the mass ratio of 1:1. The optimized nanoemulsion formulation, which exhibited highest drug permeation, consisted of 0.5% *w/w* ondansetron, 5% *w/w* oleic acid 90, 30% *w/w* S_{mix} (2:1), and 64.5% *w/w* water. The permeation studies were conducted under the occlusive conditions. Under in-use conditions, water of the formulation might evaporate quickly leaving the oil/surfactant on skin which might alter not only the thermodynamic activity of the drug but also the micro-structure of the nanoemulsion. The results of *ex vivo* permeation study revealed that the oil, S_{mix} and drug concentration significantly affect the drug permeation across the skin from the nanoemulsion. Based on *ex-vivo* and *in-vivo* data, it can be concluded that the developed nanoemulsion for transdermal delivery showed a great potential to replace available oral conventional formulation and could be used for CINV. The future perspective includes elaborated preclinical, toxicological and clinical studies for developing clinically viable formulation.

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