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Research Article

INFLUENCE OF COMPONENTS OF NANOEMULSION SYSTEM FOR TRANSDERMAL DRUG DELIVERY OF NIMODIPINE

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ABSTRACT

Nimodipine, a dihydropyridine calcium channel blocker is used in the treatment of hypertension. It is a highly lipophilic, poorly water soluble API. Due to its extensive first-pass metabolism after oral administration it has bioavailability of only around 13%. Topical transdermal option of this potential API has been reported. Chemical penetration enhancers in many such products may cause skin irritation on prolonged therapy. As nanoemulsions do not need the chemical enhancers, they are advantageous over the conventional transdermal drug delivery systems. The aim of the present study was to develop nimodipine nanoemulsion formulation for topical administration.The nanoemulsion formulation consisted of Triacetin & IPM (Isopropyl myristate) (1:1) as an internal oil phase in external aqueous phase, Tween 80 as surfactant and PEG-400 as cosurfactant. Pseudoternary phase diagram was developed to determine the effect of the surfactant to cosurfactant mass ratio (S_{mix}) on the nanoemulsion formation, a transparent region. The optimized nanoemulsion formulation was subjected to physicochemical and thermodynamic stability studies. All the selected formulations were found to be stable. Novel nimodipine nanoemulsion formulation could be designed and projected to be suitable for transdermal application.

Keywords: Nimodipine, nanoemulsion, transdermal, nonionic surfactant, pseudoternary phase diagram.

INTRODUCTION

Nimodipine is an effective dihydroperidine calcium channel blocker used in the treatment of hypertension[1]. It is a lipophilic poorly-water soluble drug and undergoes extensive first-pass metabolism after oral dosing. This results in its low bioavailability. The elimination half-life is also very short i.e. about 1-2 hours[1]. Transdermal drug delivery system provides a novel platform for lipophilic drugs, where through proper selection of the formulation not only the bio availability might be improved, the side effects of oral administration would also be avoided[2].

Nanoemulsion is transparent and thermodynamically stable liquid-in-liquid dispersion with droplet size in the range of 10-100 nm[3, 4]. It is composed of oil phase, water phase and surfactant in combination with cosurfactant. It provides high solubilization potential for lipophilic compounds. Its nanosized internal phase droplets have increased surface area, which due to extremely small drop size influence the penetration of drug through the skin. One of the important properties of nanoemulsion is that they improve therapeutic efficacy of the drug and reduce the volume of the drug delivery system, which in turns minimizes the toxic side effects[5]. The key for the successful formulation of such desirable transdermal option lies in proper selection of oil, surfactant and cosurfactant. No such work has been reported for nimodipine. The *objective* of this study was to provide a logistic screening approach for transdermal drug delivery of nimodipine.

MATERIALS AND METHODS

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 gift samples from Gattefosse 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). 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 Milli Q water purification system (Miliipore, MA). All other chemicals and solvents procured from local market in Mumbai were of analytical grade.

Screening of components

The solubility of nimodipine in various oils, surfactants and cosurfactant was determined by making an attempt to dissolve. An excess amount of drug was added in 2ml of each of the selected oils, surfactants and cosurfactants employing 5-ml stoppard vials and mixed using a vortex mixer (Dolphin, Mumbai). Combinations of the oils were also employed to determine the solubility. The vials were kept at 37±1.0 ºC in an isothermal shaker (EXPO HI-TECH, Mumbai, India) for 72 hour to arrive at equilibrium. The equilibrated samples were removed from the shaker and centrifuged at 3000 rpm for 15 min. The supernatant was filtered through a 0.45 µm membrane filter (Neha enterprises, Thane). The filtrate was determined for the dissolved API in each oil, surfactant, cosurfactant by HPLC at 238 nm.

1) Oils: Oleic acid, castor oil, olive oil, labrafil 1944 CS, soybean oil, IPM, triacetin&labrafac PG.
2) Surfactants: Labrasol, Tween 80 & Tween 20.
3) Co-surfactants: PEG-200, PEG-400, propylene glycol, capryol PGMC and Transcutol P.

Formulation of nanoemulsion

The combination of Triacetin and IPM (1:1) was selected as the oil phase. Tween 80 and PEG-400 was selected as surfactant and cosurfactant respectively. Surfactant was blended with cosurfactant in the weight ratio of (1:0, 1:2, 1:3, 1:1, 2:1, 3:1, and 4:1). These ratios identified as S_{mix} were chosen in increasing concentration of surfactant with respect to cosurfactant as well as increasing concentration of cosurfactant with respect to surfactant for detailed study of the phase diagrams needed for nanoemulsion formulation. Water phase titration method was used for the construction of pseudoternary phase diagrams. Sixteen different combinations in different weight ratios of oil and S_{mix} 1:9, 1:8, 1:7, 1:6, 1:5, 1:3, 2:8 (1:4), 1:3.5, 1:2, 3:7(1:2.3), 4:6(1:1.5), 5:5(1:1), 6:4(1:0.7), 7:3(1:0.43), 8:2(1:0.25), 9:1(1:0.1) were taken. It involved stepwise addition of water to each ratio of oil and S_{mix} and then mixing the components with the help of vortex mixers at 25 ºC. Based on the visual observation, easily flowable and transparent region was

identified as relevant to nanoemulsion. The physical state of the nanoemulsion formulation was marked on a pseudo-3-component phase diagram with one axis aqueous phase, the second one oil and the third one representating a mixture of surfactant and cosurfactant at fixed weight ratios (S_{mix} ratio).

Formulation of nimodipine-loaded nanoemulsion

Nanoemulsion region being identified with the help of pseudoternary phase diagram, different o/w nanoemulsion formulations corresponding to different S_{mix} weight ratios were selected so that the drug could be incorporated into the oil phase. Stock oil phase of nimodipine was prepared by dissolved drug in oil phase. The drug concentration was kept constant for all selected formulations. These formulations were subjected to different thermodynamic stability tests to assess their physical sturdiness.

Stability studies

Selected formulations were subjected to accelerated physical stability studies.

A) Centrifugation: Formulations were centrifuged at 3000 rpm for 25 min and observed for phase separation. The formulations that did not show any phase separation confirmed by visual observation were taken for the heating and cooling cycling.

B) Heating–cooling cycling: Six cycles between the refrigerator temperature (4 ºC) (LG, India) to and fro 45 ºC in a hot air oven (Microlab, Mumbai, India) with storage of 48 hour at each temperature were conducted and the formulation which were not showing turbidity and phase separation at these temperatures subjected to a freeze–thaw cycling.

C) Freeze-thaw cycling: These cycles were performed for the formulations between-21 ºC and 25 ºC for 48 hours. One freeze-thaw cycle consisted of storing of nanoemulsion at -21 ºC for 24 h after that they were stored at 25 ºC for another 24 h. Three such freeze thaw cycles were carried out and then the physical stability of the nanoemulsion was observed. The formulations which survived stability tests were carried forward for characterization. The composition of selected nanoemulsion formulations are given in table 1.

Characterization of nanoemulsion formulations

Microscopy

Morphology and structure of the nanoemulsion were studied using transmission electron Microscopy (TEM) employing Philips CM200 operating at 200 KV (Philips, Netherland) and capable of point-to-point resolution. To procure the TEM observations, a drop of diluted nanoemulsion was applied to a 200 mesh copper grid and left for 2 min. After this the grid was kept inverted and a drop of phosphotungstic acid (PTA) was applied to grid for 1 sec. Excess of PTA was removed by washing with water and absorbing on a filter paper. The grid was kept under IR lamp for half an hour for drying and was analyzed using the instrument operated at 200 KV.

Droplet Size

Droplet size distribution was determined by photon correlation spectroscopy that analyzes the fluctuations in light scattering due to Brownian motion of the particle, using a zetasizer 1000HS (Malvern Instuments, UK). Light scattering was monitored at 25 ºC at a 90º angle. A solid state laser diode was used as light source. The optimized nanoemulsion sample was suitably diluted with distilled water, placed in quartz couvette and subjected to droplet size analysis.

Viscosity

The viscosity was determined using Brookfield viscometer LV DV-E (Brookfield Engineering, USA) using spindle no. 2(62) in triplicate at 25 ºC.

Refractive Index

The refractive index of placebo (without the drug) and drug loaded formulations were determined by an Abbe- refractometer (Erma, Japan), by placing one drop of the formulation on the slide in triplicate at 25 ºC.

HPLC

Quantitative analysis of Nimodipine was carried out by a validated HPLC method[6]. 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.

RESULT AND DISCUSSION

All the components for topical administrations should be pharmaceutically acceptable, nonirritant and nonsensitizing to the skin and fall under GRAS (Generally regarded as Safe) category.

Screening of components

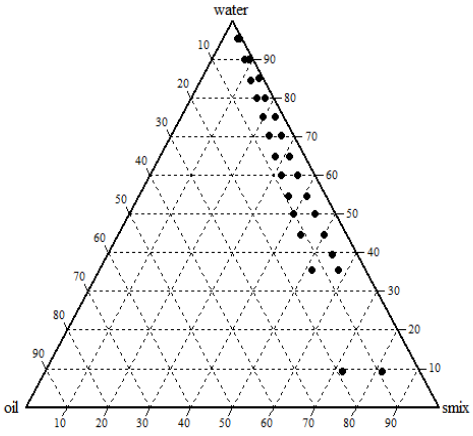
The most important criterion for screening of components is the solubility of a poorly soluble drug in oil, surfactant and cosurfactants. Selection of formulation components mainly depends on the highest solubility of the drug in oil, surfactant and cosurfactants[7, 8]. For the present study the oil, surfactant and cosurfactant selection was based on the maximum nanoemulsion region. Maximum nanoemulsion region provides flexibility to the formulator to load the drug in nanoemulsion. Drug loading per formulation and the volume of the formulations are the important factors to be considered for the therapeutic need, which minimizes the side effects and maximizes the bioavailability[9]. Thus, higher solubility of the drug in the oil phase is an important criterion, as it would help the nanoemulsion to maintain the drug in solubilized form. To achieve the maximum drug loading in the oil phase of nanoemulsion, the oil which has maximum solubilising capacity for drug candidate and gives the large nanoemulsion region is selected as an oily phase for the formulation. Oil in water (o/w) nanoemulsion is an excellent solubilizing system for hydrophobic drugs[10]. The formulation which is having the oil of low drug solubility would require more oil to incorporate the required drug dose. Thus, the higher surfactant concentration would require for oil solubilization which might increase the toxicity of the system[11]. Hence, the solubility of the drug in the oil phase becomes an important factor for the selection of oil. For the present study, oil from two different categories such as long-chain triglycerides (isopropyl myristate), and short-chain triglycerides (triacetin) in (1:1) ratio were selected as an oil phase for the development of nanoemulsion formulation.

Nanoemulsion, thermodynamically unstable results in the addition of surfactant or blend of surfactants that stabilizes the formulation. Surfactants with cosurfactant have the high solubilization capacity for oil phase by enhancing the micelle formation[12]. Based on the nanoemulsion region suitable emulsifier was selected to study the phase diagram behavior of oil. 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[13]. 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.

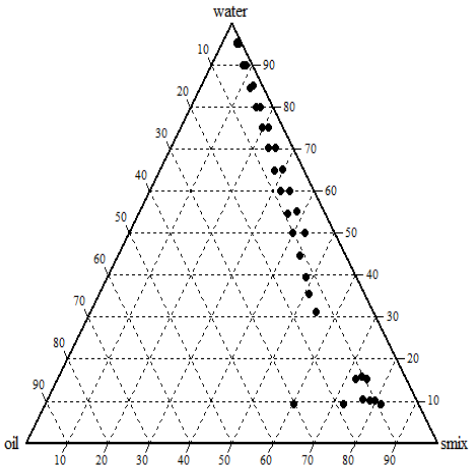
Addition of cosurfactant is necessary, which further reduces interfacial tension and increases the adsorption at the surface, where nanoemulsion region obtains at low S_{mix} concentration. They can also prevent the formation of a viscous phase[14]. Thus, the cosurfactant (PEG-400) which showed the maximum nanoemulsion region was selected for the study.

Nanoemulsion formulation with effect of mass ratio of surfactant and cosurfactant (S_{mix})

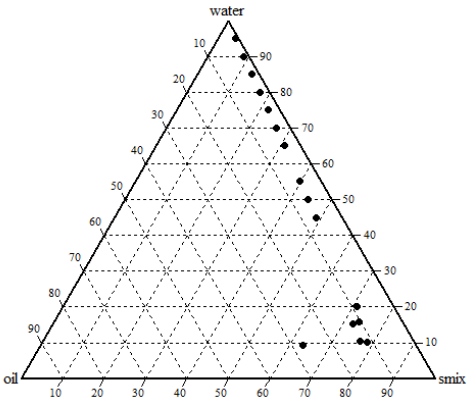
The nature and amount of surfactant and cosurfactant plays an important role in influencing the phase properties such as size distribution and position of nanoemulsion region[15]. Figure 1. [Pseudoternary phase diagrams showing nanoemulsion (shaded area) region of Triacetin and IPM (1:1) oil, Tween 80 (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.1A) has a low nanoemulsion area. Might be; the cosurfactant when present or absent at lower concentration the surfactant was not able to sufficiently reduce the o/w interfacial tension. Hence, 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. 1A) and 1:1 (fig. 1B), 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.



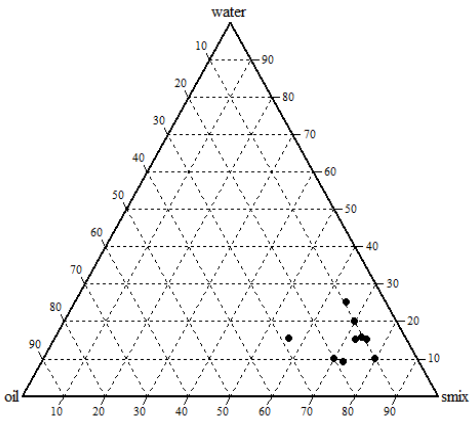
A) S_{mix} 1:0



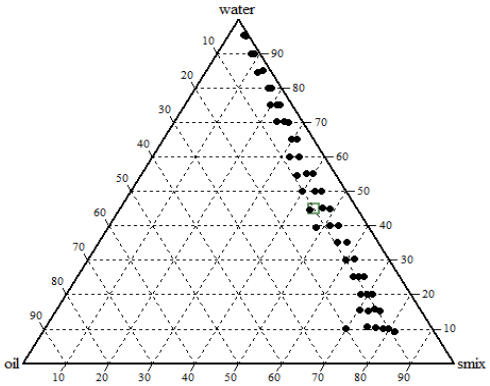
B) S_{mix} 1:1

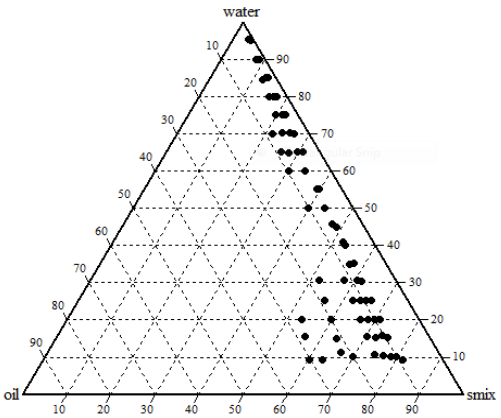


C) S_{mix} 1:2

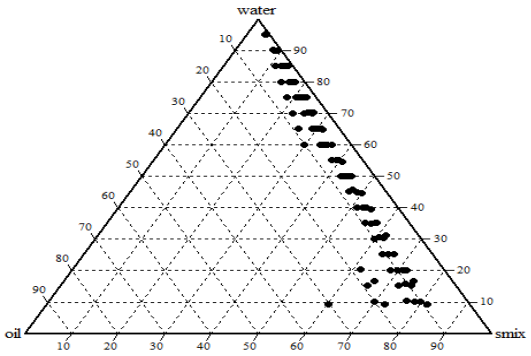


D) S_{mix} 1:3





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



G) S_{mix} 4:1

FIG. 1: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} 4:1

The maximum concentration of oil that could be solubilized by S_{mix} ratio 2:1 (fig. 1E) was 20.00% w/w by using 70.00% w/w of S_{mix}. In S_{mix} ratio 3:1 (fig. 1F), 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. 1G), 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 1C, fig1D). Hence, it was attempted up to S_{mix} 1:3.

The prepared nanoemulsion formulations were found to be transparent and easily flowable. The formulation which consists of the lowest possible S_{mix} concentration was selected for the further evaluation. The maximum drug permeation and flux is very much depended on affinity of the drug for nanoemulsion composition. The highest amount of surfactant and cosurfactant limits the maximum flux by decreasing the thermodynamic activity of the drug in the system[16]. Thus, the formulation consisting lowest S_{mix} concentration was selected. The selected nanoemulsion formulations were subjected to thermodynamic stability studies.

Stability Studies

To avoid the possibility of metastable formulation[17], 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[18]

Characterization of nanoemulsion formulations

Transmission electron microscopy (TEM)

Fig 2 is the TEM. The droplets in the nanoemulsion appear dark and the surroundings are bright, a "positive" image was seen using TEM. Some droplet sizes were measured using TEM as it is capable of point to point resolution.

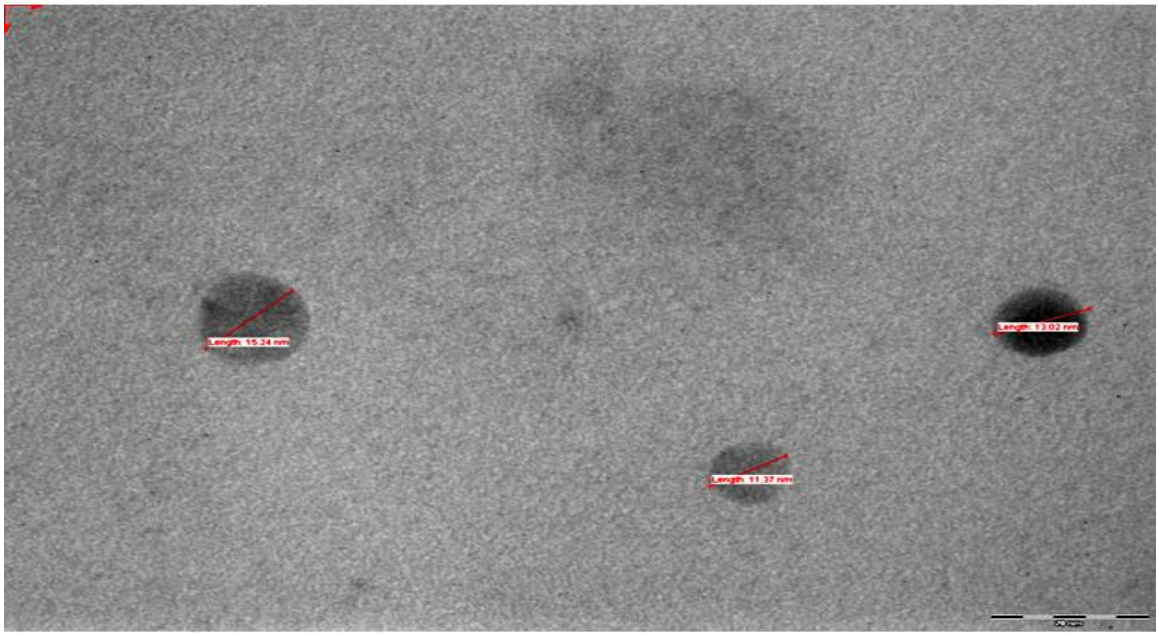


Fig 2: Transmission electron microscopic positive images of nimodipine nanoemulsion

Droplet Size

Table 3. depicted as all the formulations had droplets in the nano size range. Polydispersity is the ratio of standard deviation to the mean droplet size. Lower uniformity of a formulation could be possible with high value of polydispersibility. The low polydispersibility values were observed for all the formulations (0.101-0.394), which indicated the uniformity of droplet size within each formulation. Constant mass ratio of surfactant results in larger droplet size. The droplet size increased as the volume of the disperse phase increased in the formulation[19].

Table 1: Composition of selected Nanoemulsion Formulations

Formulation code	S_{mix} Ratio	oil(%w/w)	S_{mix}(%w/w)	Water(%w/w)
NE 1	1:1	8.00	32.00	60.00
NE 2	1:1	9.09	36.36	54.55
NE 3	2:1	8.00	32.00	60.00
NE 4	2:1	9.09	36.36	54.55

Table 2: Solubility of nimodipine in different components at 25 ºC

Components	Solubility (mg/ml)	Components	Solubility (mg/ml)
Olive oil	4.28	Triacetin	Triacetin
Propylene glycol	0.19	Capryol PGMC	81.7
IPM	8.40	Transcutol P	535.0
Oliec acid	9.78	Tween 20	192.0
Polyethylene glycol 200	14.90	Triacetin+IPM (1:1)	100.4
Soyabeen Oil	24.40	Labrasol	256.0
Castor oil	24.40	Labrasol	256.0
Labrafac PG	35.80	Polyethylene glycol 400	135.6
Triacetin + Oliec acid (1:1)	36.50	Triacetin+Labrafac (1:1)	174.0
Labrafil 1944 CS	91.90	Triacetin+Labrafil 1944 CS (1:1)	164.0

Table 3: The characteristics of the nanoemulsion formulations

Formulation code	Globule Size(nm) mean ± SD	Polydispersity mean ± SD	Viscosity(cP) mean ± SD	Refractive index mean ± SD
NE1	11.00±0.058	0.131±0.037	29.33±0.235	1.406±0.002
NE2	14.36±0.241	0.348±0.065	31.66±0.942	1.410±0.001
NE3	10.39±0.074	0.394±0.115	36.00±0.577	1.409±0.001
NE4	46.67±13.48	0.101±0.029	39.00±0.816	1.407±0.026

Viscosity

In Table 3. an increase in the viscosity of the formulation was observed. As the volume of dispersed phase increased, the S_{mix} concentration increased to stabilize decreased in mean oil droplets size and thus the continuous phase viscosity increased. Overall, very low viscosity of the nanoemulsion formulations was observed[20].

Refractive Index

Table 3. shows that the mean value of the refractive index for all the formulations was relatively similar. Refractive index indicates the isotropic nature of the formulation. Thus, it could be concluded that the nanoemulsion formulations were not only physically stable but also chemically stable without interaction between nanoemulsion components and drug.

CONCLUSION

The size and region of existence of nanoemulsion was strongly influenced by the presence of surfactant and cosurfactant in the system. Selection of components for nanoemulsion formulation was based on maximum nanoemulsion region facilitated by the different components. Crucial steps for formulation of nimodipine as nanoemulsion was carried out successfully. The results of above formulation suggested that in future, nanoemulsion formulation in the form of dermal gel can be prepared effectively. It could be concluded that nanoemulsion system can be introduced as a novel transdermal formulation for nimodipine.

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