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Research Article

INVESTIGATION OF NANOEMULSION SYSTEM FOR TRANSDERMAL DELIVERY OF GLIBENCLAMIDE

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ABSTRACT

The aim of the present study was to investigate the nanoemulsion system for enhanced percutaneous penetration of glibenclamide (GLBD). Nanoemulsions do not need the chemical enhancers; they are advantageous over the conventional transdermal drug delivery systems. Pseudoternary phase diagrams were constructed in order to optimize the surfactant, cosurfactant and surfactant: cosurfactant weight ratios (Smix). The nanoemulsion formulation consisted of Labrafac and Triacetin (1:1, ratio) as an internal oil phase in external aqueous phase, Tween 80 as surfactant and diethylene glycol monoethyl ether 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. Nine nanoemulsion formulations were selected and characterized. The nanoemulsion formulations had small droplet size (<117nm), uniform size distribution (PI, < 0.247), and low viscosity (<73.0mP). All the selected formulations were found to be stable. Novel GLBD nanoemulsion formulation could be designed and projected to be suitable for transdermal application.

Keywords: Nanoemulsions; Transdermal Delivery; Pseudo Ternary Phase Diagrams; Glibenclamide.

INTRODUCTION

Nanoemulsions (NE) are quaternary systems composed of an oil phase, a water phase, surfactant frequently in combination with cosurfactant[1-3]. These spontaneously formed systems possess precise physicochemical properties such as transparency, optical isotropy, low viscosity and thermodynamic stability. In stable nanoemulsion, droplet diameter is usually within the range of 10-100 nm (100–1000 A°) [4]. Due to their unique physicochemical properties, NE offer advantages over traditional topical and transdermal drug delivery formulations. Many studies have shown that NE formulations possess improved transdermal and dermal delivery properties both in vitro[5-17], as well as in vivo[18-22]. The high solubilizing capacity of NE enables them to increase the solubility of poorly water-soluble drugs. Both, increase in solute concentration and the tendency of the drug to favor partitioning into the stratum corneum make NE a useful vehicle to enhance transdermal drug permeability[23]. As demonstrated by a recent publication[18], the transdermal permeation rate of a lipophilic drug significantly increased from NE as compared to macroemulsions. In macroemulsions the free mobility of the active material between the internal (disperse) phase to the external (continuous) phase within the structure of the formulated system is limited due to the strong interactions between the surfactants that form tight interfacial film. In NE, the co-surfactant lowers the interfacial tension of the surfactant film, resulting in a more flexible and dynamic layer[3, 14]. The drug in this energy rich system can diffuse across the flexible interfacial surfactant film, a thermodynamic process that increases partitioning and diffusion into the stratum corneum. This article is intended to demonstrate the feasibility of new o/w NE system for transdermal delivery of glibenclamide (GLBD).

Glibenclamide is a second generation sulphonylureas oral hypoglycemic agent used for the management of diabetes mellitus. It causes hypoglycemia by stimulating release of insulin from pancreatic b cells and by increasing the sensitivity of peripheral tissue to insulin[24].Owing to its high portion of hepatic first pass metabolism (~50%) [25], its low molecular weight (494 Da), its moderate lipophilicity (Log P, 4.8) as well as its clinical effectiveness in low doses[26, 27] (5mg to 15mg), the percutaneous application of GLBD provides, therefore, a preferred alternative to the oral dosage form.

Transdermal administration of GLBD might offer some advantages over oral route in subject with the treatment of hyperglycemia in non-insulin dependent diabetes mellitus (NIDDM), but has been associated with severe and sometimes fatal hypoglycemic reactions and gastric disturbances like heartburn, nausea, vomiting, anorexia and increase appetite after oral therapy because of high inter individual variation[28, 29]. Since these drugs are usually intended to be taken for a long period, patient compliance is also very important. Transdermal drug delivery system (TDDS) provides a means to sustain drug release as well as reduce the intensity of action and thus reduce the side effects associated with its oral therapy. The NE system is a promising vehicle due to powerful ability to deliver drug through skins[13]. The stable NE system consisted of oils as combination of labrafac and triacetin (1:1), commonly used nonionic surfactant (Tween 80), non-irritant cosurfactant (diethylene glycol monoethyl ether) and water was prepared, its physicochemical properties and transdermal permeation ability of GLBD were characterized.

MATERIALS AND METHODS

Materials

GLBD was a gift sample from Cipla (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 and olive oil were purchased from genuine chemicals (Mumbai, India). Triacetin (glycerin triacetate), tween 80, tween 20 and polyethylene glycol 200 (PEG-200) were purchased from Ozone chemicals (Mumbai, India). Polyethylene glycol 400(PEG-400), propylene glycol (PG) and n-butanol were purchased from E-Merck (Mumbai, India).Isopropyl myristatae (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 used in the study were of analytical grade.

UHPLC/ESI-qTOF/MS Analysis of GLBD in Samples from Receptor

Chromatographic conditions UHPLC system (Waters ACQUITY UPLC, Waters Corp., USA) coupled with Waters Q-TOF Premier TM (Synapt

Mass Spectrometry, Serial No. JAA272, Manchester, UK) mass spectrometer was used for pulmonary studies. BEH C18, 100.0 mm × 2.1 mm; 1.7 µm (ACQUITY UPLC, Waters Corp., MA, USA) column was used as stationary phase. The mobile phase for UHPLC analysis consisted of acetonitrile: 2 Mm ammonium formate: formic acid (70:30: 0.1% v/v) which was degassed prior to use. The total chromatographic run time was 4.0 min. The flow-rate was set at 0.2 mL min−1 and 10 µL of sample solution was injected in each run. Quantitation was performed using Synapt Mass Spectrometery (Synapt MS) of the transitions of m/z 494.0 → 369.03 for GLBD with a scan time of 1.0 min scan time, and 0.02 s inter-scans per transition. The optimal MS parameters were as follows: capillary voltage 3.0 kV, cone voltage 20 V, source temperature 120 ⍰C and de-solvation temperature 350 ⍰C. The optimum values for compound-dependent parameters like trap collision energy (Trap CE) and transfer collision energy were set to 13.5 and 4.0 respectively for fragmentation. Nitrogen was used as the de-solvation and cone gas with a flow rate of 500 and 50 L h−1, respectively. Argon was used as the collision gas at a pressure of approximately 5.3 × 10−5 Torr. The accurate mass and composition for the precursor ions and for the fragment ions were collected in multi-channel analysis (MCA) mode were acquired and processed using Mass Lynx V 4.1 software. The MS scan and chromatogram for GLBD showed protonated daughter [M+H] + ions at m/z 494.11 → 369.03 and retention time at 1.99 min shown in Fig. 1. The drug was analyzed in filtrate by UHPLC/ESI-qTOF/MS. The study was done in triplicate

Preparation of Nanoemulsions

Determination of Solubility of GLBD in Oils, Surfactants and Co-surfactants

To find out the suitable oil which can be used as the oil phase in NE and provide excellent skin permeation rate of GLBD, the solubility of GLBD in various oils (oleic acid, IPM, olive oil, castor oil, triacetin, labrafac and combination of labrafac and triacetin (1:1) ratio was measured. The solubility of GLBD in various surfactants (sorbitan monolaurate, sorbitan monoleate, polyoxyethylene sorbitan monolaurate, Polyoxyethylenes orbitan monooleate, capryol PGMC, labrafil 1944 CS and labrasol) and cosurfactants (diethylene glycol monoethyl ether, PEG 200, PEG 400 and propylene glycol) was also determined. An excess amount of GLBD was added in 2.0 mL of the selected oil, surfactant and cosurfactant in stoppered vials (capacity 5.0 mL) and then preliminary mixing was carried out over magnetic stirrer for few minutes. Later on, these vials were kept in mechanical bath shaker for 72 hours at 37 ± 0.5 ^{0}C. The equilibrated samples were centrifuged at 10,000 rpm for 15 min. The supernatant was separated, filtered and after appropriate dilution with methanol, solubility was determined by validated UHPLC/ESI-qTOF/MS.

Construction of Pseudo-Ternary Phase Diagram

In order to optimize surfactant and cosurfactant, phase diagrams for each surfactant and cosurfactant combinations were constructed by using aqueous titration method. The ratio of each of selected surfactant to cosurfactant (Smix) was kept constant (1:1) while oil to Smix ratio was taken 1:9. Surfactant and cosurfactant were selected on the basis of the number of NE points demonstrated by phase diagrams. After selection of surfactant and cosurfactant, their optimum concentration ranges were determined by detailed study of phase diagrams using different ratios of Smix (1:0, 1:1, 2:1, 3:1, 1:2, 1:3). For each Smix ratio, oil: Smix ratio was varied. Total sixteen different combinations of oil and Smix (1:9, 1:8, 1:7, 1:6, 1:5, 1:4, 1:3.5, 1:3, 1:2.3, 1:2, 1:1.5, 1:1, 1:0.7, 1:0.43, 1:0.25, 1:0.1) were made so that maximum ratios was covered for the study to delineate the boundaries of phases precisely formed in the phase diagrams. Slow titration with aqueous phase was performed to each weight ratio of oil and Smix and visual observation were carried out for transparency, flowability and physical state of NE. For preparation of drug loaded NE, GLBD (5 mg/mL) was added to the oil phase of the NE.

Formulation of GLBD 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 GLBD was added to the mixture of oil, surfactant cosurfactant with varying component ratio as described in Table 1, and then appropriate amount of water was added to the mixture drop by drop and the nanoemulsion containing GLBD was obtained by vortexing the mixture at ambient temperature[30]. The drug concentration was kept constant for all selected formulations. These formulations were subjected to different thermodynamic stability tests to assess their physical durability.

Thermodynamic Stability of Nanoemulsions

To assess the thermodynamic stability of drug loaded NE, clarity, phase separation, droplet size and drug content were evaluated before and after subjecting to following stress tests as previously reported[31].

• Heating cooling cycle: NE formulations were subjected to six cycles between refrigerator temperature (4°C) and 45°C (storage not less than 48 h at each temperature). Stable formulations were then subjected to centrifugation test[32].

• Centrifugation: Formulations were centrifuged at 3500 rpm for 30 min and those that did not show any phase separation were taken for the freeze thaw stress test.

• Freeze thaw cycle: Formulations which passed centrifugation test were subjected to three freeze thaw cycles between -21 °C and +25 °C (storage not less than 48 h at each temperature).

Characterization of Nanoemulsions

Transmission Electron Microscopy (TEM)

Morphology of the NE was studied using TEM (Philips, Netherland) operating at 200 KV 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. Combination of bright field imaging at increasing magnification and of diffraction modes was used to reveal the form and size of the NE. In order to perform the TEM observations, the diluted NE was deposited on the holey film grid and observed after drying.

Droplet Size and Size Distribution

Droplet size was determined by photon correlation spectroscopy that analyzed the fluctuations in light scattering due to Brownian motion of the particles[33], using a zetasizer 1000HS (Malvern Instruments, UK).The formulation (0.1 mL) was dispersed in 50 mL of water in a volumetric flask, mixed thoroughly with vigorous shaking and light scattering was monitored at 25°C at a 90° angle. A solid state laser diode was used as light source. Polydispersity index (PI), for the formulations was determined as ratio of standard deviation to the mean droplet size of the formulation.

Viscosity

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

Refractive Index and pH

Refractive index of NE was determined using an Abbes type refractometer ((Erma, Japan),) at 25 ± 0.5 °C. The apparent pH of the formulation was measured by pH meter (Elico, India) in triplicate at 25 ± 1ºC.

Conductivity

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

RESULT AND DISCUSSION

Screening of Oil

Being a moderately lipophilic drug, it was very important to find out an appropriate solvent to dissolve GLBD, because only the dissolved drug can permeate through skin. In order to screen appropriate

solvent/s for the preparation of NE, the solubility of GLBD in various oils, surfactants and co-surfactants was measured. Solubility of GLBD in labrafac and triacetin (1:1) ratio was found to be 23.02 ±0.39 mg/mL, which was the best among the oils investigated was selected as an oil phase in the present study. Based on preliminary solubility studies, the surfactants; tween 80 (34.00 ±0.39 mg/mL), and co-surfactants; di-ethylene glycol mono-ethyl ether (97 ±0.41 mg/mL), which was the best among the surfactants and co-surfactants shown in Fig. 2. For the present study the oil, surfactant and cosurfactant selection was based on the maximum nanoemulsion region in the pseudo ternary phase diagram. Maximum nanoemulsion region provides flexibility to the formulator to load the drug in nanoemulsion. Constructed pseudo-ternary phase diagrams are self explanatory about the presence of NE region which assists easy selection of ingredients proportions for preparation of stable formulation. Large NE region would also facilitate the selection of formulation with low surfactant and cosurfactant concentration, desirable for preparing non irritating formulations.

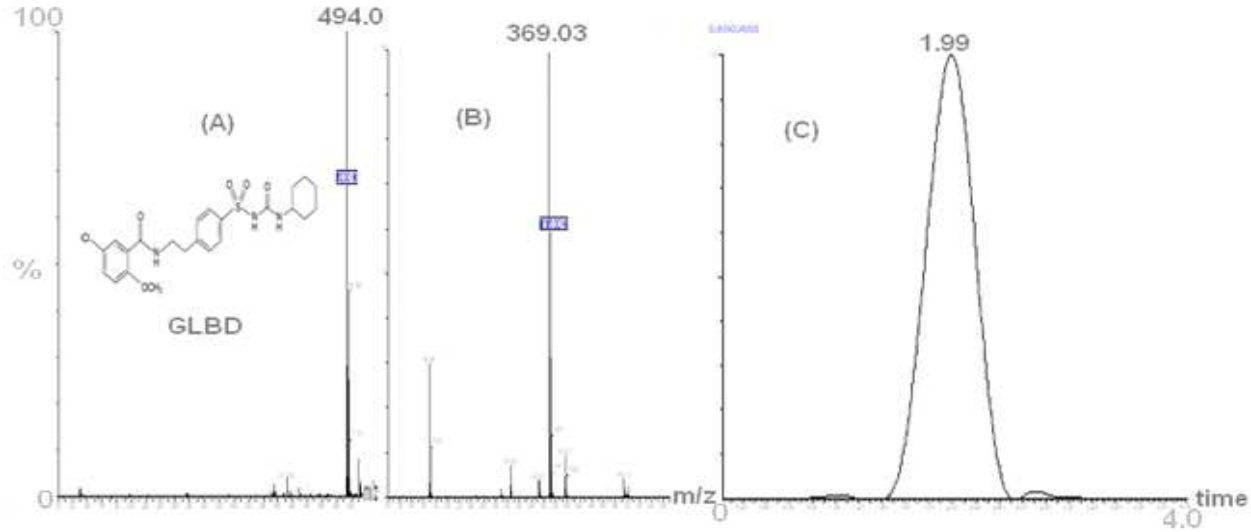

Fig. 1: Mass spectrum of: (A) GLBD precursor ion (protonated precursor [M+H]+ ions at *m/z* 494.0); (B)GLBD product ion (major fragmented product ion at *m/z* 369.03); (C) The Peaks of the GLBD

Screening of Surfactant/Cosurfactant

Aqueous titrations were done by taking polysorbate 20, tween 80, span 20, span80 and caprylocaproyl macrogol-8-glyceride as surfactant with different cosurfactant diethylene glycol mono-ethyl ether, PEG 200, PEG 400 and propylene glycol. Sixteen possible combination were made and in each combination a constant surfactant to cosurfactant ratio (1:1) was taken, while oil to Smix ratio was kept at 1:9 since higher concentration of Smix is favorable for maximum NE formation[4,31]. Each combination was titrated with water and the resultant physical state of NE was marked on a pseudo ternary phase diagram with one axis representing the water, one representing oil and the third representing a Smix. 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[34]. Thus co-surfactant diethylene glycol mono-ethyl ether showed the maximum nanoemulsion region. After studying the results, it was found that the combination consisted of tween 80 as a surfactant, and diethylene glycol mon-oethyl ether as a cosurfactant was selected for the study.

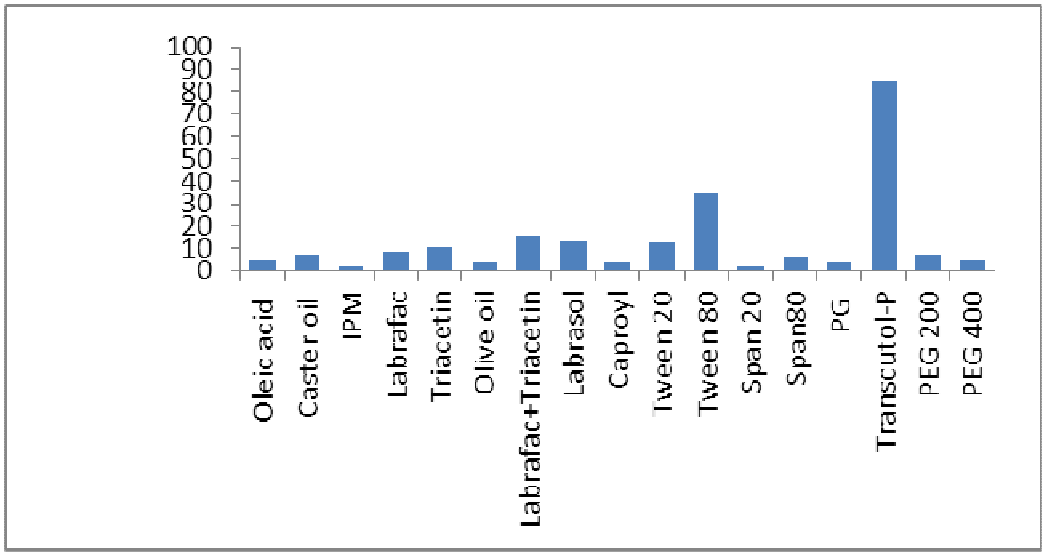

Fig. 2: Solubility of GLBD in different oils, surfactant and cosurfactant. IPM indicates Isopropyl myristate, PG Propylene Glycol and PEG =Polyethylene Glycol

Phase Diagram Study

Physical appearance of all NE formulations showed no distinct conversion boundaries from w/o to o/w at all Smix ratios. The rest of the region on the phase diagram represents the turbid and conventional emulsions based on visual observation. Significant difference was seen in ternary phase diagrams of NE constructed with different Smix ratio in Fig. 3, a-f.

It was observed, when tween 80 was used alone without diethylene glycol mono-ethyl ether (Smix ratio 1:0), very low amount of labrafac and triacetin could be solubilized at high concentration of tween 80 (Fig. 3, a). This could be attributed to the fact that transient negative interfacial tension and fluid interfacial film is rarely achieved by the use of single surfactant, usually necessitating the addition of a cosurfactant[3, 30]. At equal amounts of tween 80 and diethylene glycol mono-ethyl ether (Smix ratio 1:1), the NE region in the phase diagram increased significantly (Fig. 3, b) compared to that obtained at Smix ratio 1:0 (Fig. 3, a). The presence of diethylene glycol mono-ethyl ether (cosurfactant) decreases the bending stress of interface and makes the interfacial film sufficiently flexible to take up different curvatures required to form NE over a wide range of compositions [3,35]. However, when concentration of diethylene glycol mono-ethyl ether with respect to tween 80 was increased (Smix ratio 1:2 and 1:3) the NE area was decreased (Fig. 3, c and d, respectively) compared to Smix ratio 1:1 (Fig. 3. b). The decrease in the NE area is possibly due to presence of low concentration of tween 80 which reduces the amount of micelles and consequently decreases the solubilization capacity of NE[15]. Moreover, NEs formed at Smix ratio 1:3 were unstable and showed phase separation within 24 h (data not shown). In contrast to this, when concentration of diethylene glycol mono-ethyl ether with respect to tween 80 was decreased (Smix ratio 2:1), the NE area was increased (Fig. 3 e) compared to Smix ratio 1:1 (Fig. 3 b). However, at further lower diethylene glycol mono-ethyl ether concentrations (Smix ratio 3:1), the NE area was increased (Fig. 3, f), compared to Smix ratio 1:2 and 1:3 (Fig. 3, c and d).In brief, system at Smix ratio 2:1 formed large isotropic NE region than the systems at other Smix ratios.

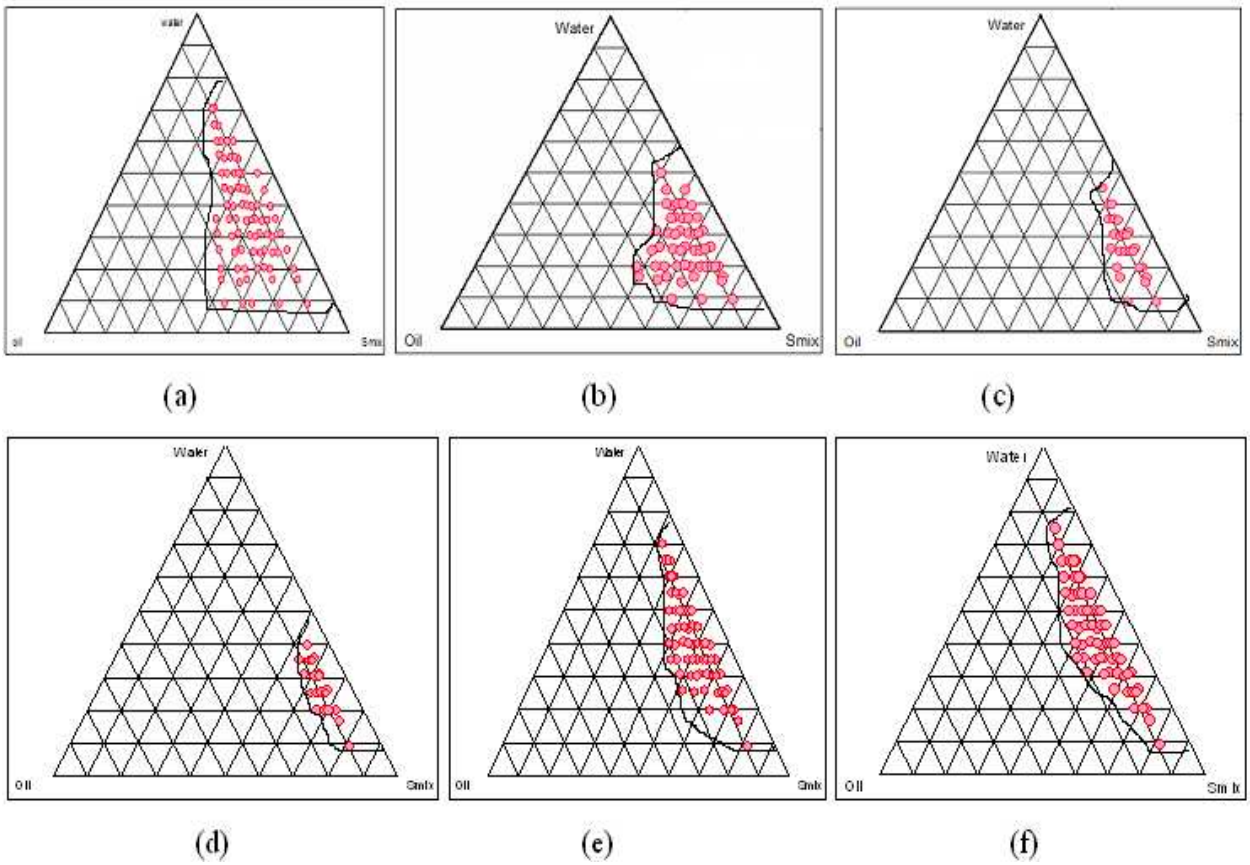

Fig. 3: Pseudo-ternary phase diagrams showing the o/w nanoemulsion (shaded area) regions of existence with Labrasol and Triacetin(1:1) (oil), Tween-80 (surfactant), Transcutol-P (cosurfactant) at Smix ratios; (a) 1:0, (b) 1:1, (c) 1:2, (d) 1:3, (e) 2:1, (f) 3:1, (%w/w).

Selection of Formulations from Phase Diagrams

Following criteria were chosen for the selection of formulations.

- GLBD was added to the mixture of oil, surfactant and cosurfactant with varying component ratio as described in Table 1, and then appropriate amount of water was added to the mixture drop by drop and the nanoemulsion containing GLBD was obtained by vortexing the mixture at ambient temperature[30].
- Formulations with Smix ratio 1:0, 1:2 and 1:3 were not selected because the formulations were unstable and showed phase separation.
- Based on the phase diagrams, three Smix ratios 1:1 (NE-A), 2:1 (NE-B) and 3:1 (NE-C) were optimized. From the selected Smix ratios, NE compositions with 33 % (NE-A1, NE-B1, and NE-C1), 45 % (NE-A2, NE-B2, NE-C2) and 55 % (NE-A3, NE-B3, NE-C3) Smix ratios were selected from the region of existence (Table 1).

Characterization of Nanoemulsions

In this work, the influence of concentration of NE components and GLBD on the characteristics of NE was studied The TEM analysis revealed that NEs droplets were spherical in shape, discrete with size in nanometer range (< 117nm) (Fig. 4).

Table 1: Composition of Selected Nanoemulsion Formulations

Smix Ratio [a]	Formulation Code [b]	Percent w/w of Components in Formulation		
		Oil (%)	Water (%)	Smix (S + CoS) (%)
Formulation NE-A	NE-A1	10	57	33
Smix ratio = 1:1	NE-A2	15	40	45
	NE-A3	18	27	55
Formulation NE-B	NE-B1	10	57	33
Smix ratio = 2:1	NE-B2	15	40	45
	NE-B3	18	27	55
Formulation NE-C	NE-C1	10	57	33
Smix ratio = 3:1	NE-C2	15	40	45
	NE-C3	18	27	55

[a] Surfactant/cosurfactant ratio;

[b] NE represents nanoemulsion; A, B and C represents Smix ratio 1:1, 2:1 and 3:1, respectively; Suffix 1,2 and 3 represents Smix concentration 33, 45 and 55 %, respectively.

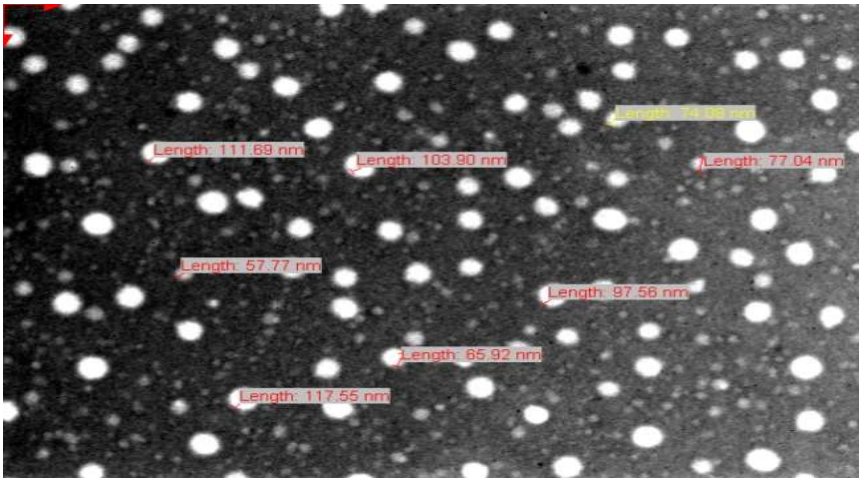

Fig. 4: Transmission electron microscopic positive image of GLBD nanoemulsion

Values of polydispersity index (PI), which is a measure of uniformity of droplet size within the formulation, were also calculated. A small droplet sizes are very much prerequisite for drug delivery as the oil droplets tend to fuse with the skin thus providing a channel for drug delivery[36]. All the NE formulations exhibited a narrow size distribution (PI < 0. 0.247) (Table 2). The results of particle size analysis (Table 2) were in agreement with the droplet size measured by TEM photograph (Fig. 4). The mean droplet size of drug free NE-B formulations ranges from 60.00 to 75 nm, which was lower as compared to the drug free NE-A (80.02 to 96.03 nm) and NE-C formulations (101.67 to 117.05 nm) (Table 2). It is hypothetically described that at the optimum Smix ratio (2:1 for present study) the diethylene glycol monoethyl ether was exactly inserted into the cavities between the tween 80 molecules, causing the interfacial film to condense and stabilize, resulting in smallest droplet diameters (60.00±2.756) with lowest poly dispersity value (0.131±0.034). Higher the PDI, lower the uniformity of the droplet size in the formulation[37]. Viscosity of all the NE formulations was very low as expected for o/w emulsion[38] (Table 2). When formulations with different Smix ratios were compared, the minimum viscosity values were obtained for NE formulations (29.14±2.82 to 86.00±0.577mP) (Fig 4). Refractive index is the net value of the components of NE and indicates isotropic nature of formulation. The data in Table 3 indicates that the mean value of the refractive index for all the formulations was approximately similar. The conductivity measurements (0.135±0.08–0.259 ±0.13 mS/cm) indicated the nature of NEs to be of oil-in-water type. All the NE formulations had pH values ranging from 6.2 to 6.74, favorable for topical application[39]. It was observed that incorporation of drug did not significantly affect the pH values of NEs.

Table 2: Physical Characteristics of Nanoemulsion Formulations (Mean ± SD, n = 3)

Formulation	Droplet Size (nm)	Polydispersity	Viscosity (mP)	Refractive Index	Conductivity (ms/cm)
NE-A1	80.02±1.751	0.208 ±0.041	62.89±3.09	1.406±0.002	0.242 ±0.16
NE-A2	89±012.250	0.221±0.022	52.66±0.942	1.410±0.023	0.209 ±0.04
NE-A3	96.03±2.852	0.231±0.017	46.00±0.577	1.406±0.008	0.139±0.03
NE-B1	69.02±3.053	0.151±0.041	34.00±0.816	1.407±0.026	0.259 ±0.13
NE-B2	60.00±2.756	0.131±0.034	29.14±2.82	1.402±0.006	0.219 ±0.12
NE-B3	74.36±0.241	0.185±0.065	41.33±0.235	1.404±0.011	0.172±0.07
NE-C1	101.67±13.48	0.214±0.115	72.66±0.942	1.409±0.001	0.239 ±0.21
NE-C2	110.39±0.074	0.201±0.029	86.00±0.577	1.408±0.002	0.189 ±0.32
NE-C3	117.05±2.253	0.247±0.052	69.00±0.816	1.398±0.002	0.135±0.08

Thermodynamic Stability of Nanoemulsions

Stress test including heating cooling cycle, centrifugation and freeze thaw cycles showed that all the formulations had a good physical stability. After three months, GLBD was found to be stable with recovery > 97 % for all the formulations. No significant change in the mean values of the refractive index of the formulations was observed during 3 months (data not shown). Thus, it can be concluded that the NE formulations were not only physically stable but also chemically stable.

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 GLBD as nanoemulsion was carried out successfully. The GLBD thermodynamically stable o/w NE systems were prepared and characterized. The results of above formulation suggested that in future, nanoemulsion formulation in the form of dermal gel can be prepared effectively for both *ex-vivo* and *in vivo* studies. It could be concluded that nanoemulsion system can be introduced as a novel transdermal formulation for GLBD.

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