Formulation Design and Development of Self-Nanoemulsifying Drug Delivery Systems Containing a Hydrophobic Selective β1-Adrenoreceptor Blocker

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Abstract:

The present work was aimed to design and develop self-nanoemulsifying drug delivery systems (SNEDDS) with the objective to overcome the problems associated with the delivery of talinolol, a hydrophobic and poorly bioavailable drugs having pH dependant solubility. The solubility of talinolol in various oils, surfactants, co-surfactants and aqueous phases were determined to identify and select the best combination with proper dose incorporation. Various surfactants and co-surfactants were screened for their ability to emulsify the selected oil. The area of nanoemulsification formation for the selected systems was identified by constructing the pseudoternary phase diagrams. The resulted nanoemulsions were found to be transparent without any phase separation and precipitation of drug. The influence of drug incorporation and pH of the dilution medium was also assessed on the phase behavior and droplet size of the selected systems. The developed formulations needed less than 45% of surfactant and yielded nanoemulsion of droplet size in the range of 20-150 nm, which was not affected by the pH of the dilution medium. Whereas, the corresponding monophasic region in the respective phase diagram decreased under the influence of drug incorporation and pH of the dilution medium. Furthermore, the droplet sizes measured by transmission electron microscopy analysis were in agreement with the analysis using photon correlation spectroscopy. The release rate of designed SNEDDS formulation was investigated using an in vitro dissolution test showed a complete drug release within 15 minutes irrespective of the pH of dissolution medium as compared with the plain drug, which showed a limited dissolution rate.

Keywords: Talinolol, self-nanoemulsifying drug delivery systems, pseudoternary phase diagrams, pH dependant solubility, droplet size.

1. INTRODUCTION

Drug solubility and stability have long been a major challenge for the design and development for effective formulation of drugs. To enhance the solubility, dissolution, stability, absorption and bioavailability of drugs, self-nanoemulsifying drug delivery systems (SNEDDS) are one of the most promising techniques for poorly water soluble and lipophilic drugs [1]. The nano-size ranged droplets influence the transport property of the formulation and would enhance the interfacial area associated with the drug [2,3]. SNEDDS are isotropic liquid mixtures of oil, surfactant, co-surfactant and drug that form fine, thermodynamically stable oil-in-water nanoemulsion when introduced into aqueous medium under gentle agitation [4]. Oil, surfactant and co-surfactants are the most important components

of SNEDDS composition. The use of surfactants in these kinds of formulations are known to improve the bioavailability of the drugs by activation of various mechanisms which includes: (i) by maintaining the drug in solution form by avoiding and/or by improving the drug dissolution [5], (ii) by increasing intestinal epithelial permeability of drugs [6], and (iii) by increasing tight junction permeability of drugs [6].

Talinolol, a Class IV drug (poor bioavailability due to both poor solubility and poor permeability) as per biopharmaceutical classification system, is a selective β1-adrenoreceptor blocker with moderate lipophilic properties. It is used in the therapeutic dosage range (50-300 mg/day) [7]. Talinolol has a higher and longer degree of β1 adrenoreceptor occupancy than propranolol. Talinolol also exerts antioxidant effects in smokers and in patients with coronary heart disease [8]. Its oral absorption is incomplete and erratic [9]. The solubility of talinolol is pH dependent and lower at higher pH [10]. Despite its reasonable lipid solubility the bioavailability of talinolol is about 55% in humans [11]. The low bioavailability of talinolol is mainly attributed to its poor water solubility (0.02 mg/ml), which may also be responsible for its poor bioavailability, as dissolution is a rate-limiting factor in intestinal absorption of poorly water soluble drugs [12]. An approach, which will increase drug solubility as well as improves the dissolution profile, is highly desirable for optimizing the therapeutic performance of talinolol.

Formulation approaches like controlled release tablets were adopted to improve the bioavailability of talinolol [9]. A system comprises of buffers, salts and surfactants are anticipated to improve the bioavailability of talinolol by altering its liquid crystal form [10]. Daniel Wagner et al., (2003) [10] have evaluated potential of different dissolution media upon dissolution and solubility of talinolol. Interestingly, they have also observed that an optimum concentration of surfactants can improve the solubility and dissolution profile of talinolol. Furthermore, it has been widely reported that the use of emulsions of medium chain mono-, di- and triglycerides can improve the oral bioavailability of

poorly water soluble drugs [13].

SNEDDS being a versatile technology have the potential to increase the bioavailability of Class II (solubility limited poor bioavailability) and Class IV drugs (poor bioavailability due to both poor solubility and poor permeability) because of the existence of micro domains of different polarity within the same single-phase solution. The drug partitions between disperse and continuous phase, and when the system comes into contact with a semi permeable membrane, the drug can be transported through the barrier thus increasing the bioavailability of Class III drugs (permeability limited poor bioavailability). The use of SNEDDS/ nanoemulsions as drug delivery systems can improve the efficacy of drug, allowing the total dose to be reduced and thus minimizing side effects.

In the present study it was hypothesized that, SNEDDS can improve the solubility and dissolution by altering the crystal form of talinolol in different dissolution media [10]. Thus, the increasing solubility of hydrophobic drug like talinolol can also enhance its absorption in gastrointestinal tract [14]. Furthermore, the design and development of talinolol loaded SNEDDS would be an efficient, convenient and more patient compliant approach in comparison to controlled or immediate release tablets, as SNEDDS can be filled in hard gelatin capsules due to their anhydrous nature enabling its administration as unit dosage form.

The clinical usefulness of the SNEDDS is evident from the commercially available formulations containing cyclosporin A, ritonavir and Saquinavir. Self-nanoemulsifying systems have been investigated or recommended for delivering poorly water-soluble drugs such as ubiquinone [4], curcumin [15], itraconazole [16], vinpocetine [17], fenofibrate [18], alpha-asarone [19], atorvastatin [20], halofantrine [21], ontazolest [22], progesterone [23], retinol acetate [24] and cefpodoxime proxetil [25]. In a study the percentage release of simvastatin from a self-emulsifying system was reported 1.5-2 times high than the conventional tablet [26]. A few other studies have reported enhancement in the bioavailability of poorly soluble drugs when formulated as SNEDDS [27,28]. These

investigations further help in successful design and development of SNEDDS that can deliver talinolol in unit dosage form and would release talinolol rapidly, independent of pH, in the form which can be easily absorbed throughout the gastrointestinal tract.

First step towards development of SNEDDS is the selection of suitable oil, surfactant and co-surfactant. This selection depends upon the solubility of drug dose in these components and its optimum proportion to get a clear transparent nanoemulsion. In addition to this, the selection of surfactant(s) is done by measuring the percent transmittance of different mixtures of surfactant(s) and selected oils [25]. The selection of talinolol loaded SNEDDS was done by the visual inspection [13,14] of the dispersion that gives a clear transparent nanoemulsion without precipitation of talinolol. Finally, the prepared nanoemulsions were also evaluated for their stability. The effect of drug incorporation and the effect of pH of the dissolution medium on phase behavior were reported to avoid the metastable formulations.

2. MATERIALS AND METHODS

2.1. Materials

Talinolol was a generous gift from Pioneer Agro Industries (Thane, India). Polyglyceryl-6-dioleate (Plurol Oleique 479), Lauroglycol 90, Labrafac lipophile WL-1349, Caprylic/capric triglyceride polyethylene glycol-4 complex (Labrafac PG), oleoyl macroglycerides EP (Labrafil 1944 and 2125) was obtained as gift samples from Gattefosse India Pvt. Ltd. (Mumbai, Inida). Hard gelatin capsules were from Associated Capsules (Mumbai, India). Triacetin, Miglyol 812, castor oil, ethyl oleate, isopropyl myristate, soybean oil, and olive oil were obtained from Loba chemie Pvt. Ltd. (Mumbai, India). Ethanol, PEG 200, PEG 400, propylene glycol, Tween 80 and Tween 20 were purchased from s.d. fine chemicals (Mumbai, India). The materials investigated were of GRAS and were within there acceptable limits. Acetronitrile high-performance liquid chromatography (HPLC) grade was purchased from E. Merck (Mumbai, India).

Double distilled water was prepared freshly from a Milli-Q water purification system (Millipore, Billerica, MA), whenever required. All other chemicals used in the study were of analytical grade and were used as received.

2.2. Solubility study

The solubility of talinolol in various oils, buffers, surfactants, co-surfactants and their combinations were determined by using shake flask method [25, 26]. Briefly, an excess amount of talinolol was added to each vial containing 5 ml each of the selected vehicle, i.e., either oil, surfactant, co-surfactant or buffer. After sealing, the mixtures were vortexed for 10 minutes in order to facilitate proper mixing of talinolol with the vehicles. Mixtures were then shaken for 72 hours in a water bath shaker (Remi, Mumbai, India) maintained at room temperature. Mixtures were centrifuged at 5000 rpm for 10 minutes followed by filtration through 0.22 µ nylon membrane filters (Millipore, Bangalore, India). Filtrate was suitably diluted with methanol and talinolol dissolved in various vehicles was quantified by a validated HPLC method developed in house. The method was validated according to International Conference on Harmonization (ICH) guidelines. Finally, the amount of drug and solubility was determined using $E_{1cm}^{1\%}$.

2.3. Evaluation of effective surfactant and co-surfactant

The selection of surfactant(s) for emulsification ability is done by measuring and comparing the percent transmittance of different mixtures of surfactant(s) and selected oils [25] with an objective to explain the basis for selection of components for nanoemulsion formulations from the pseudoternary phase diagrams [25]. Oil selection was done on the bases of their ability to solubilize talinolol. Briefly, equal quantity of surfactant was added to the selected oil. The mixture was gently heated at 45-50°C

for homogenizing the components. From this isotropic mixture, perfectly weighed 150 mg was diluted into 250 ml of double distilled water to yield a fine emulsion. The ease of formation of emulsion was monitored by noting the number of volumetric flask inversions required to give uniform emulsion. The time taken for the formation of fine emulsion was also noted. The resulting emulsions were observed visually for the relative turbidity. The emulsions were allowed to stand for 2 hours and their % transmittance was assessed at 400-700 nm by UV-1601 UV-Visible double beam spectrophotometer (Shimadzu, Japan) using double distilled water as blank.

The turbidimetric method was used to assess relative efficacy of the co-surfactant to improve the nanoemulsification ability of the surfactants and also to select best co-surfactant from the large pool of co-surfactants available for peroral delivery. Selected surfactant(s) was mixed with co-surfactant(s) i.e. 1:1 Smix mixture. Then, the equal quantity of selected oil was added to this mixture (50:50) and the mixture was homogenized with the aid of the gentle heat (45–50°C). This isotropic mixture was accurately weighed and diluted into 250 ml of double distilled water to yield fine emulsion. The time taken and ease of formation of emulsions was noted by noting the number of 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 hours and their transmittance was measured at 400-700 nm by UV-1601 UV-Visible double beam spectrophotometer (Shimadzu, Japan) using double distilled water as blank. As the ratio of co-surfactants to surfactant(s) is the same in this case, the turbidity of resulting nanoemulsions will help in assessing the relative efficacy of the co-surfactants to improve the nanoemulsification ability of the selected surfactant(s) for the selected oil. Similarly, the nanoemulsification ability and the efficacy of co-surfactant were also determined by varying the surfactant(s) to co-surfactant ratio i.e. from 1:1 to 1:2 and 1:3.

2.4. Construction of pseudoternary phase diagrams

Pseudoternary phase diagrams of selected surfactant(s), co-surfactant and oil were plotted; each of them, representing an apex of the triangle [14]. Ternary mixtures with varying compositions of surfactant, co-surfactant and oil were prepared. In this case, the concentration of surfactant, oil and co-surfactant was varied from 5 to 50% (w/w), 0 to 100% (w/w) and 0 to 45% (w/w), respectively. For any mixture, the total of surfactant, co-surfactant and oil concentrations always added to 100% (w/w).

Different such mixtures with varying surfactant, co-surfactant and oil concentrations were prepared by keeping one of these components constant, each time. The percentage limit of surfactant, co-surfactant and oil used herein was selected by considering their acceptable safe dose and decided on the basis of the requirements stated by Pouton (2000) for the spontaneously emulsifying systems [29]. Compositions were evaluated for nanoemulsion formation by diluting 150 mg of each of the mixtures into 250 ml of double distilled water and visually observed for phase clarity, precipitation and flowability. The concentration of double distilled water at which the turbidity to transparency and vice-versa transitions occurred, was observed. The droplet size of the resulting dispersions was determined by photon correlation spectroscopy (PCS). Measurement was done using a Zetasizer 1000 HS (Malvern Instruments, Worcestershire, UK). Light scattering was monitored at 25°C at a 90° angle. Dispersions, having droplet size 150 nm or below were considered desirable. The area of nanoemulsion formation using a phase diagram was identified for the respective systems in which nanoemulsions with desired droplet size were obtained.

2.5. Effect of talinolol incorporation and pH of the aqueous phase on pseudoternary phase diagrams

The drugs as well as pH of the vehicle have considerable influence on the phase behavior of the spontaneously emulsifying systems [30-32].

The effect of talinolol and pH of the aqueous phase on the phase behavior and the area of nanoemulsion formation was studied. In present investigation, 5% w/v of talinolol was dissolved in selected oily phase, and various compositions were prepared in the similar fashion as described in the above section. The influence of the pH of aqueous phase on the phase behavior and area of nanoemulsion formation was investigated by diluting the formulations with various vehicles viz. water, buffer pH 1.2, buffer pH 3.0, buffer pH 6.8 and buffer pH 7.4. The mean droplet size of the resulting dispersions was measured by using PCS and the data obtained was used to identify the area of nanoemulsion formation.

2.6. Formulation of SNEDDS

Among the different formulae SNEDDS were selected from the pseudoternary phase diagrams. The drug dose incorporation in the particular combination of components was calculated. The amount of oil, surfactant and co-surfactant was weighed in their safety limits and were mixed together to get a clear transparent talinolol loaded SNEDDS.

2.7. Evaluation of talinolol loaded SNEDDS

Developed SNEDDS were evaluated for robustness to dilution, nanoemulsion droplet size analysis, transmission electron microscopy, effect of talinolol loading and *in vitro* dissolution profile.

2.8. Robustness to dilution

Robustness of talinolol loaded preconcentrate (SNEDDS) formulations (150 mg) to dilution was studied by dropwise addition to 50, 100 and 1000 times into various dilution media [25] viz. water, buffer pH 1.2, buffer pH 3.0, buffer pH 6.8 and buffer pH 7.4. This was done at room temperature with a gentle stirring at 100 rpm, magnetically. Visual observations were made immediately after dilution for the assessment of self-nanoemulsification efficiency, appearance i.e. transparency (clarity), phase separation, and precipitation of drug. Time taken for the formation of clear nanoemulsion was

also noted for each dilution. In addition, the diluted nanoemulsions were stored for 24 hours and the same observation was made.

2.9. Nanoemulsion droplet size analysis

Each developed formulations (150 mg) were diluted into various dilution medias (250 ml) including double distilled water, buffer pH 1.2, buffer pH 3.0, buffer pH 6.8 and buffer pH 7.4. The mean droplet size and polydispersity index of the resulting nanoemulsions were determined by PCS. Measurements were obtained at an angle of 90° at 25°C. Nanoemulsions were diluted in respective vehicles to ensure that the light scattering intensity was within the instrument's sensitivity range. The resultant nanoemulsions were also allowed to stand for 6 hours at room temperature to assess dilution stability.

2.10. Transmission electron microscopy

Morphology and structural observation of the talinolol loaded SNEDDS formulations were studied using transmission electron microscopy (TEM, H7500, Hitachi, Japan) operating at 100 kV capable of point-to-point resolution. For observation by TEM, a drop of SNEDDS (0.5 ml) was then directly placed on the copper electron microscopy grids supported by Formvar films. The excess was drawn off with filter paper. Furthermore, it was stained in 0.5% phosphor tungstic acid solution for 30 seconds and observed after drying. Combination of bright field imaging at increasing magnification was used to reveal the form as well as size of the formed nanoemulsion.

2.11. Effect of talinolol loading

The increase or decrease in the amount of talinolol would influence the droplet size of the resultant nanoemulsions, if talinolol were participating at interface of nanoemulsion [25]. In order to investigate role of talinolol, various formulations were prepared containing varying amount of talinolol from 5 to 20% (w/w). Talinolol loaded SNEDDS (150 mg) were diluted into 250 ml of

different media viz. double distilled water, buffer pH 1.2, buffer pH 3.0, buffer pH 6.8 and buffer pH 7.4 and the mean droplet size of resulting nanoemulsions was determined by PCS.

2.12. In vitro dissolution profile

5% w/v of talinolol loaded SNEDDS was filled in size '0' hard gelatin capsules and used for release studies; results were compared among those of pure drug (5% w/v). *In vitro* release profile of SNEDDS was studied using USP XXIII apparatus II at 37±0.5°C with a rotating speed of 50 rpm in 500 ml of dissolution media namely, double distilled water, pH 1.2, 3.0, 6.8 and 7.4 buffers so as to evaluate the effect of pH on *in vitro* dissolution of talinolol. During the study, 5 ml of aliquots were removed at predetermined time intervals (5, 10, 15 and 20 minutes) from the dissolution medium, filtered through 0.22 µ nylon membrane filters (Millipore, Bangalore, India) and replaced with fresh dissolution media. The dissolution studies were also performed for a dummy SNEDDS formulation (without drug), which was kept as blank for the analysis of drug loaded SNEDDS. This was done to nullify the effect of emulsion particles so as to determine only the drug in the aqueous phase. The amount of talinolol released was analyzed using a validated UV spectrophotometeric method developed in house at a λ_{max} of 242 nm.

3. RESULTS AND DISCUSSION

3.1. Solubility study

Solubility studies for talinolol were aimed to select and identify the suitable oil, surfactant(s) and co-surfactant(s), for the design and development of SNEDDS. One of the most essential consideration while designing and developing self-emulsifying formulation is avoiding precipitation of the drug upon dilution in the gut lumen *in vivo*. Therefore, the identification of the suitable oil, surfactant or co-surfactant having maximal solubilizing potential for drug under investigation is very important to achieve optimum drug loading [29,33]. It is even

more important for talinolol, as its target dose is high.

Solubility of talinolol in various buffers, oils, surfactants and co-surfactants is presented in Figures 1. Solubility studies clearly indicated that talinolol has pH dependant solubility as shown in Figure 1A. Amongst the various selected oils, triacetin (Figure 1B) was found to solubilize the highest amount of talinolol and was selected for further study. The selection of surfactant and co-surfactant in the further study was governed by their emulsification efficiency (Table 1 and 2) as well as their ability to solubilize talinolol (Figure 1C and D).

3.2. Evaluation of surfactant and co-surfactant for its emulsifying ability

The percent transmittance values of various dispersions are given in Table 1. Emulsification studies clearly distinguished the ability of various surfactants to emulsify triacetin. These studies indicated that Tween 20 (HLB: 16.7) and Tween 80 (HLB: 15) had very good ability to emulsify triacetin followed by Labrafac L WL-1349 (HLB: 1), whereas, Labrafil 2125 (HLB: 4) and Labrafil 1944 (HLB: 4) appeared to be a poor emulsifier for triacetin. This indicates that the HLB values of the surfactants used plays an important role and there was a great difference in their emulsification ability. This observation is in line with the investigations reported by Malcolmson et al. (1998) [34] and Warisnoicharoen et al. (2000) [35] who concluded that microemulsification is also influenced by the structure and chain length of the surfactant. Tween 20 and Tween 80 rendered very good nanoemulsions requiring short time for nanoemulsification and were selected for further investigation.

In addition, surfactants must lower the interfacial tension to facilitate the dispersion process during the formation of nanoemulsion from SNEDDS. They provides a flexible film around the droplet that can readily collapse and also provides a curvature at the interfacial region for the desired different types of nanoemulsions like o/w type, w/o type and/or bicontinuous type, depending upon the lipophilicity of the surfactant [2,32].
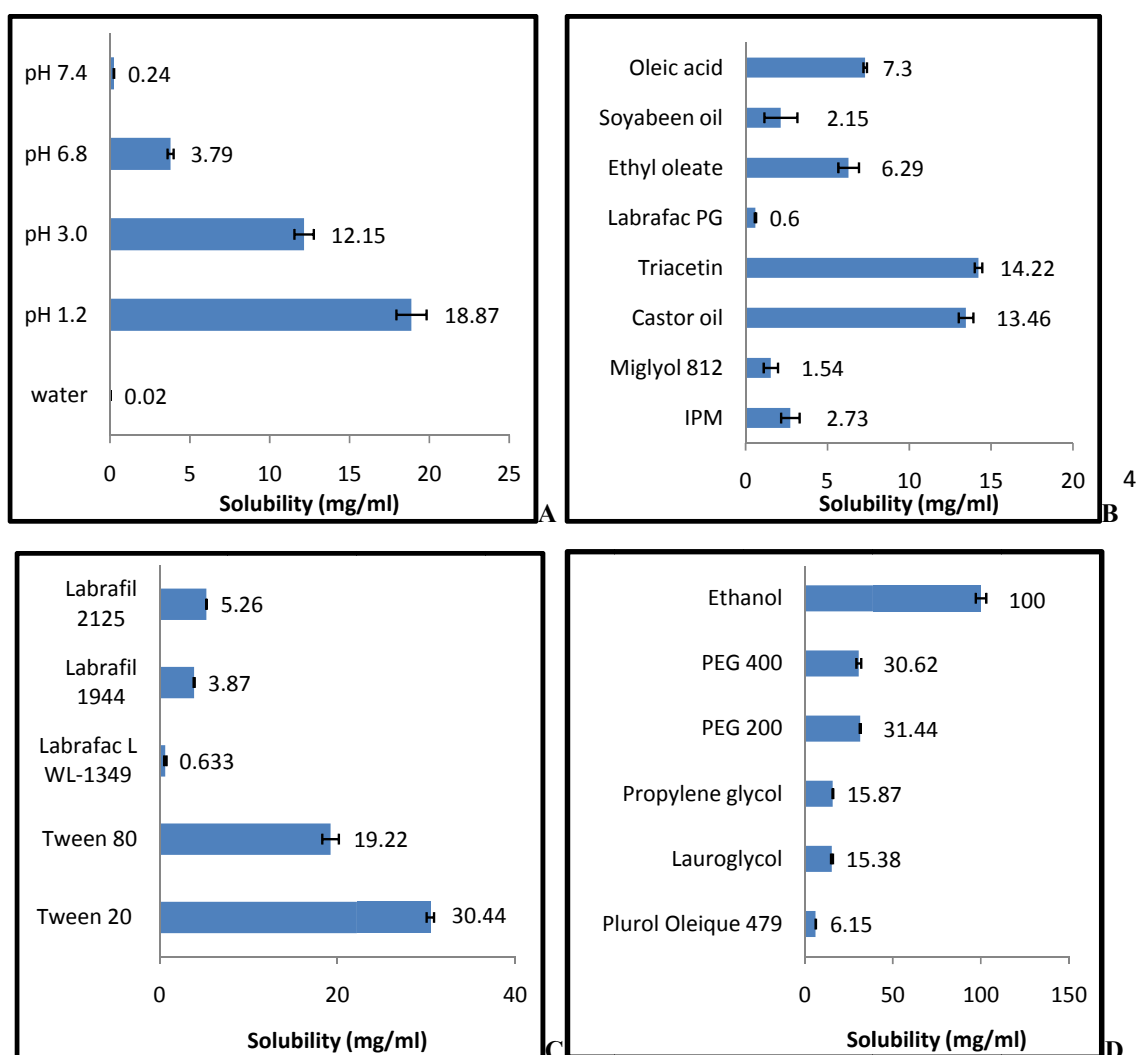
Figure 1: Solubility of talinolol in various buffers (A), oils (B), surfactants (C) and co-surfactants (D). Data are expressed as mean ± S.D. (n=3). IPM indicates isopropyl myristate. PEG indicates polyethylene glycol.

Table 1: Emulsification efficiency of various non-ionic surfactants for triacetin

Surfactant	% Transmittance[*]
Tween 20	99.8 ± 0.2
Tween 80	99.7 ± 0.3
Labrafac L WL-1349	97.3 ± 0.5
Labrafil 1944	93.1 ± 0.6
Labrafil 2125	67.7 ± 1.1
2.5% Brij 721	55.5 ± 1.7

Data are expressed as mean ± S.D. (n=3).

Table 2: *Emulsification studies on surfactant/co-surfactant combinations for triacetin*

Co-surfactant	% Transmittance[*]	
	Tween 20	**Tween 80**
Ethanol	99.7 ± 0.4	99.8 ± 0.2
Propylene glycol	98.4 ± 0.2	98.3 ± 0.4
Lauroglycol 90	93.1 ± 0.5	93.8 ± 0.2
Plurol Oleique 479	92.2 ± 0.8	89.5 ± 0.8
Polyethylene glycol 200	90.6 ± 0.6	64.7 ± 1.1
Polyethylene glycol 400	87.5 ± 1.0	66.7 ± 1.1

Data are expressed as mean ± S.D. (n=3).

Furthermore, the turbidimetric method was used to judge relative efficacy of the co-surfactants to improve the nanoemulsification ability of the surfactant and also to select best co-surfactant, both hydrophilic and lipophilic, from large pool of co-surfactants available for peroral delivery. All the co-surfactants increased the spontaneity of the nanoemulsion formation as it leads to greater penetration of the surfactant monomers, thereby further decreasing the interfacial tension [3]. Interestingly, ethanol and propylene glycol as co-surfactants appeared to be equivalent in improving nanoemulsification ability of Tween 20 and Tween 80. In case of lipophilic co-surfactants such as Plurol oleique 497 and Lauroglycol 90, good correlation was observed between the structure i.e. the chain length of co-surfactant and the transmittance values of resulting dispersions. Larger the chain length of the co-surfactant lesser was the transmittance value. This correlation was also applicable to ethanol, propylene glycol, PEG 200, PEG 600, Lauroglycol 90 and Plurol oleique 497 (Table 2).

Lauroglycol 90 was less effective as co-surfactants. This was attributed to the presence of lauric acid backbone, which is longer in chain length than ethanol and propylene glycol as co-surfactant. But Lauroglycol 90 was more efficient than Plurol oleique, which has oleic acid backbone, which is longer in chain length than lauric acid. This observation is also in line with the investigations reported by Malcolmson et al. (1998) [34] and Warisnoicharoen et al. (2000) [35].

Ethanol appeared to be the best among all the hydrophilic co-surfactants which can further be validated with the help of droplet size analysis. Lipophilic co-surfactants despite of their good potential were not investigated further as systems with higher lipid content may cause stability problem. Among lipophilic co-surfactants, Lauroglycol 90 and Plurol Oleique 479, Lauroglycol 90 exhibited good nanoemulsification ability. However due to less solubilizing potential of lipophilic co-surfactants for talinolol (Figure 1D.), were not used for further studies. Ethanol, a hydrophilic co-surfactant having good solubilizing potential for talinolol was selected and Tween 20-Ethanol-Triacetin and Tween 80-Ethanol-Triacetin systems were developed for further studies.

3.3. Construction of pseudoternary phase diagrams

The ternary phase diagrams of Tween 20-Ethanol-Triacetin and Tween 80-Ethanol-Triacetin systems are shown in Figures 2A and B, respectively. The circles in the phase diagram indicates the area, which was explored for locating nanoemulsification region under a given combination of surfactant, co-surfactant and oily phase. The circles shaded in pink color indicates the region in which nanoemulsions of desired droplet size ranging from 20-150 nm were obtained. From Figures 2A and B, it is evident that both the systems (Tween 20-Ethanol-Triacetin and Tween 80-Ethanol-Triacetin) having almost the same nanoemulsification region. Tween 80-Ethanol-Triacetin system yielded nanoemulsions for the compositions that had as low as 25% (w/w) of oily
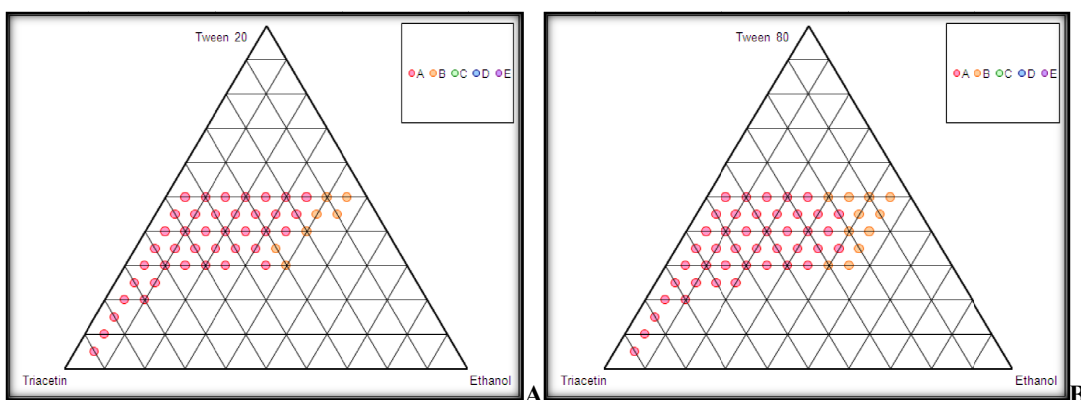
Figure 2: *Pseudoternary phase diagram of Tween 20 (A) and Tween 80 (B) systems without talinolol incorporation, circles showing nanoemulsion area. The regions having pink circles indicate the desired size range of nanoemulsions (20-150 nm). The region having orange circles indicates the nanoemulsions of having droplet size ranging from 200-500 nm.*
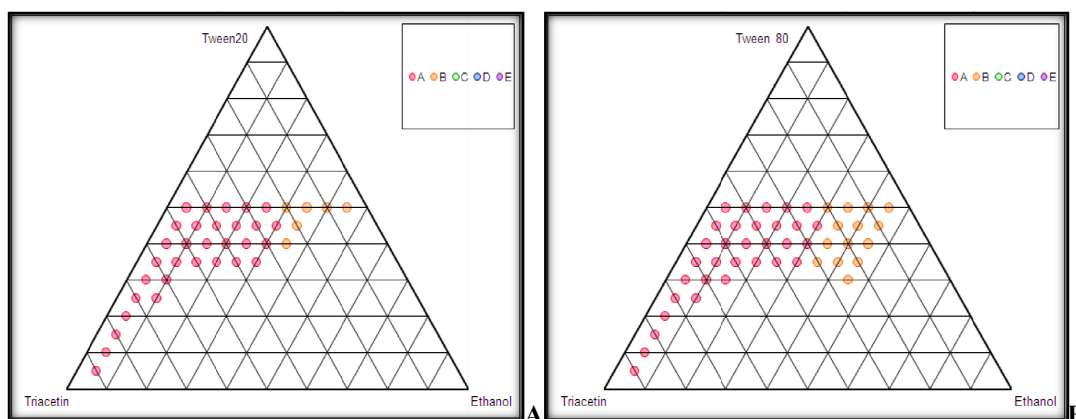

Figure 3: *Pseudo-ternary phase diagram of Tween 20 (A) and Tween 80 (B) systems showing the effect of talinolol incorporation, circles showing nanoemulsion area.*
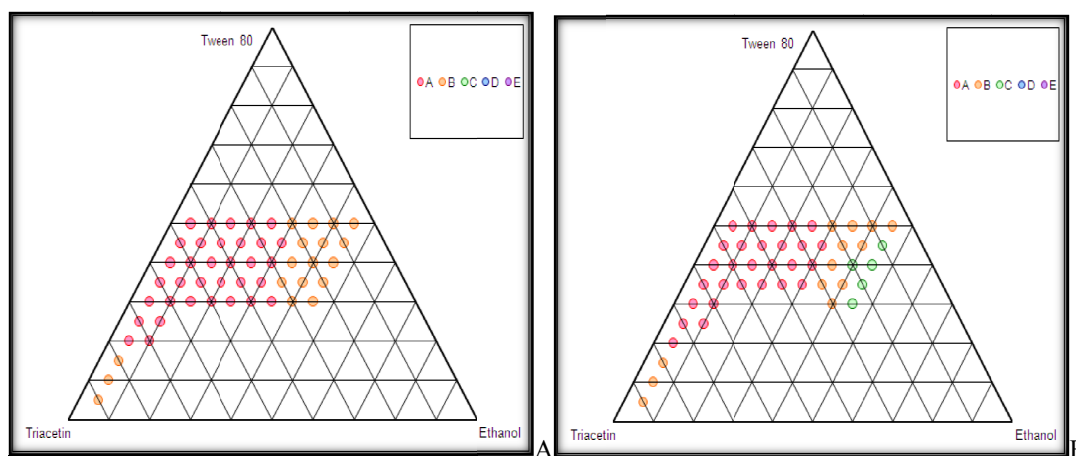

Figure 4: *Pseudo-ternary phase diagram of talinolol loaded Tween 80 showing the effect of pH 6.8 (A) and pH 7.4 (B) buffers as dilution medium, circles showing nanoemulsion area. The region having green circles indicates the nanoemulsions of having droplet size greater than 500 nm.*

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Table 3: *Droplet size and polydispersity index of talinolol SNEDDS at different pH conditions*

Dissolution medium	Water	Buffer pH 1.2	Buffer pH 3.0	Buffer pH 6.8	Buffer pH 7.4
Droplet size (nm)[*]	93.2	68.2	110.8	121.4	124.2
Polydispersity index[†]	0.690	0.666	0.636	0.699	0.548

[*]Droplet size expressed as mean ($n = 2$) where relative standard deviation was <10%.
[†]Data expressed as mean ($n = 2$).

phase, comprising of oil (25% w/w) + surfactant (35% w/w) + co-surfactant (45% w/w). Whereas, Tween 20-Ethanol-Triacetin system yielded nanoemulsions for compositions having about 40% (w/w) of oily phase, comprising of oil (40% w/w) + surfactant (30% w/w) + co-surfactant (30% w/w). These compositions can also be used to solubilize various other hydrophobic drugs and can become potential platform systems. Both the systems provide a larger nanoemulsion region with desired droplet size and a greater capacity for drug incorporation in the oily phase, which is most desirable for talinolol.

In addition, surfactant and co-surfactant adsorbed at the interface, providing a mechanical barrier to coalescence as well as reducing the interfacial energy. So, the degrees to which the surfactant changes the dispersion entropy and lowers the surface tension of the interface and increases the interfacial area turn out to be the bases for the free energy change of nanoemulsion formulations [3]. Thus, with a considerable constructive entropy change a large reduction in surface tension is companied leads to a negative free energy of formation [3]. This leads to a spontaneous and stable formation of nanoemulsion formulation. Therefore, the selection of oil, surfactant and co-surfactant, and the mixing ratio of oil to Smix (surfactant and co-surfactant), plays a vital role in the development of nanoemulsions.

3.4. Effect of talinolol incorporation and pH of the aqueous phase on pseudoternary phase diagrams

The pseudoternary phase diagrams indicating effect of talinolol incorporation on phase behavior

and area of nanoemulsion existence are shown in Figures 3A and B. It was expected that talinolol would influence the phase behavior and the area of nanoemulsion formation as in these formulae [25]. Talinolol substituted one-fourth amount of triacetin as compared to the systems without talinolol. Phase diagram studies indicated that there was remarkable influence of talinolol on the area of nanoemulsion formation for both the Tween 20 and Tween 80 based systems. Incorporation of talinolol in triacetin led to a reduction in the area of nanoemulsion formation for both Tween 20 and Tween 80 based SNEDDS when compared to the area in Figure 2A and B, respectively. Talinolol, due to its low aqueous solubility, is likely to participate in the nanoemulsion by orienting at the interface. The reduction in the area of nanoemulsion formation could be due to talinolol influenced interaction of surfactant and co-surfactant with oil [30-32].

Furthermore, the effect of pH of the aqueous phase on ternary phase diagrams was also studied. In case of Tween 80 based system there was a profound affect of pH of the dilution medium on the area of nanoemulsion formulation. The area of nanoemulsion formation was found same for buffer pH 1.2 and pH 3.0. This behavior supports the aforementioned hypothesis about the orientation of talinolol. As talinolol is having much more solubility at buffer pH 1.2 and pH 3.0 than water, it likely to migrate more in the external phase leading to reduction in the amount present at interface. This indicates that an effective concentration of surfactant and co-surfactant is available for nanoemulsion formation at buffer pH 1.2 and pH 3.0, simulating the pH of stomach and duodenum. Where as, in case of buffer pH 6.8 and pH 7.4, a remarkable
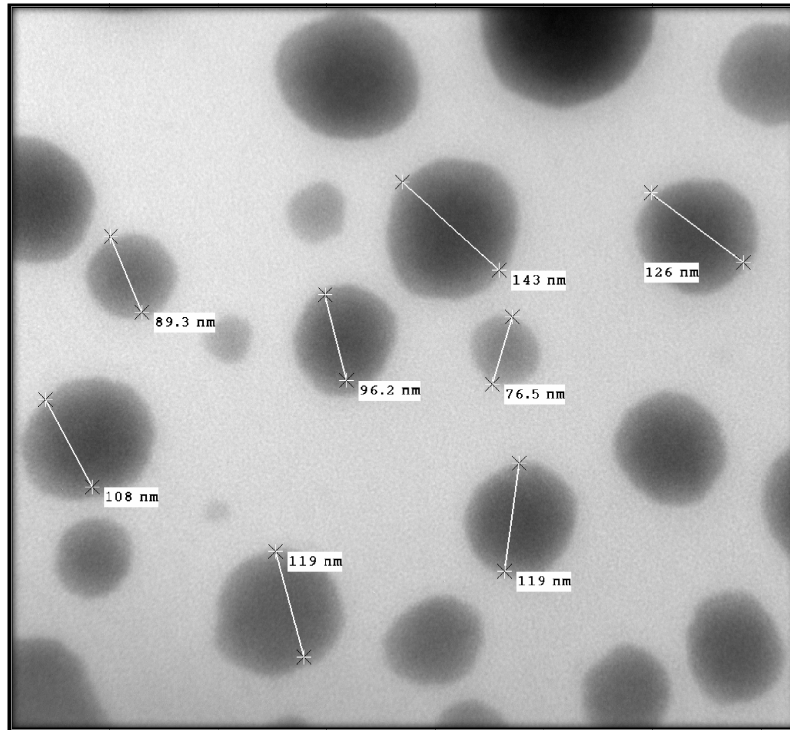
Figure 5: *Transmission electron microscopic positive image of talinolol loaded SNEDDS showing spherical shape and size of some oil droplets (x 100,000).*
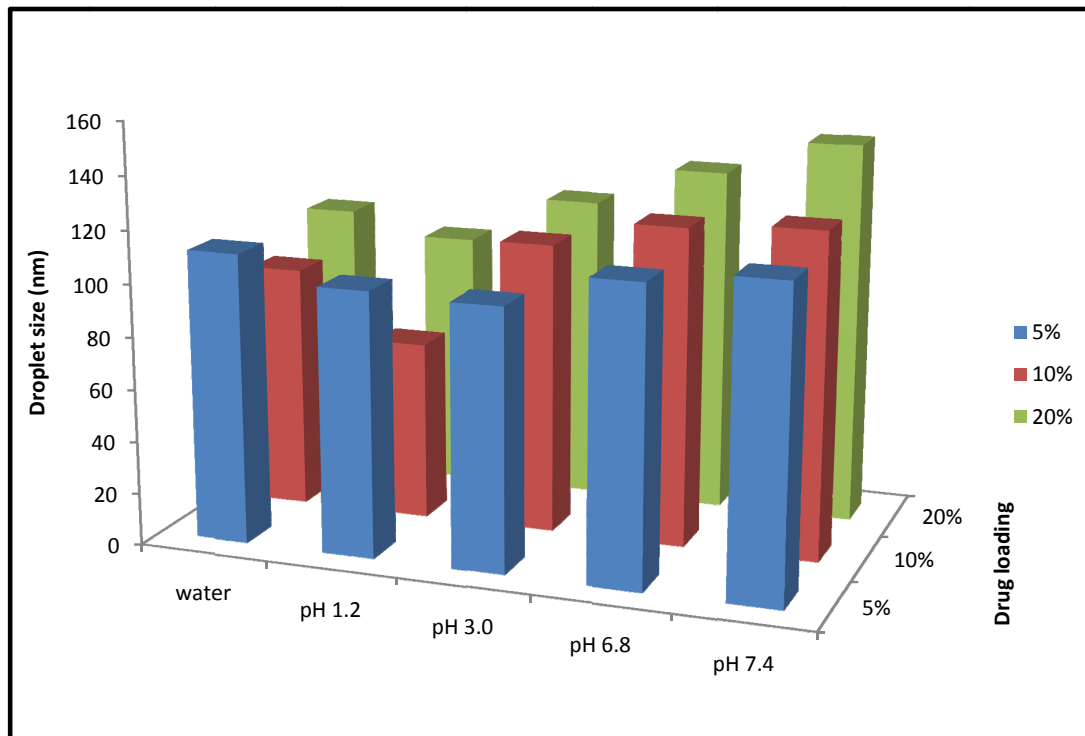

Figure 6: *Effect of talinolol loading on mean droplet size of SNEDDS. Data are expressed as mean (n=2).*

Table 4: *In vitro dissolution profile of talinolol loaded SNEDDS and pure talinolol*

Time (min)	% Cumulative release[*]									
	Water		Buffer pH 1.2		Buffer pH 3.0		Buffer pH 6.8		Buffer pH 7.4	
	SNEDDS	Pure drug	SNEDDS	Pure drug	SNEDDS	Pure drug	SNEDDS	Pure drug	SNEDDS	Pure drug
5	99.92 ± 0.19	0.001±0.002	100.01 ± 1.05	0.22±0.15	100.32 ± 0.88	0.31±0.52	89.93 ± 1.53	0.16±1.87	94.69 ± 2.73	0.003±1.23
10	100.02 ± 1.33	0.001±0.004	101.11 ± 0.49	2.53±2.35	101.29 ± 0.12	3.18±1.05	99.89 ± 3.19	1.54±5.60	99.61 ± 0.12	0.05±3.87
15	101.81 ± 1.11	0.002±0.004	102.01 ± 1.61	5.31±1.02	101.93 ± 1.22	6.21±2.10	100.27 ± 0.98	3.56±1.88	100.22 ± 1.13	0.06±1.98

[*]Data expressed as mean ± S.D. ($n = 3$).

reduction in area of nanoemulsion region of desired droplet size ranging from 20-150 nm was observed as shown in Figure 4A and B, respectively; when compared to the area in Figure 2B and 3B.

The area of nanoemulsion formation was least for water as dilution medium. The effect of pH of the aqueous phase on pseudoternary phase diagrams of Tween 20 based system was same as that of systems based on Tween 80.

3.5. Selection of best SNEDDS formulae

From pseudoternary phase diagrams, the compositions which completely solubilize the desired dose of drug and which could accommodate the optimum safe concentration of surfactants and co-surfactants were selected for the study. The final formulation was selected based on the drug loading efficiency, amount of surfactant and oil used and consistency in mean droplet size at varying pH. Exactly 5% w/w of the talinolol was dissolved in the final composition of SNEDDS and it was kept constant in all the selected formulations. Further, the amount of the lowest safe concentration of oil and surfactant that can be ingested orally was considered. It is widely reported that large amount of surfactants cause mild or acute toxicity [3], hence, it is essential to use the safe concentration of surfactants in the formulations. The best talinolol loaded SNEDDS comprises of surfactant, co-surfactant and oil at a ratio of 35:15:50% w/w.

3.6. Evaluation of talinolol loaded SNEDDS

Nanoemulsions resulting from dilution of talinolol loaded SNEDDS formulations with various dilution media were robust to all dilutions i.e. transparent and did not show any phase separation or precipitation even after 2 days of storage.

The mean nanoemulsion droplet size of talinolol loaded SNEDDS after dilution with various dilution media is given in Table 3. Even though, talinolol loaded SNEDDS showed fairly similar desired droplet size in the nano range of 20–150 nm, when diluted with various dilution media of different pH; the droplet size increases as the pH of the dilution medium increases. This is attributed to the solubility of talinolol present at the interface of the nanoemulsion droplets [1,2,32]. In addition, it is also observed that the droplet size increase as the concentration of oil increases in the formulation, because of the concurrent decline in the concentration of surfactant and co-surfactant. The time required for formation of nanoemulsions after dilution with various dilution media was less than 1 minute. The resulting nanoemulsions were transparent in appearance and they did not show any signs of phase separation and drug precipitation even after 10 days. The talinolol loaded SNEDDS turned into nanoemulsion when diluted with different dissolution media. Furthermore, in a TEM positive image, the talinolol loaded SNEDDS appeared dark and the surroundings were bright (Figure 5). It showed the clear spherical shape and uniform droplet size of SNEDDS. Some droplet sizes were measured, as TEM is capable of point-to-point resolution. These droplet sizes were in agreement with the results measured from droplet size analysis using PCS as given in Table 3.

The amount of talinolol incorporation also influenced the droplet size of nanoemulsions

obtained after diluting talinolol SNEDDS with various dilution media. The droplet size increased with the increase in the % of talinolol loading as shown in Figure 6. The droplet size was least in buffer pH 1.2 as compare to other dilution media, in all the three concentrations of drug loading. This may be due to the highest solubility of talinolol in buffer pH 1.2 (Figure 1A).

In vitro dissolution profile of developed talinolol loaded SNEDDS in various dissolution media was found to be significantly higher i.e. 100% of talinolol was released within 10-15 minutes irrespective of the pH of dissolution medium as compared with that of pure drug (20% of drug release within 10-15 minutes). The amount of talinolol released is given in Table 4. It could be suggested that the SNEDDS formulation resulted in spontaneous formulation of a nanoemulsion having a small droplet size, which permitted a faster rate of drug release into the aqueous phase, more rapidly than that of the pure drug. Thus, this higher availability of dissolved talinolol from the developed SNEDDS could lead to high absorption and improved oral bioavailability.

4. CONCLUSION

Talinolol loaded SNEDDS formulation containing Tween 20 (35% w/w), Ethanol (15% w/w) and Triacetin (50% w/w) as surfactant, co-surfactant and oil, respectively has been designed and developed based upon rapid and high drug release, desired droplet size, minimum polydispersity, lower surfactant and co-surfactant concentration and high drug solubility. The method employed in the investigation for screening of components of SNEDDS helped in understanding the emulsification efficiency of various surfactants for selected oil using different surfactants and co-surfactants. Studies on pseudoternary phase diagrams indicated that the concentration of talinolol and the pH of dilution medium significantly affect the area as well as the droplet size of the nanoemulsion formation. Studies also showed how SNEDDS can be designed and developed for the delivery of hydrophobic compounds with high drug loading and proper infinite dilution can be achieved without drug precipitation.

SNEDDS of talinolol could accommodate high dose of talinolol (up to 200 mg) depending upon the composition of SNEDDS and exhibited rapid release independent of pH of dissolution media. The present investigation showed that the SNEDDS formulation may be used as a possible alternative to traditional oral formulations of talinolol to improve its absorption and availability.

5. ACKNOWLEDGMENT

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