ORIGINAL ARTICLE

Design and evaluation of self-nanoemulsifying drug delivery systems for nebivolol hydrochloride

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Nebivolol hydrochloride (NEB) is third generation beta-blocker, approved by the FDA to treat a hypertension. It's a racemic mixture of a d-Nebivolol and l-Nebivolol. Oral delivery of the NEB shows a lower bioavailability due to its poor solubility and permeability. In present study, self nano emulsifying drug delivery is formulated to increase the bioavailability of drug by increasing solubility and permeability through the gastro intestinal membrane. Excipients are selected on basis of results obtained from solubility studies of drug in various surfactants and oils. Selected system of oil, surfactant and co-surfactant were screened for their miscibility and emulsification ability. The ternary phase diagram was constructed using system Capmul MCM EP as oil, Tween-60 as surfactant, Transcutol HP and PEG-400 as co-surfactant. Five compositions were prepared from the self-emulsifying area of the ternary diagram, loaded with NEB and then tested for robustness to dilution, pH effect on globule size, mean globule size and polydispersity index (PDI), zeta potential, viscosity and drug release for the selection of optimized formulation. Further, the effect of viscosity and pH on the globule size and PDI of optimized SNEDDS was studied. *In-vitro* drug release study was performed using dialysis bag method. *Ex-vivo* drug release studies were also carried out to determine the permeability of drug loaded SNEDDS through the stomach and intestinal membrane. The optimum concentration of a system determined Capmul MCM EP 25%, Tween-60 50%, Transcutol HP 12.5%, PEG-400 12.5% with a globule size of 124.5 nm, cloud point at 770C and zeta potential of -5.123 mV. *In-vitro* drug release study and *ex-vivo* permeation study showed significant increase in dissolution rate and permeability respectively, as compared to the drug suspension and marketed preparation (NEBISTAR™).

Key words: *Dissolution, ex-vivo drug release, nebivolol hydrochloride, self-nanoemulsifying drug delivery systems, solubility studies*

INTRODUCTION

Approximately 40% of new drug candidates have poor water solubility and oral delivery of such drugs is frequently associated with implications of low bioavailability, high intra- and inter-subject variability and lack of dose proportionality.[1] Various approaches such as pH adjustment,[2] co-solvency,[3] particle size reduction,[4] solid dispersion,[5,6] hydrotropy,[7] micellar solubilization,[8] complexation,[9] lipid based formulations[3] are used to increase the solubility and bioavailability of such drug molecules. Among these most common approaches, lipid-based formulations such as incorporation of drug into oils[10] and in these self-nanoemulsifying formulations[11,12] are mostly preferred.

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Self-nanoemulsifying drug delivery systems (SNEDDS) are isotropic mixtures of drug, lipids and surfactants, usually with one or more hydrophilic co-solvents or co-emulsifiers that form fine oil in water nanoemulsions upon mild agitation in an aqueous medium with a droplet sizes ranging 20-200 nm.[13,14] The digestive motility of the stomach and intestine providing the agitation required for self-emulsification *in-vivo*.[15,16] Long chain triglyceride (LCT) and medium chain triglyceride (MCT) oils with different degrees of saturation were mainly used as oily-phase of SNEDDS, whereas non-ionic compounds with a relatively high hydrophilic-lipophilic balance (HLB) are the most widely recommended as surfactants.[12] The MCT are directly transported by the portal blood to the

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systemic circulation, whereas the LCT are transported via the intestinal lymphatic's.[17,18] Nano-emulsion within the intestine is capable of maintaining otherwise a poorly soluble drug in solution[19] and small droplet size of it provide a large interfacial surface area for drug absorption.[15,20] Nano-emulsions have a higher solubilization capacity than simple micellar solutions. Thermodynamic stability of nanoemulsions offers advantages over unstable dispersions, such as emulsions and suspensions, because they can be manufactured with little energy input (heat or mixing) and have a long shelf life.[21,22] SNEDDS usually include studies to ensure that the drug does not precipitate upon dispersion or digestion, since re-dissolution of the precipitate is believed to decrease absorption.[23]

In SNEDDS, surfactant enhance permeation across the intestinal membrane, reduce or eliminate food effect and enhance drug bioavailability,[24,25] also reduce a hepatic clearance of the drug, in addition to increasing its solubility.[26] For a suitable self-emulsifying vehicle in formulation, it is important to assess; (a) the drug solubility in various components, (b) the area of self-emulsifying region in the phase diagram and (c) droplet size distribution following self-emulsification.[27] As liquids produce some disadvantages such as gastrointestinal irritation due to the large quantity of surfactants in the formulations, it is important to design adequate dosage form for the administration of these lipid systems.[28] Various alternatives such as hard gelatin capsules,[29,30] pellets[31] and tablets[32] are used to solve this problem.

Nebivolol, a third-generation beta blocker, is approved by the Food and Drug Administration for the treatment of hypertension.[33] The medication's unique chemical structure is composed of a racemic mixture of d-nebivolol and l-nebivolol.[34] Nebivolol exerts nitric oxide mediated vasodilatation in addition to conventional beta-blocking effects. Nebivolol undergoes extensive first-pass metabolism through the cytochrome (CYP) P450 2D6 enzyme system. There is a great difference in bioavailability of drug in extensive metabolizers (12%) and for poor metabolizers (96%).[34-37] Nebivolol shows the lower aqueous solubility which leads to the lower bioavailability. The bioavailability can be increased by increasing its solubility and reducing first pass metabolism.

SNEDDS have been reported for their potential in enhancement of absorption of drug, oral bioavailability and thus the therapeutic efficacy, as well as in controlling the release rate of poorly water soluble drugs.[29,38-42] The present study is aimed on development of SNEDDS of nebivolol, in order to increase the aqueous solubility of drug which may further result in bioavailability enhancement.

EXPERIMENTAL

Chemicals and reagents

Nebivolol hydrochloride was obtained from Torrent Pharmaceuticals, Gujarat, India. Capmul PG12, Capmul MCM EP, Captex 170, Captex 300 and Acconon E were obtained as gifts from Abitech Co. (USA). Polyethylene glycol (PEG)-8 caprylic/capric glycerides (Labrasol®), Oleoyl macrogol 6-glycerides (Labrafil® M1944CS) and diethylene glycol monoethyl ether (Transcutol HP®) were kindly donated by Gattefosse Co. (Canada). Polyoxy 35 castor oil (Cremophor EL®) was obtained from BASF Co. (India). Propylene glycol, PEG-200, PEG-400, Tween-60, Span-20, Span-80 and Tween-80 were purchased from Loba Chemie Pvt. Ltd., Mumbai (India). All other chemicals used were of analytical grade.

Solubility studies

Solubility studies of drug in different excipients (oils, surfactants, co-surfactants). Briefly an excess amount of nebivolol hydrochloride was placed in to an eppendorf tube containing 2 ml solvent either oil (Capmul PG12, Capmul MCM EP, Captex 170, Captex 300 and Acconon E) or surfactant (Span-80, Tween-20, Tween-60, Tween-80, Labrasol, Solutol HS-15, Chremophore EL and Triton X-100) or co-surfactant (Labrafil CS 1499, PEG-200, PEG-400, Propylene Glycol and Transcutol). The resultant mixture was heated on a water bath at 40°C and stirred vigorously using vortex mixer (CM 101 Cyclomixer, Remi Equipments Pvt. Ltd., Mumbai, India) for 5 min to facilitate the solubilization. The resultant mixture was continuously agitated on a rotary shaker at 40°C for 72 h. After reaching equilibrium the sample were centrifuged (R-8C Laboratory Centrifuge, Remi Equipments Pvt. Ltd., Mumbai, India) at 10,000 rpm for 15 min. The supernatant was suitably diluted with methanol and NEB dissolved was quantified using ultraviolet (UV)-spectrophotometer (UV-1800, Pharma Spec. Shimadzu, Japan) at 280 nm placing a blank.[29] Blank was prepared by dissolving respective oil or surfactant in methanol with same dilution as for the samples.[39]

Screening of surfactant

The solubility of NEB was determined in different surfactant and then surfactants were screened on their ability to emulsify the selected oil phase (Capmul MCM EP, Capmul PG12 and Capmul C8). To determine the emulsification ability, 20 µl of surfactant was added to 20 µl of the selected oil phase, mixed thoroughly and then 10 µl of this mixture was diluted to 10 ml with distilled water. The ease of formation of emulsion was monitored by the number inversions of volumetric flask required to produce uniform emulsion. Emulsion was allowed to stand for 2 h and its transmittance was measured at 638.2 nm using UV-Vis spectrophotometer (UV-1800, Pharma Spec, Shimadzu, Japan) against distilled water as blank.[39]

Screening of co-surfactant

The solubility of NEB was also determined in various co-surfactants. Co-surfactants were screened based on their efficacy to improve the nano-emulsification ability of the selected surfactants. For this, 40 µl of surfactant was mixed with 20 µl of the co-surfactant (surfactant: Co-surfactant = 2:1). The selected oil (60 µl) was added to this mixture and the

mixture was gently heated in a water bath to allow proper mixing. Ten µl of this mixture was diluted to 10 ml with distilled water and the ease of formation of emulsions was monitored by the number of inversions required to produce uniform emulsion. The emulsions were allowed to stand for 2 h and their transmittance was measured at 638.2 nm using UV-Vis spectrophotometer (UV-1800, Pharma Spec, Shimadzu, Japan) against distilled water as blank.[39]

TERNARY PHASE DIAGRAM CONSTRUCTION

Ternary diagrams of surfactant, co-surfactant and oil were plotted using Chemix, each of them representing an apex of the triangle.[43] Ternary mixtures with varying compositions of surfactant, co-surfactant and oil were prepared. The surfactant concentration was varied from 30% to 75% (w/w), oil concentration was varied from 20% to 75% and co-surfactant concentration was varied from 0% to 30% (w/w).[44] For any mixture, the total of surfactant, co-surfactant and oil concentrations always added to 100%. For example, in the experiment, first mixture consisted of 75% of surfactant (Tween-60), 25% of the oily phase (Capmul MCM EP) and 0% of co-surfactant (PEG-400: Transcutol HP = 1:1). In further experiments, the co-surfactant was increased by 5% for each composition, oily phase concentration was kept constant and the surfactant concentration was adjusted to make a total of 100%. Forty-two such mixtures with varying surfactant, co-surfactant and oil concentrations were prepared in this investigation. The percentage of surfactant, co-surfactant and oil used herein was decided on the basis of the requirements stated for the spontaneously emulsifying systems.[45] Compositions were evaluated for nanoemulsion formation by diluting 10 µl of each of the 42 mixtures to 10 ml with double distilled water. Percent transmittance of resulting dispersions was measured spectrophotometrically at λ_{max} 638.2 nm. The area of nanoemulsion formation was identified for the respective system in which nano-emulsions with globule size 200 nm or below considered desirable.[46]

Preparation of NEB loaded SNEDDS

Five different formulation of NEB loaded SNEDDS were prepared by composition shown in Table 1. Accurately weighed of surfactant (Tween-60) and co-surfactant (PEG 400: Transcutol HP) were mixed in a vial on magnetic stirrer. The weighed amount of drug (NEB)[47] was dissolved in the selected amount of oil (Capmul MCM EP) in a separate beaker. The oil phase was added drop wise to the surfactant co-surfactant mix and stirring was continued for 1 h to obtain NEB loaded liquid SNEDDS.

Optimization of SNEDDS

Optimized drug loaded formulation was selected among five SNEDDS formulation (F1, F2, F3, F4 and F5) based on the robustness to dilution, globule size analysis, zeta potential, potential determining ions (PDI), cloud point measurement, *in-vitro* drug release.

Table 1: Composition of SNEDDS formulations

Formulation	F-1	F-2	F-3	F-4	F-5
Amount of drug (mg/5 ml)	38.857	37.428	37.714	55.428	47.714
Oil (Capmul Pg-12) (%)	25	30	30	25	35
Surfactant (Tween-60) (%)	50	60	55	55	60
Co-surfactant (TSP: PEG-400) (%)	25	10	15	20	05

SNEDDS: Self-nanoemulsifying drug delivery systems, PEG: Polyethylene glycol, TSP: Transcutol HP

CHARACTERIZATION OF SNEDDS

Percent transmittance

The NEB SNEDDS were reconstituted with double distilled water and the resulting nanoemulsion was observed visually for any turbidity. Thereafter, it's percent transmittance was measured at 638.2 nm using UV-Vis spectrophotometer against double distilled water as the blank. The studies were conducted at 50, 100, 250 and 1000 times dilution.[39]

Measurement of mean globule size, zeta potential and PDI

The globule size and zeta potential of the reconstituted NEB SNEDDS were determined using Malvern Zetasizer (Nano ZS 90, UK). The samples were put in "folded capillary cells" and results obtained for size, PDI and zeta-potential were recorded.[41]

Robustness to dilution

Five formulations of NEB SNEDDS were diluted with HCl buffer (pH 1.2) and phosphate buffer (pH 6.8) and the transmittance and globule size of the resultant nanoemulsion were measured.[46]

Viscosity

The viscosity of NEB SNEDDS was determined with Brookfield viscometer (digital viscometer + Pro) at 20 rpm at room temperature (25°C).[48]

Cloud point measurement

The five SNEDDS formulations were compared for cloud point value. Each formulation was diluted with water in the ratio of 1:100 and placed in a water bath with gradual increase in temperature. At the cloud point, drop in the percent transmittance of sample from the zero point was measured spectrophotometrically.[49]

In-vitro drug release studies

In-vitro release of NEB SNEDDS was carried on (Electrolab TDT-08 L Mumbai) by dialysis method. After NEB SNEDDS was instilled into the dialysis bag (MWCO 10,000), the dialysis bag was firmly sealed and was placed in 250 ml, pH 1.2 and pH 6.8 buffer (containing 0.5% of Tween-80) as the dissolution medium at 37°C. The revolution speed of the paddle was maintained at a rate of 100 rpm.[49] Aliquots were

withdrawn from the flask at periodic time intervals, replaced with equivalent amounts of fresh media and analyzed spectrophotometrically at λ_{max} 284 nm.

Ex-vivo drug release study

Ex-vivo drug release was studied using Male Sprague-Dawley rats (250-300 g). Rats were humanely sacrificed and then stomach and small intestine were isolated and thoroughly washed with phosphate buffer saline (PBS) to remove the mucous and lumen contents. NEB SNEDDS diluted separately with HCl buffer pH 1.2 and phosphate buffer pH 6.8 were filled in the stomach and intestine respectively. Equivalent amount of plain NEB suspensions in HCl buffer pH 1.2 and phosphate buffer pH 6.8 respectively were used for comparison. Both the ends of the tissues were tied properly to avoid any leakage and were put into beakers containing 40 ml of PBS (pH 7.4) as the acceptor phase with continuous aeration supply under gentle stirring at 37 ± 2°C. Samples were withdrawn from the acceptor phase at periodic time intervals and subjected to spectrophotometric analysis. All the experiments were performed in triplicate.[39]

Stability studies

The SNEDDS formulations were filled into empty hard gelatin capsules (size 0) and subjected to stability studies at 25°C/60% relative humidity (RH) and 40°C/75% RH. Samples were charged in stability chambers (Thermolab, TH 200S, Mumbai) with humidity and temperature control. They were withdrawn at specified intervals for analysis over a period of 3 month.[40] Drug content of the capsules was analyzed using a previously developed and validated stability-indicating high performance liquid chromatographic method.

RESULTS AND DISCUSSION

Solubility studies

The solubility of the NEB was tested in different oils and surfactant which are commonly utilized in SEDDS and SNEDDS formulation. The results of solubility studies of drug in various oils, surfactants and co-surfactants are as shown in Table 2. The graphical representation [Figure 1] revealed that drug shows highest solubility in Capmul G12 followed by Capmul MCM EP and Capmul C8. These three oils were selected for further study of emulsification ability and miscibility with other ingredient.

Table 2: Solubility of NEB in different excipients

Category	Name of excipient	Solubility (mg/ml)*
Oil	Acconon E	7.6383±0.0151
	Capmul MCM EP	7.1716±0.0235
	Capmul Pg-12	14.2443±0.0182
	Coconut oil	2.172±0.0
	Captex 300	0.643±0.0215
	Captex 170	0.643±0.02157
	Capmul C8	7.7066±0.160
	Soya bean oil	0.9953±0.0149
	Linseed oil	1.2453±0.0122
	Cottonseed oil	0.649±0.0095
	Peanut oil	0.584±0.0091
	Sesame oil	0.455±0.0230
Surfactant	Triton X-100	35.152±0.020
	Tween-60	63.081±0.0166
	Labrasol	37.461±0.0265
	Tween-80	54.664±0.0224
	Solutol HS 15	49.958±0.0117
	Chremophore EL	37.344±0.0137
Co-surfactant	PEG 200	76.350±0.0274
	PEG 400	48.259±0.0131
	Span 20	84.443±0.0208
	Propylene glycol	71.939±0.0149
	Transcutol HP	25.660±0.0143
	Transcutol HP: PEG 400	72.598±0.0149
	Labrafil 1944 CS	41.366±0.0404

*Expressed as a mean±SD (*n*=3). NEB: Nebivolol Hydrochloride, PEG: Polyethylene glycol

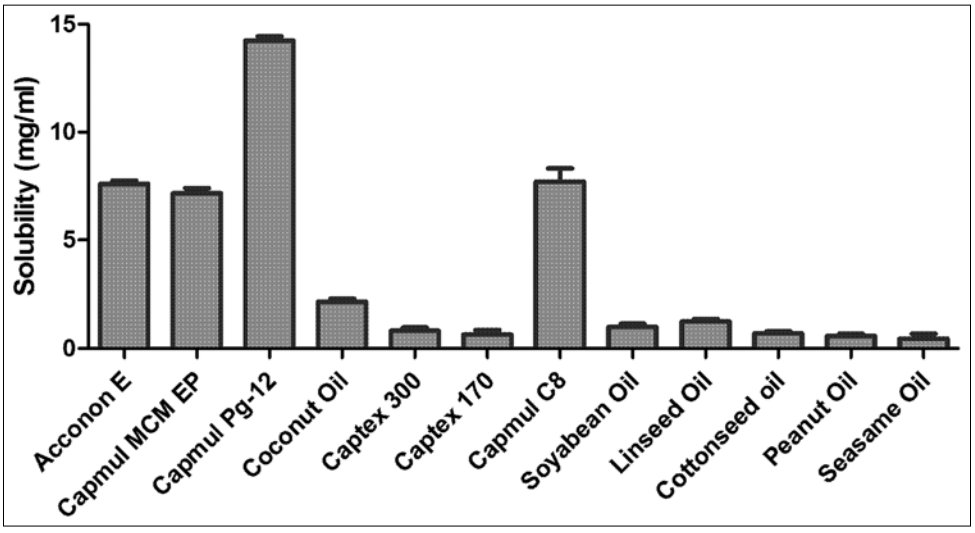

Figure 1: Solubility of nebivolol hydrochloride in various oils. *Expressed as a mean ± SD (n = 3)

Screening of surfactant

Non-ionic surfactants are considered less toxic than ionic surfactants[50] and are generally accepted for oral ingestion. In present study, non-ionic surfactants such as Tween-80, Labrasol, Chremophore EL, Triton X-100 and Tween-60 are screened. These surfactants are reported to possess different bioactive effects like effects on tight junction of cell on an intestinal membrane by Labrasol[43] and inhibitory effects of Chremophore EL and Tween-80[40,51,52] on P-gp and CYP enzymes. The surfactants were compared for their emulsification efficiencies using selected oily phases and the results of percent transmittance values of different mixtures are presented in Table 3 which shows Tween-20 gives the highest transmittance value with Capmul MCM EP followed by the Tween-60 and Labrasol. Capmul PG-12 showed poor emulsification with Tween-60 and Labrasol.

Screening of co-surfactant

Addition of a co-surfactant to the surfactant-containing formulation was reported to improve dispersibility and drug absorption from the formulation.[41] It has been noted that well-formulated SNEDDS is dispersed within seconds under gentle stirring conditions.[52] In view of current investigation five co-surfactants namely propylene glycol, Transcutol HP, PEG-400, Labrafil and Transcutol HP: PEG-400 (1:1) were compared as shown in Table 4. Capmul MCM EP as oil and Tween-60 as surfactant formed a good emulsification with all co-surfactants, with Transcutol HP: PEG-400 (1:1) showing maximum transmittance (98.6%) followed by Transcutol HP (96.4%). All dispersions exhibited instantaneous emulsion formation with only one flask inversion. Detailed study of the systems Capmul MCM EP as oil, Tween-60 as surfactant and Transcutol HP: PEG-400 (1:1) as co-surfactant was carried out via ternary phase diagrams.

TERNARY PHASE DIAGRAM CONSTRUCTION

The phase diagram of different batches of SNEDDS was constructed by varying the concentration of the selected oil, surfactant and co-surfactant as shown in Figure 2. It was observed that the Capmul MCM EP,-Tween-60, Transcutol HP: PEG-400 (1:1) system yielded nano-emulsion (globule size <200 nm) for compositions which have high oil phase up to 50%. System having low or none surfactant shows the higher globule size (>200 nm). As the amount of co-surfactant increases in the combination there is decrease in globule size. In an o/w SNEDDS, a non-ionic emulsifier with a high HLB is like Tween-60 is to be used for its drug compatibility, strong self-nano-emulsion ability, low toxicity and hemolysis.[53] As the amount of oil increases along with

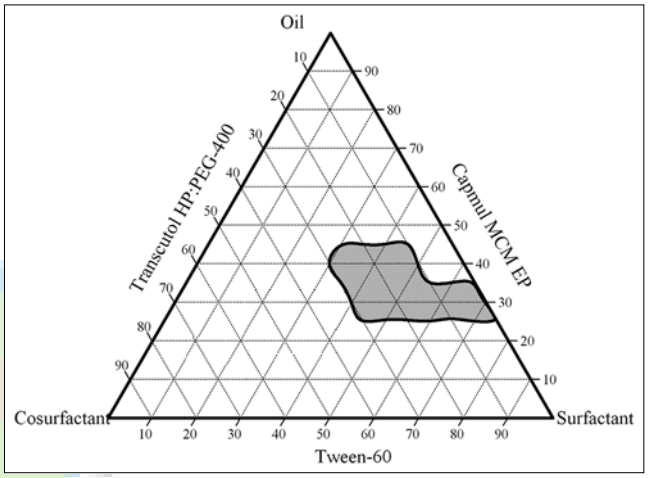

Figure 2: Ternary phase diagram of Capmul MCM EP, Tween-60 and Transcutol HP:PEG-400 (1:1)

Table 3: Emulsification efficiency with different surfactants and selected oils

Surfactant	Percentage of transmittance (at 638.2 nm)*			HLB value
	Capmul Pg-12	Capmul MCM EP	Acconon E	
Triton X-100	73.459±0.02953	82.139±0.0160	74.569±0.0425	13.5
Tween-60	68.707±0.0205	78.602±0.0372	72.157±0.0100	14.9
Labrasol	36.723±0.0105	43.126±0.0263	38.571±0.0414	14
Tween-80	54.099±0.0234	66.191±0.0635	59.357±0.0100	15
Solutol HS 15	25.673±0.0235	29.647±0.0115	26.465±0.0326	14-16
Chremophore EL	56.552±0.0306	74.266±0.0242	69.445±0.0468	14

*Expressed as a mean±SD (*n*=3). SD: Standard deviation, HLB: Hydrophilic-lipophilic balance

Table 4: Emulsification efficiency with different co-surfactants and selected surfactants

Co-surfactant	Percentage of transmittance (at 638.2 nm)*			HLB value
	Tween-60	Solutol HS 15	Triton X-100	
PEG 200	51.443±0.025	66.38±0.014	64.146±0.014	5-6
PEG 400	65.958±0.026	96.877±0.018	86.928±0.012	8-9
Span 20	52.339±0.024	55.270±0.025	49.316±0.032	8.6
Propylene glycol	66.575±0.013	58.833±0.023	83.246±0.021	11.6
Transcutol HP	68.939±0.016	73.852±0.016	59.953±0.013	
Transcutol HP: PEG 400	75.657±0.034	98.877±0.018	72.592±0.022	-
Labrafil 1944 CS	55.968±0.011	78.960±0.012	53.466±0.019	4

*Expressed as a mean±SD (*n*=3). SD: Standard deviation, HLB: Hydrophilic-lipophilic balance, PEG: Polyethylene glycol

co-surfactant there is lesser change in droplet size compare to surfactant effect. The SNEDDS having fine globule size have preferred effect on drug release and absorption. Due to smaller globule size, it possesses the higher interfacial surface and to produce the high surface area high amount of surfactant required. SNEDDS with the high amount of surfactant mixture forms stable emulsion and possess the high self-emulsification capability.

Preparation of NEB loaded SNEDDS and optimization of SNEDDS

Five formulations (F-1, F-2, F-3, F-4, F-5) of NEB loaded SNEDDS were prepared as shown in Table 1. Optimization of the SNEDDS formulations (F1, F2, F3, F4 and F5) was done based on the result obtained from the following tests.

CHARACTERIZATION OF SNEDDS

Percent transmittance

The transmittance values of all five formulation above 90%, confirming the self-nano-emulsification efficiency of the SNEDDS. Results are shown in Table 5.

Measurement of mean globule size, zeta potential and PDI

The droplet size of the emulsion is a crucial factor in self-emulsification performance because it determines the rate and extent of drug release as well as absorption.[54] The smaller the droplet size, the larger the interfacial surface area will be provided for drug absorption[56-58] and it is reported that larger droplets are less neutralized by mucin than smaller droplets.[58]

Particle size after nano-emulsification is the most important property of SNEDDS. Mechanisms of particle size effect on drug absorption may include improved release and facilitated lymphatic transport.[56,59-61]

As shown in Table 6, as the surfactant concentration increases globule size decreases. This indicates that oil phase is needed to dissolve the drug while higher surfactant concentration helps in stabilizing globule. The globule size distribution and polydispersity index revealed that, among all, F-1 formulation shows the closer globule size distribution and also produces the finest emulsion.

In some cases, the mean droplet size may increase with increasing surfactant concentrations. This phenomenon could be attributed to the interfacial disruption elicited by enhanced water penetration into the oil droplets mediated by the increased surfactant concentration and leading to ejection of oil droplets into the aqueous phase.[56] This signifies the uniformity of droplet size within the formulation. The higher the value of polydispersity, the lower is the uniformity of the droplet size in the formulation.[62]

It is necessary to assess zeta potential of the SNEDDS as it can identify the charge of oil globules in the emulsion. Zeta potential is a very important factor in characterizing emulsification efficiency. The significance of zeta potential is that its value can be related to the stability of colloidal dispersions. For the smaller droplet, a high zeta potential will confer stability in the solution or dispersion by resisting aggregation. It has been noted that the zeta potential played an important role in the interactions with mucus of the gastrointestinal tract.[57,63] Colloids with high zeta potential (negative or positive) are electrically stabilized. Negative values of zeta potential of the formulations [Table 6], indicated that the formulations were negatively charged and therefore gives indication of stable system.

Effect of pH on dilution

SNEDDS formulations were exposed to different folds of dilution in different media in an attempt to mimic the *in-vivo* conditions where the formulation would encounter

Table 5: Effect of dilution on different SNEDDS formulations using double distilled water as a dilution media

Dilution	Percentage of transmittance (at 638.2 nm)*				
	F1	F2	F3	F4	F5
50 times	81.275±0.0267	56.137±0.0284	56.813±0.0105	35.371±0.0073	24.254±0.0125
100 times	94.141±0.0125	74.452±0.0296	64.796±0.1098	51.064±0.0137	53.774±0.0077
250 times	97.907±0.0145	80.673±0.0195	79.527±0.0176	88.121±0.0070	85.773±0.0095
1000 times	99.815±0.0596	94.344±0.0264	93.358±0.0087	95.391±0.0155	92.292±0.0090

*Expressed as a mean±SD (*n*=3). SNEDDS: Self-nanoemulsifying drug delivery systems, SD: Standard deviation

Table 6: Physical properties of SNEDDS formulations

Test parameter	SNEDDS formulation				
	F1	F2	F3	F4	F5
Z-average diameter*	124.68±0.145	148.29±0.198	168.58±0.147	174.23±0.231	0.182.64±0.157
PDI*	0.125±0.0137	0.195±0.0119	0.254±0.201	0.173±0.0124	0.169±0.0214
Zeta potential (mV)	−5.74	−4.84	−4.18	−2.53	−3.75
Viscosity (cPs)	24	36	39	59	48
Cloud point (°C)	77-78	76-77	75-76	75-77	74-75

*Expressed as a mean±SD (n=3). SD: Standard deviation, SNEDDS: Self-nanoemulsifying drug delivery systems, PDI: Potential determining ions

gradual dilution. Physical integrity of nano-emulsion formed and drug solubilization capacity after dilution of SNEDDS must be assessed and ensured as it gives an idea about its performance *in-vivo*.[56,64] It has been observed that the pH of dilution media [Table 7] does not shows any effect on the percent transmittance of formulations.

Viscosity

The viscosity of SNEDDS formulation is important to fill it in hard gelatin capsules. If the SNEDDS has very low viscosity, it may enhance the probability of leakage from the capsule and the system with very high viscosity may create problem in pourability.[65] The viscosity value [Table 6] of formulation <10,000 cps, is generally considered as suitable for developed SNEDDS which can be filled in hard gelatin capsules by commercial liquid filling equipment's.[65] The viscosity values are also known to provide a linking on whether the system is w/o or o/w type.[66] It is also reported that viscosity affected the droplet size and rate of drug diffusion.[67,68]

Cloud point measurement

The cloud point is an essential factor in SNEDDS consisting of non-ionic surfactants and it is responsible for the successful formation of a stable nano-emulsion.[13] At a temperature higher than cloud point, irreversible phase separation occur due to dehydration of ingredient, which may affect drug absorption. Phase separation can occur due to dehydration of polyethylene oxide moiety of the non-ionic surfactant. Due to this the drug release from formulation gets affected. To avoid this cloud point of the formulation should be over 37°C.[41]

In this study, cloud points of all formulations [Table 6] were very high, F1 formulation shows the higher cloud point at that temperature shows a rapidly fall in transmittance. All formulation shows the cloud point above the 37°C hence it is stable at intestinal temperature.

In-vitro drug release studies

When SNEDDS encounter aqueous medium, different forms of solubilized drug are formed, that encompass free molecular state, drug in nano-emulsion and drug in micellar solution. Under these circumstances, it is necessary to separate free drug molecules from those entrapped in the nano-emulsion droplets or micelles to assess the real release pattern.[49] Thereby, conventional release testing is not adequate to this system. For that purpose dialysis bag method is reported.[49,69]

Type of systems is characterized by higher percentage of hydrophilic surfactants. This high proportion is usually concomitant with higher probability of surfactant migration into surrounding aqueous media upon dispersion.[52,64]

The high surfactant concentration released is supposed to form micelles that trap free drug inside, with subsequent hindrance in drug release. It is reported that dialysis bag with molecular weight cut-off of 10,000 circumscribes escape of nano-emulsion into release medium.[56] The initial step shows a burst release which can be attributed to the surface associated drug, followed by a slower sustained release phase that nano-sized droplets of emulsion can enhance the release of poorly soluble drugs.[29] The measured release rate from SNEDDS was significantly faster than that from the conventional tablet [Figure 3].

Ex-vivo drug release studies

The cumulative percent drug release of SNEDDS, marketed preparation and pure drug from rat stomach and intestine is shown in Figure 4. It was observed that the release of the drug was enhanced from the reconstituted SNEDDS, F-1 formulation, as 95% drug was released within 60 min for SNEDDS in comparison to 44% drug release in 120 min from plain drug suspension and 50% drug release in 120 min form marketed preparation. It can be notated that absorption of the drug from the intestine can be enhanced with SNEDDS, fulfilling our objective of increasing intestinal absorption for enhancing the bioavailability of NEB. It can be attributed to oil droplet absorption of various lipid absorption mechanisms: Such as passive diffusion, pinocytosis or endocytosis,[45] Their small droplets size also provides a large interfacial surface area for drug release and absorption and hence bioavailability.[29]

Stability studies

The developed formulations were found to be physically and chemically stable for 3 months at 30 ± 2°C/65 ± 5% RH and 40 ± 2°C/75 ± 5% RH [Table 8]. No change in the

Table 7: Effect of dilution on different SNEDDS formulations

Dilution medium	Dilution	Percentage of transmittance (at 638.2 nm)*				
		F1	F2	F3	F4	F5
Phosphate buffer pH 1.2	50 fold	64.678±0.194	44.547±0.637	37.667±0.808	51.253±0.375	23.651±0.254
	100 fold	87.237±0.127	22.892±0.918	20.737±0.412	63.265±0.655	47.178±0.657
	250 fold	95.501±0.504	68.161±0.788	61.655±0.116	86.254±0.568	57.457±0.583
	1000 fold	98.270±0.257	94.467±0.527	85.768±0.517	92.381±0.823	83.475±0.871
Phosphate buffer pH 6.8	50 fold	67.269±0.814	54.579±0.504	55.766±0.324	47.106±0.854	19.333±0.658
	100 fold	81.331±0.303	77.756±0.609	79.296±0.599	76.339±0.503	63.567±0.289
	250 fold	89.675±0.984	65.658±0.571	91.617±0.315	84.637±0.616	59.467±0.218
	1000 fold	98.823±0.116	93.666±0.664	83.185±0.115	89.946±0.543	82.467±0.624

*Expressed as a mean±SD (*n*=3). SNEDDS: Self-nanoemulsifying drug delivery systems, SD: Standard deviation

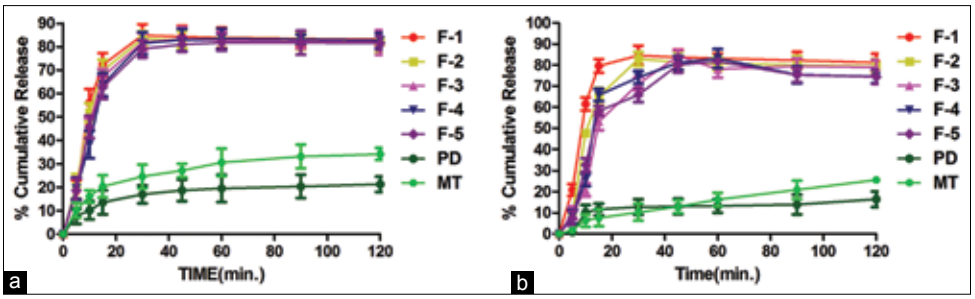

Figure 3: *In-vitro* drug release of different self-nanoemulsifying drug delivery systems formulation using: (a) pH 1.2 buffer as a dilution media and (b) pH 6.8 buffer as a dilution media

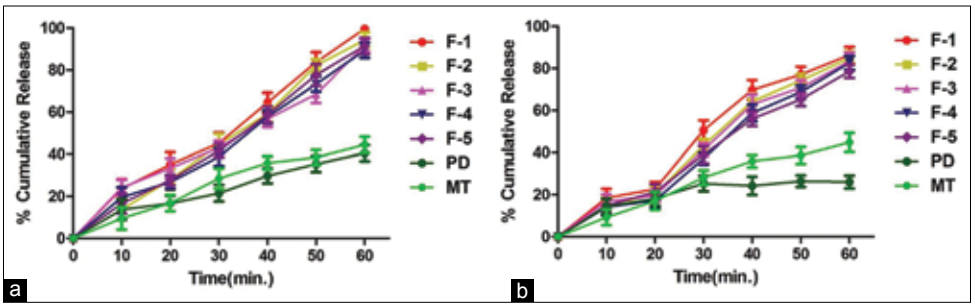

Figure 4: *Ex-vivo* drug release studies (a) from rat stomach of reconstituted self-nanoemulsifying drug delivery systems (SNEDDS), PD: Pure drug, MT: Marketed preparation. (b) From rat intestine of reconstituted SNEDDS, PD: Pure drug, MT: Marketed preparation

Table 8: Stability studies of SNEDDS

Days	Temperature condition	Globule size	PDI	Percentage of transmittance	Drug content
0	2-8°C	124.54±0.197	0.125±0.0137	99.81±0.11	99.73±0.025
	30±2°C/65±5% RH	124.54±0.197	0.125±0.0137	99.81±0.11	99.73±0.025
	40±2°C/75±5% RH	124.54±0.197	0.125±0.0137	99.81±0.11	99.73±0.025
30	2-8°C	124.08±0.041	0.141±0.0176	99.78±0.14	99.54±0.014
	30±2°C/65±5% RH	123.87±0.154	0.164±0.0137	99.84±0.08	99.27±0.007
	40±2°C/75±5% RH	124.57±0.208	0.178±0.0112	99.45±0.24	99.14±0.011
60	2-8°C	124.12±0.107	0.149±0.0184	99.82±0.17	99.38±0.009
	30±2°C/65±5% RH	123.94±0.039	0.186±0.0125	99.88±0.09	99.06±0.017
	40±2°C/75±5% RH	124.74±0.315	0.198±0.0125	99.71±0.12	98.92±0.019
90	2-8°C	124.22±0.133	0.151±0.0201	99.79±0.13	99.31±0.012
	30±2°C/65±5% RH	124.41±0.011	0.208±0.0095	99.84±0.11	98.88±0.013
	40±2°C/75±5% RH	124.94±0.177	0.217±0.0175	99.68±0.11	98.77±0.004

*Expressed as a mean±SD (*n*=3). SD: Standard deviation, SNEDDS: Self-nanoemulsifying drug delivery systems, PDI: Potential determining ions, RH: Relative humidity

physical appearance of SNEDDS was observed during the stability studies and the SNEDDS remained clear with no signs of precipitation. This indicated that the drug remained solubilized even at accelerated stability conditions (40 ± 2°C/75 ± 5% RH). The Z-average size was similar at both the storage conditions for SNEDDS. Furthermore, no significant changes were observed in the droplet size of for SNEDD at both the storage conditions. Also, no significant decrease in the NEB content was observed indicating that NEB remained chemically stable in the SNEDDS. Thus, it can be concluded that the NEB SNEDDS would remain physicochemically stable at long-term stability conditions (30 ± 2°C/65 ± 5% RH) as well as accelerated conditions (40 ± 2°C/75 ± 5% RH) for 3 months.

CONCLUSION

The present study has clearly showed the potential utilization of SNEDDS for formulating NEB with improved aqueous solubility, stability and *in-vitro* drug release. The SNEDDS with relatively high drug content was prepared which self-emulsified easily with mean emulsion droplet size of 124.5 nm. Stability study and cloud point study confirmed that the SNEDDS had no dilution effect and was stable at pH 1.2 and 6.8 buffer without any precipitation of drug and without any change in emulsion droplet size.

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