Formulation Studies on Novel Self-Solidifying Self-Nanoemulsifying Drug Delivery Systems of Nebivolol Hydrochloride

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Abstract: Nebivolol HCl (NBL) a third generation beta blocker poses lack of oral bioavailability (12%) owing to its low solubility and first pass biotransformation in liver. The present research was undertaken to prepare solid Self-nanoemulsifying drug delivery system (S-SNEDDS) of NBL which will present NBL at molecular level in nanoemulsion form throughout GIT. Increased solubility along with intestinal lymphatic transport of lipid rich nanoemulsified drug bypassing hepatic first pass may enhance bioavailability. Based on solubilization of the drug and spontaneity of self-emulsification, Pecool as an oily phase, Cremophore RH 40 and Gelucire 50/13 as surfactants and ethanol as co-surfactant/co-solvent were selected as the excipients to produce NBL loaded S-SNEDDS. Total 9 formulations were made with different ratios of the excipients and the optimized formulation was selected on the basis of solidification of SNEDDS on refrigeration and maintenance of the solid state. Spherical shaped morphology of oil globules was confirmed by TEM analysis. On dilution S-SNEDDS showed nanoparticles of size 180-190nm with a Polydispersity index 0.4-0.8 and Zeta potential -5.17, -7.56mV. The DSC and X-ray diffraction patterns of the S-SNEDDS show the amorphous state of NBL in the lipid matrix. Developed S-SNEDDS showed pH-independent drug dissolution which in SIF was fourfold greater as compared to plain drug. The intestinal permeability by everted sac technique showed threefold increase in transportation of NBL from S-SNEDDS formulation compared to NBL solid suggesting that S-SNEDDS of NBL is an excellent and practical approach of enhancing the oral bioavailability through improved solubility.

Keywords: Cremophore RH 40, gelucire 50/13, nebivolol hydrochloride, oral bioavailability, pceool, solid self-emulsifying drug delivery system.

INTRODUCTION

Recently many compounds with prevailing pharmacological activity have been in the arena of drug discovery by surprising movement of modern technology comprising rapid and novel in silico drug design inputs [1]. Many of the new chemical entities (NCEs) in contemporary discovery libraries and development programs have unfavorable physical and chemical properties with respect to drug absorption. In particular, up to 70% of discovery compounds and 40% of forthcoming candidates are insoluble in water. An increasing number of drugs are categorized as Class-II drugs by being poorly water soluble and highly lipophilic and hence more biologically permeable, by Biopharmaceutical Classification System (BCS). Frequently class-II drugs show suboptimal oral bioavailability, greater intra- and inter-subject drug-response differences and impaired dose proportionality [2]. Consequent to this fact, greater number of drug candidates fail to reach the market, even though they exhibit prospective pharmacodynamic activity. These capable molecules present idiosyncratic formulation and development challenges and often suffer from poor bioavailability because the rate and extent of absorption from the gastrointestinal tract (GIT) of such drugs are highly influenced by dissolution process [3]. Many strategies have been developed to enhance the oral absorption of class II drugs via improving the rate of dissolution of the drugs or by presenting the drug in a more absorbable form in the gut [4].

The SNEDDS present drugs in dissolved state in isotropic mixtures of oils of natural, semisynthetic or synthetic origin, surfactants and co-surfactants. Once administered orally, SNEDDS emulsify immediately on exposure to GIT fluids to form o/w emulsion with droplet size in the range of 20–200nm. Subsequently the absorption of the poorly water soluble drug would be rapid and complete due to its presence in the molecular state throughout its stay in the gastrointestinal tract [5].

Nebivolol hydrochloride (NBL) is a third generation beta blocker (highly selective for the β-adreno receptors) cardiovascular drug, reported to have only 12% oral bioavailability which is the result of not only the drug’s poor aqueous solubility (4mg/mL) but also by its extensive first pass metabolism as NBL is a substrate for Cytochrome P-450 enzymes. The other reason for its poor bioavailability is its pH-dependent solubility i.e. its solubility in gastric fluid (acidic) is significantly more than intestinal fluid (alkaline) so that the drug gets precipitated in intestinal region before getting completely absorbed [6, 7].

The objectives of the present study were designing and formulating Solid Self-nanoemulsifying drug delivery system (S-SNEDDS) of low-water soluble drug NBL with the intention to increase its solubility profile and to characterize the obtained formulation in respect to in vitro and ex vivo parameters.

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The rationality of SNEDDS of NBL is not only the inhibition of precipitation of NBL in intestinal fluid (alkaline environment), but also the protection of NBL from hepatic first pass metabolism. The latter may result due to circumventing the hepatic first pass effect by intestinal lymphatic transport of the lipid rich nanoemulsified drug.

MATERIALS AND METHODS

Materials

NBL was a generous gift from Watson Pharma Pvt. Ltd., Maharashtra, India. Captex 200, Captex 500, Captex 355 EP/NF, Capmul MCM EP, Capmul MCM C8 EP, Caprol PEG 860 (Abitech Corporation, USA) Labrafac Lipophile 1349, Maisine 35-1, Lauroglycol 90, Labrafil M 1944 CS (LM 1944), Labrafil M 2125 CS (LM 2125), Labrasol, Labrafac CC, Transcutol P, Gelucire 50/13 (Gel 50/13), Pcecel, Capryol PGMC, Lauroglycol 90, Lauroglycol FCC, Plurol Oleique CC 497 (PO 497), (Gattefosse, Mumbai, India), Poloxamer L-407, Poloxamer L-188 (PoL-188), Cremophore RH 40 (Cr-RH 40), Cremophore EL(BASF, Mumbai, India), oleic acid pure, ethyl oleate, olive oil, coconut oil, shark liver oil, sunflower oil, soyabean oil, clove oil, arachis oil, castor oil, ground nut oil, PEG 200, propylene glycol, N-butanol, ethanol, Tween 20, Tween 80, Span 80, Span 20, Tea tree oil, isopropyl myristate were purchased from SD fine chem. and hard gelatin capsules (Associated Capsules, Mumbai, India) were obtained as gift samples. The other pharmaceutical aids were used as received. Freshly prepared double distilled water was used in all formulations and experiments.

Methods

HPLC Analysis Details

An HPLC system (Agilent 1200 series quaternary pump system with Chemstation software, Version B.04.03) along with ZORBAX Eclipse XDB C18 4.6 X 150mm, 5µm column. The mobile phase consisted of a mixture of methanol: water (70:30 v/v), at a flow rate of 1mL/min that led to retention time of 2.55min when detection was carried out at 368.2nm by UV.Visible spectrophotometer against distilled water as blank [9].

Solubility Profile of NBL in Various Vehicles

The solubility of NBL in various natural and modified oils such as oleic acid pure, ethyl oleate, olive oil, coconut oil, shark liver oil, sunflower oil, soyabean oil, clove oil, arachis oil, castor oil, tea tree oil, isopropyl myristate, ground nut oil, Captex 200, Captex 500, Captex 355 EP/NF, Capmul MCM EP, Capmul MCM C8 EP, Maisine 35-1, Pcecel, Labrafil Lipophile WL 1349 and Caprol PEG 860, in co-surfactants such as Lauroglycol 90, LM 1944, LM 2125, Transcutol P, Lauroglycol FCC, PO 497, propylene glycol, N-butanol and ethanol, in surfactants such as Labrasol, Labrafac CC, Gel 50/13, Capryol PGMC, Poloxamer L-407, PoL-188 Cr-RH 40, Tween 20, Tween 80, Span 80 and Span 20 and different buffer solutions such as 0.01N HCl, Simulated gastric fluid (SGF) and Simulated Intestinal Fluid (SIF) was determined at two stages. In first stage, the approximate solubility of NBL in various vehicles was checked by visual observations to select the promising vehicles from the pool which can solubilise higher amounts of NBL. In the second stage, the solubility of NBL in selected vehicles was measured using routine shake flask method. Briefly, in each vial containing 1g of the selected vehicles an excess amount of NBL (based on approximate solubility data) was added. These mixtures were heated at 50-60 °C on water bath. Mixing of the systems was performed using a vortex mixer. Mixtures were then shaken for 48 hrs in a water bath shaker maintained at 37 °C ± 2 °C and then centrifuged at 3000rpm for 5 min, followed by filtration through membrane filter. The concentration of NBL was then quantified by HPLC [9].

Emulsification Efficiency Study

Selection of Surfactant(s) and Co-Surfactant(s)

The emulsification ability of various surfactants to spontaneously emulsify the selected oily phase was studied to choose the best surfactant(s) among the surfactants tested. The available co-surfactants were screened in terms of their effectiveness in potentiating the emulsification of the selected oily phase along with the surfactants. The spontaneity of emulsion formation was assessed by counting the times of volumetric flask inversions (FI) required to give a homogeneous emulsion on visual examination. After 2 hrs standing, the transmittance of resulting emulsions was measured at 638.2nm by UV-Visible spectrophotometer against distilled water as blank [9].

Preparation of Surfactants Combination (S-comb)

Four different surfactant combinations (S-comb), at 1:1(w/w) ratio were prepared. Each of these combination contains one liquid state surfactant either Labrasol or Cr-RH40 and one solid state surfactant either Gel 50/13 or Poloxamer L-188.

S-comb 1: Labrasol + Gel 50/13
S-comb 2: Labrasol + PoL-188
S-comb 3: Cr-RH40 + Gel 50/13
S-comb 4: Cr-RH40 + PoL-188

Four co-surfactants/co-solvents viz. Ethanol, PO-497, LM-2125 and LM-1944 were evaluated and compared for their efficiency (spontaneity) to emulsify oily phase Pcecel along with the above mentioned S-comb individually.

Optimization of Gel 50/13 Concentration

The concentration of selected solid state surfactant i.e. Gel 50/13 was optimized on the basis of its ability to solidify Self-emulsifying (SE) mixture and spontaneity in dispersion and emulsification of solid SE mixture.

Gel 50/13 and SE mixture (Pcecel: Cr-RH40: Ethanol at 1:1:1 w/w) was mixed at five different ratios (w/w) viz. 30:70, 35:65, 40:60, 50:50 in the glass vial, heated to melt Gel 50/13 above its melting points (~50 °C) on a hot plate to get a clear solution followed by vortexing (5-10 min) to ensure homogeneity. The vials containing molten mixtures were then allowed to solidify at two different storage conditions i.e. initially at room temperature (28±2 °C) and then in
refrigerator (4 °C). The time (min) required to solidify each mixture in both storage temperatures was noted.

An SE mixture which gets solidified easily either at room temperature or in refrigerator was further investigated for its ability to maintain the solid state at room temperature and at 40 °C for 15 days separately. The physical consistencies of the mixtures were then assessed visually.

The spontaneity of dispersion and subsequent self-emulsification of the solid SE mixture were assessed by visual observations and grouped based on the emulsification speed, uniformity, and apparent stability of the resultant emulsion. Briefly, with the help of spatula 50mg solid SE mixture was added into a glass beaker containing 250mL of distilled water. This test was performed at room temperature, and the solution was stirred magnetically at low speed. The time required for complete dispersion and emulsification of mixture was noted [10].

The produced nanoemulsion was then assessed for clarity (appearance) by visual observations and by measuring % transmittance at 638.2 nm against distilled water [9].

### Formulation of NBL Solid-SNEDDS (NSS)

A series of nine different formulation systems as given in Table 1 were developed by changing the ratio of oily phase to surfactant. The oily phase was prepared by mixing Peceol with Ethanol at 4:1 w/w ratio. In all formulation systems, concentration of Gel 50/13 (40% w/w) and level of NBL (5.5mg; equivalent to 5mg of NBL base) were kept constant.

- NBL was added in the vial containing the respective amount of oily phase, this oily mixture was heated at 40 to 50 °C in water bath followed by sonication in bath sonicator (5-10 min) to ensure the complete solubilisation of drug.
- Respective quantity of Cr-RH 40 and Gel 50/13 (Prewarmed in separate vial at 50-60 °C) in molten state was added and homogenized on cyclomixer for 10-15 min to ensure homogeneity.
- The molten mixtures (505mg equivalent to 5.5mg of NBL) were then manually filled in hard gelatin capsules of size “1” with the help of micropipette.

- Capsules filled with Liquid state SE mixture were allowed to solidify at room temperature or in refrigerator, after ensuring the complete solidification. Capsules were stored at room temperature for not less than 24 hrs prior to *in vitro* dissolution study [11, 12].

### Optimization of NBL Solid-SNEDDS (NSS) Formulations

#### Saturated Solubility of NBL

To select the few best systems with maximum NBL solubility capacity, saturation solubility of NBL in all nine SNEDDS formulation systems was estimate. The same process as mentioned in solubility study of NBL in vehicle (stage two) was followed for this experiment.

#### Ability of Self-Solidification and Spontaneity of Self-Emulsification

The formulations were assessed by visual observations for their physical state. The formulations which do not solidify and/or do not maintain solid state at room temperature were not considered for further evaluation.

The spontaneity of self-emulsifying properties of NBL SNEDDS formulations was observed visually and the formulations were categorized on the basis of rapidity of emulsification, uniformity, and apparent stability of the emulsion formed. Simultaneously, the tendency to disperse the solid mass to produce fine nanoemulsion was judged qualitatively as “good” when solid masses disintegrated and dispersed easily in water and was rated “bad” when there was incomplete disintegration/dispersion of solid mass and they remained as solid plugs, especially when stirring was stopped.

The formulations were then grouped as clear (transparent/ transparent with a bluish tinge) or non clear (turbid); stable (absence of precipitation at the end of 24 hrs), or unstable (precipitating within 24 hrs) [11, 13]. All experimental trials were carried out in duplicate.

#### Stability to Dilution Study

S-SNEDDS of NBL were exposed 50,100 and 1000 fold dilution in distilled water, 0.01N HCl, SGF and SIF. The dispersion of solid mass was achieved by magnetic stirring at

### Table 1. Compositions of various NBL Solid-SNEDDS formulation batches.

<table>
<thead>
<tr>
<th>Components (mg) per unit formula</th>
<th>NBL S-SNEDDS formulation code</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NSS₁</td>
</tr>
<tr>
<td>NBL</td>
<td>5.5*</td>
</tr>
<tr>
<td>Peceol containing Ethanol (4:1)</td>
<td>75</td>
</tr>
<tr>
<td>Cr-RH 40</td>
<td>224.5</td>
</tr>
<tr>
<td>Gel 50/13</td>
<td>200</td>
</tr>
<tr>
<td>Total</td>
<td>505</td>
</tr>
</tbody>
</table>

*Equivalent to 5mg of NBL base.
100 rpm for 5 min. The resultant nanoemulsion was assessed for appearance and the % transmittance (%T) value was measured after 2 hrs at 638.2nm against distilled water as blank using UV-visible spectrophotometer [9].

Globule Size, Polydispersity Index and Zeta Potential Analysis

The solutions obtained on 1000 fold dilution of the formulations in either distilled water, SGF or SIF were placed in an electrophoretic cell for measuring the globule size, Polydispersity index and Zeta potential of globules of resultant nanoemulsion.

Evaluation of Optimized Formulations of NBL S-SNEDDS (NSS4)

Morphology of Globules by Transmission Electron Microscopy (TEM)

Transmission electron microscopy was employed to study the morphology of the resulting nanoemulsion (1000 fold dilution in distilled water) droplets.

Solid-State Characterization of S-SNEDDS

DSC Thermogram

Thermal properties Gel 50/13, physical mixture of NBL and Gel 50/13 in 1:1 (w/w) ratio and NSS4 were investigated using METTLER TOLLEDO, DSC-822e. The obtained thermograms were then compared with the thermogram of plain NBL powder.

Powder X-Ray Diffraction Pattern

The physical state of NBL in S-SNEDDS was characterized by powder X-ray diffraction scattering (PXRD) measurements using X-ray diffractometer (Bruker D8 Advance diffractometer, Germany with X-Pert Data Collector, PAN analytical). PXRD was performed on samples of plain NBL, neat Gel 50/13, molten mixture of NBL and Gel 50/13 at 1:1(w/w) ratio and NSS4.

Scanning Electron Microscopy (SEM) Study

The outer macroscopic structure of Plain NBL, Gel 50/13, molten mixture of NBL and Gel 50/13 at 1:1(w/w) ratio and NSS4 were investigated by scanning electron microscope (JSM-6510LV; Jeol, Japan).

In vitro Dissolution Method

In vitro dissolution of plain NBL and NSS4 (equivalent to 5 mg of NBL base) filled in hard gelatin capsules were studied using USP apparatus I at 37±0.50 °C with 100 rpm in three different dissolution media namely 0.01N HCl, SGF and SIF to expose the impact of pH of dissolution medium on drug release. Aliquots of 5mL were removed at different time intervals (10, 20, 30, 45 and 60 min) from 900mL dissolution medium and replaced with fresh buffer to maintain the sink condition. The aliquots filtered through membrane filter (0.45µ x 45mm) was analysed for NBL content by HPLC method.

Dissolution profile of conventional marketed tablet formulation of NBL (equivalent to 5mg of NBL base) was evaluated in same dissolution media mentioned above. Dissolution of marketed tablet was performed in USP apparatus II with the speed of 75 rpm 37±0.5 °C and compared with the developed S-SNEDDS of NBL.

Ex vivo Intestinal Permeability Study by Everted Sac Technique

All animal experiments were performed with prior approval of study protocols by the Institute animal Ethics Committee, Ultra College of Pharmacy, Madurai, India (UCP/IAEC/2012/065) and the use and care of experimental animals were according to the Indian National Science Academy (INSA) guidelines. Either sex Wistar rat (150-200g), after being fasted for about 20 hrs with access only to tap water, were anesthetized. A midline incision was made in the abdomen and the entire length of the small intestine was removed carefully and placed in cold KRPB (Krebs-Ringer-Phosphate-Buffer, pH 7.2) solution which was continuously aerated with the help of an electrical aerator. This intestinal segment was then cut and the overlying longitudinal and circular muscle layers was properly isolated without damaging the mucosal layer; the cleaned cut segment was then washed four to five times with KRPB solution, ligated with silk thread to one end of a glass rod, and carefully everted on the glass rod. The everted gut sac, filled with 2mL of KRPB solution (both ends were closed by silk thread) was placed inside the 100mL glass beaker containing 50mL of either NBL solution (100µg/mL) or nanoemulsion of NSS4 in KRPB solution (100µg/mL), continuously bubbled with atmospheric air and maintaining the temperature 37±0.5 ºC. The KRPB solution outside the sac was the mucosal fluid, and the solution inside the sac was the serosal fluid. An aliquot of drug solution was withdrawn from the serosal compartment after 60 min, volume (mL) of serosal fluid removed after 60 min was noted. Amount of NBL from NSS4 nanoemulsion and plain NBL solution permeated across the intestine (from mucosal surface to serosal sac) was determined using UV-Visible spectrophotometer after appropriate dilutions using suitable blank [14, 15]. Experiment was repeated in triplicate for each test solution containing NBL. Relative permeability was calculated using following formula,

Relative permeability (µg/cm²) = [Concentration of drug in µg/mL (in serosal fluid) X Volume in mL (of serosal fluid)] / Mucosal surface area in cm².

Stability Studies

Chemical and physical stability of NSS4 was assessed for a period of 6 months under storage conditions; 40±2 °C/75±5% RH as per ICH guidelines. The formulation in molten state was filled in glass vials and hard gelatin capsules and allowed to get solidified at room temperature. After ensuring the solidification of formulations, vials were sealed by using rubber cork and aluminum cap by crimping. Samples were removed at 0, 30, 60, 90 and 180 days of interval. Stability samples removed from vials were assessed for physical appearance, drug content, dispersion and self-emulsification behavior, globule size, while stability samples of formulations filled in capsules were assessed for compatibility with
capsule shell and dissolution efficiency at 20 min (Q<sub>20</sub> min) in SGF by comparing with % cumulative drug release shown by 0<sup>th</sup> day stability formulation at 20 min in SGF by considering it as 100% [16, 17].

RESULTS AND DISCUSSION

Solubility Profile of NBL in Various Vehicles

The solubility of NBL in buffer solutions (Fig. (1)) indicated that NBL has pH dependent solubility. Amongst the buffers tried, solubility of NBL was found maximum in 0.01N HCl. Similar observation has been made with the weak base talinolol (pKa = 9.4), which is soluble more in acidic pH and less soluble in neutral pH as it can be converted into uncharged in neutral conditions [15]. Consequently basic drugs may have complete solubility in the stomach; however they may precipitate in the intestine due to the pH shift to the alkaline side. It is highly important to minimize this precipitation in order to maintain the dissolved nature of the basic compounds in neutral media, which is essential for the absorption of the basic drugs with low solubility in neutral pH [15].

The results from the primary screening of oils explain the inability of natural oil to solubilise the NBL. From the primary screening (data not shown), four oils namely Peceol, oleic acid, ethyl oleate and Cap MCM C8 EP (approximate solubility of NBL is more than 15 mg/g) were selected for the quantitative estimation of solubility of NBL. The results of quantitative estimation of solubility suggested that, the maximum solubility of NBL was found in Peceol (23.12±1.21 mg/g), these observations indicated that NBL might be not only poorly water soluble but also poorly oil soluble. This solubility property of NBL might have been the reason for its poor oral absorption and very low bioavailability (12%). The same observations were reported for the drug silymarin [18].

Peceol is glycerol monooleate (type 40), a mixture of mono- and diglycerides of oleic acid, which are more identical to those chemical units resulted in lipid digestion in the intestine [19]. Peceol has been reported to increase the bioavailability of the low water soluble drug compounds like ontaoza (10 fold increase in bioavailability) [20] by enhancing the lymphatic drug transport. Kristina S et al reported the potential of Peceol to increase the oral bioavailability of model drug Verapamil by inhibiting the pre-systemic drug metabolism by regulating and inhibiting the P-gp protein expression [19]. For present study, Peceol was selected as oily phases to produce SNEDDS of NBL, due to its higher potential to solubilise NBL and its bioactive role which could enhance the oral bioavailability of NBL.

NBL showed poor solubility in surfactant and co-surfactant tested except for LM 1944 which showed potential to solubilise NBL (31.25mg/g) (Fig. (2)). Amongst all vehicles tried, the higher solubility of NBL was found in Ethanol (42.26±2.56mg/g).

![Fig. (1). Solubility profile of NBL in different vehicles,*Data expressed as mean ± SD (n=3).](image)

![Fig. (2). Solubility of NBL in selected oily phases, surfactants and co-surfactants, *Data expressed as mean, error bar shows SD (n=3).](image)
Emulsification Study

Selection of Surfactant and Co-Surfactant

Surfactants were evaluated to explore their efficiency to emulsify selected oily phase; Peceol on aqueous dilution. The results of emulsification study reveals the inability of different surfactants to emulsify Peceol, the maximum emulsifying ability was observed in Cr-RH40 which produced emulsion with 89.25%T and required 10 FI, followed by Gel 50/13 (87.56% T, 15 FI). Labrasol and PoL-188 produced nanoemulsion which are similar in their appearance with about 85%T but to accomplish it they differed by required number of flask inversion (10 and 25 FI respectively). These results are identical to the findings of Birader SV et al. [21] who reported that the emulsion produced using Peceol is milky in appearance, high turbid and coarse droplets. The reason for such behavior of the Oily phase, Peceol might be due to its structure (hydrocarbon chain length; C21-C22) or the molecular weight. Structurally Peceol is glycerol monooleate (type 40) with lower HLB value 3. Based on the above results, four different surfactants namely Cr-RH 40, Labrasol, Gel 50/13 and PoL-188 were chosen along with the co-surfactant to emulsify Peceol.

It was observed that spontaneity of emulsification of selected surfactants was enhanced by addition of nearly all co-surfactant (reduced the required number of FI), but the %T of produced emulsion was not improved significantly. The findings made it clear that the single surfactant with co-surfactant will not be efficient to emulsify Peceol and an additional surfactant would be required to do so. Hence, combinations of surfactants were tried along with the individual co-surfactant to emulsify Peceol efficiently.

Surfactants in Combination (S-Comb.)

Interestingly, it was reported that PoL-188 and Gel 50/13 were used to produce solid-self emulsifying drug delivery system without addition of external solidifying agents as these agents are amphiphilic in nature and are at solid state in room temperature. Shah AV et al. [12] reported a novel approach of preparing solid lipid-based formulations where the conversion of solutions of the drug in liquid lipid-surfactant mixtures was effected by mixing them with a solid PoL-188 matrix by heating the contents at 70 °C and the hot mixtures were filled into hard gelatin capsules. Upon cooling at room temperature, the formulation got solidified.

In the other study [22], solid self-emulsifying system for the model drug Probucol, a practically water-insoluble compound with high partition coefficient (Log p = 11) was formulated by using Gel 50/13 to serve as solidifying as well as emulsifying agent for triglycerides. The solid carrier Gel 50/13 is primarily a mixture of PEG 1500 mono- and diesters with palmitic (C16) and stearic (C18) acid with an HLB value of ~13 (13). Drug candidate gets solubilised in solution of liquid triglyceride, this drug containing solution can be incorporated in the solid matrix as a dispersed but separate phase. After oral administration, the solid formulation would self-emulsify without the need for any additional surfactant. It was also reported that the Gel 50/13 was used for improvement of solubility and dissolution profile by the development of solid dispersion of hydrophobic drugs like Clonazepam [23], Indemethacin [24] and very recently for NBL [25] with the potential of improving oral bioavailability. These preliminary studies indicated that Gel 50/13 and PoL-188 could serve as a suitable solidifying and emulsifying agent in SNEDDS formulations containing NBL, so that PoL-188 and Gel 50/13 were used as the carrier to prepare solid dosage forms using hard gelatin capsules.

Since all the four potential surfactants (individually and in combination with co-surfactant) show the equal but moderate ability to emulsify Peceol (%T less than 95), it was decided that the surfactant having the solidifying ability (Gel 50/13 and PoL-188) to be used in combination with one of the other two surfactants namely Labrasol and Cr-RH40 with the intention that the produce system will self-solidify, which will avoid the incorporation of external solidifying agent to reduce the total volume of unit dosage form to be fill directly in to size “0” or size “1” hard gelatin capsule and secondly these solidified SNEDDS spontaneously produce nanoemulsion on aqueous dilution.

Selection of Co-Surfactant(s) for S-Comb.

Efficiency of co-surfactants was investigated for the S-Comb. Amongst the four combination tried it was observed that Labrasol in combination with Gel 50/13 and PoL-188 was found incapable to emulsify Peceol and produced emulsions were lower in %T. On the other hand, Cr-RH40 produced a fine nanoemulsion in combination with both Gel 50/13 as well as PoL-188 with nearly all co-surfactants tried, amongst the co-surfactants PO 497, LM and Ethanol produced fine nanoemulsion with more than 96%T.

So, as far as the emulsification (in combination with solid surfactant) is concerned, as compared to Labrasol, Cr-RH40 was found to be better choice to formulate NBL SNEDDS.

Combination, Cr-RH40: PoL-188 along with LM 2125 as co-surfactant, emulsify Peceol and produces fine nanoemulsion with highest %T 94.23, but this combination required more number flask inversion (20 FI) to disperse the PoL-188 flakes completely.

Combination, Cr-RH40: Gel50/13 in presence of Ethanol as co-surfactant spontaneously emulsifies Peceol in water and produce clear and transparent nanoemulsion with the highest %T 98.75 with less than 5 flask inversion.

In comparison to PoL-188, Gel 50/13 was found the better choice as solidifying carrier since it is reported that PoL-188 is incapable to solidify triglycerides in higher concentration, on the other hand Gel 50/13 could solidify as high as 80% of triglycerides lipid [12].

So based on this investigations and the obtained results it was finalized to use Gel 50/13 for its better and spontaneous emulsification ability along with Cr-RH40 and triglycerides solidification tendency. Secondly many researchers reported the used Gel 50/13 to produce solid dispersion to increase the solubility and dissolution profile of poorly soluble drug candidate [26].

The approach of blending water soluble co-solvents like ethanol with oil to produce self-nanoemulsifying system, also improves the solubilising potential of the formulation for drugs with a log p value between 2 and 4 [27]. The same
strategy was used in the reformulation of commercial product NeoRAL in which ethanol has been used as co-solvent. A few other examples of commercial lipid based products where ethanol is used as co-solvents are NeoRAL soft gelatin Capsule, NeoRAL Oral Solution, Gengraf hard gelatin Capsule, Sandimmune soft gelatin Capsule and Sandimmune oral solution [22]. NBL has log p in between 2 and 4 (log p =-3.14). NBL is solubilised in ethanol (solubility 42.12mg/g) to a greater extent and ethanol acts as co-surfactant and produce fine nanoemulsion with the surfactant combination i.e. Cr-RH40 and Gel 50/13. Based on the obtained result ethanol was selected as Co-surfactant/solvent to be blended with oily phase Pecceol for formulating S-SNEDDS of NBL.

Optimization of Gel 50/13 Concentration

Ability to Solidify SE Mixture and Spontaneity of Emulsification of SE Mixture

Five different ratios of Gel 50/13 to SE mixture, from 30:70 to 60:40 (w/w) were investigated for this study. Results of this experiment are summarized in Table 2. It was observed that Gel 50/13 can solidify SE mixture as high as about 1.5 times at room temperature within 1 h and maintain SE mixture in solid state for longer duration at room temperature and even at 40 °C. This system (40:60 w/w) disperses spontaneously (within 5 min) in water and produce nanoemulsion with more than 93% T.

When ratio of Gel 50/13:SE was tried at 60:40 w/w and 50:50 w/w, it was observed that these systems get solidified easily at room temperature and maintain the solid state even at 40 °C, but had a limitation of poor dispersion on aqueous dilutions, requires more than 15 min to get dispersed.

Concentration of SE mixture above 1.5 times of the Gel 50/13 concentration (system 35:65 w/w) could not solidify easily at room temperature and required freezing to get solidified, but after removing from refrigerator and storing at room temperature, the consistency of this system changes from solid to semi solid.

In case, the preparation consists of 1 part of Gel 50/13 and more than 1.85 parts of SE mixture (system 30:70 w/w), could not completely solidify neither at room temperature nor in refrigeration.

From the above findings it was concluded that to make a spontaneously dispersing and stable solid SNEDDS formulations of NBL consisting of Pecceol, Cr-RH40 and Ethanol, concentration of Gel 50/13 should be maintained at 40% w/w to the total mass of formulation i.e. ration of Gel 50/13 to SE mixture 1:1.5.

Formulation of NBL-SNEDDS

Based on solubility, spontaneity of emulsification study and solidification ability Pecceol: Cr-RH40: Ethanol: Gel 50/13 was selected as best system to produce S-SNEDDS of NBL. Ethanol was selected as co-solvent for its solvent capacity which increases the solubility of NBL and its ability to enhance the spontaneity of surfactant combination (Cr-RH40 + Gel 50/13). Pecceol (Oil) is blended with ethanol in the ration 4:1 w/w, as reported in literatures the concentration of ethanol should not be more than 15 % w/w to the total

Table 2. Optimization of Gel 50/13: SE mixture ratio.

<table>
<thead>
<tr>
<th>Storage conditions</th>
<th>[Gel 50/13] : [Pecceol + Cr-RH 40 + Ethanol; 1:1:1]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30: 70</td>
</tr>
<tr>
<td></td>
<td>Solidification ability</td>
</tr>
<tr>
<td>At room temperature (28±1 ºC) Before cooling</td>
<td>Not solidify even after 24 hrs</td>
</tr>
<tr>
<td>Cooling at refrigerator (4 ºC)</td>
<td>Partially solidify</td>
</tr>
<tr>
<td>At room temperature (28±1 ºC) After cooling</td>
<td>Completely liquid with increased viscosity</td>
</tr>
<tr>
<td>At 40 ºC for 15 days After cooling</td>
<td>Not Performed</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dispersibility test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dispersibility test</td>
</tr>
<tr>
<td>Time required to produce uniform emulsion (Min)</td>
</tr>
<tr>
<td>Appearance</td>
</tr>
<tr>
<td>% T</td>
</tr>
<tr>
<td>Grade</td>
</tr>
</tbody>
</table>
formulation. A maximum amount of ethanol (11% w/w) was present in formulation NSS\textsubscript{3} and was lowest (3% w/w) in NSS\textsubscript{1}.

Optimization of NBL S-SNEDDS Formulations

Saturated Solubility of NBL

The saturated solubility of NBL in different SNEDDS formulations was found in the range of 7.54 to 11.07mg/g. The solubility of NBL was observed to increase along with an increase in the concentration of oily phase, since the oily phase is made up of Pecceol blended with ethanol and NBL’s solubility in ethanol is maximum. All the anhydrous formulation were able to solubilise 137% to 201% of the target dose of NBL (5.5mg, equivalent to 5mg of NBL base), in the other words all these formulation systems contain the NBL solubility less than its 80% saturated solubility.

Self-solidification Ability

SNEDDS formulations of NBL were evaluated for their solid state stability at various storage conditions. The formulations containing high concentration of Cr-RH40 (NSS\textsubscript{4}, NSS\textsubscript{8}, NSS\textsubscript{3}, and NSS\textsubscript{4}) solidified readily even at room temperature or in refrigerator and maintained their solid state in both the storage conditions tried i.e. at room temperature and at 40 °C.

On the other hand, formulations with lower concentration of Gel 50/13 and high concentrations of Pecceol and ethanol (NSS\textsubscript{3}, NSS\textsubscript{3}, and NSS\textsubscript{4}) did not solidify at room temperature for even after 24 hrs on standing. These formulations get solidified after placing inside the refrigerator but solid state could not be maintained even for 1 hr after removing it from refrigerator and placing at room temperature or 40 °C storage conditions.

Formulations consisting of moderate amount (20% to 25%) of Cr-RH40 (formulation code NSS\textsubscript{5} and NSS\textsubscript{6}) showed solidification at room temperature as well as in refrigeration conditions and maintained their solid state at room temperature but got converted to liquid again at 40 °C.

Based on rapid solidification and maintenance of solid consistency even at 40 °C, four formulations (NSS\textsubscript{1}, NSS\textsubscript{2}, NSS\textsubscript{3} and NSS\textsubscript{4}) were chosen for further studies.

Spontaneity for Emulsification

It is well reported that there is no significant difference in the nanoemulsions prepared using nonionic surfactants (Cr-RH 40 and Gel 50/13), dispersed in either water or simulated gastric or intestinal fluid [10].

Formulation NSS\textsubscript{1} consisting of high concentration of surfactant, Cr-RH 40 and correspondingly lower amount of oily phase, Pecceol, took more than 5 min to get dispersed in aqueous phase and produced non transparent white bluish emulsion, the reason being the high concentration of surfactant causing production of micelle which are large in globule size subsequently reducing the transmittance of the emulsion [27]. This formulation showed precipitation of drug on aqueous dilution standing for 24 hrs at room temperature. This may be due to further lowering of solvent capacity of the formulation with lower oily constituent Pecceol on dilution with aqueous fluids. Moreover, the surfactant contributed in higher amount for the solubilisation of NBL in anhydrous form of SNEDDS formulation and on high dilution the surfactants present at the surface of the oily nano sized globules may desorb which may also have caused the precipitation of the poor water soluble drug.

(NSS\textsubscript{4}) took 5-6 min for dispersion of the solid flakes but after which the system spontaneously produced nanoemulsion, due to a high concentration of surfactant. This observation is well in agreement with the theory reported by Pouton et al [3] explaining that an increase in concentration of surfactant up to certain level reduces the interfacial tension thereby reducing the globule size of the dispersion but further increments in the concentration of the surfactant (above its CMC) caused production of micelle resulting in globule of larger size.

On the other hand formulations NSS\textsubscript{3} and NSS\textsubscript{4}, where oil and surfactants were present in optimum ratio (1 : 1.75 and 1 : 1.25 respectively) spontaneously dispersed (less than 5 min) in aqueous media and produced fine nanoemulsion, which appeared as clear or slightly bluish indicating the globule size of this dispersion is within the nano range. Both these formulations maintain the NBL in dissolved state even after 24 hrs of standing indicating the potential of formulations to inhibit precipitation of NBL, this might be because of sufficient amount of oily phase; Pecceol with higher NBL solubility does not lose the solvent capacity even after dilution. With these types of formulations, precipitation of drugs is not expected in gut before the drug gets absorbed and super saturation could definitely accentuate absorption by improving the thermodynamic stability of drug [27].

Based on these findings from dispersion time, appearance and drug precipitation test, only NSS\textsubscript{1} and NSS\textsubscript{4} formulations were selected for further studies.

Stability to Dilation Study

Both the formulation systems (NSS\textsubscript{3} and NSS\textsubscript{4}) were found robust to various dilution folds using dilution medium different in pH. NSS\textsubscript{3} produced fine nanoemulsion which was slightly bluish or white bluish in appearance with more than 91%T. On the other hand NSS\textsubscript{4} produce slightly bluish and even clear and transparent nanoemulsion on dilution with transmittance value more than 94%T.

Globule Size, Polydispersity Index and Zeta Potential

Table 3 depicts the result of globule size analysis of NBL S-SNEDDS (NSS3 and NSS\textsubscript{4}) after 1000 fold dilution, it was observed that the oil droplet produced by NSS\textsubscript{4} (170.23nm in water) are smaller in diameter than that of NSS\textsubscript{3} (187.57nm in water). Zeta potential of droplets produced from both formulations was observed with negative charge irrespective of the dilution medium and it was in the range of -5.21mV to -7.56mV (Fig. (3), Fig. (4)). Based on these findings formulation system NSS\textsubscript{4} was selected as optimized formulation and evaluated.
Table 3. Globule size analysis of NSS₃ and NSS₄.

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Dilution/Dissolution media</th>
<th>Distilled water</th>
<th>0.1 N HCl (SGF)</th>
<th>Phosphate buffer pH 6.8 (SIF)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Globule Size (nm)³</td>
<td>P.I. *</td>
<td>Zeta potential mV</td>
<td>Globule Size (nm)³</td>
</tr>
<tr>
<td>NSS₃</td>
<td>187.57±3.27</td>
<td>0.421</td>
<td>-5.21</td>
<td>185.26±4.87</td>
</tr>
<tr>
<td>NSS₄</td>
<td>170.23±4.19</td>
<td>0.346</td>
<td>-5.56</td>
<td>165.56±3.15</td>
</tr>
</tbody>
</table>

*Values are expressed as mean ± SD (n=2).
³Values are expressed as mean (n=2).

Fig. (3). Globule size distribution of optimized SNEDDS of NBL (NSS₄) in SGF.

Fig. (4). Zeta potential of optimized SNEDDS of NBL (NSS₄) in distilled water.
Evaluation of Optimized NBL Solid-SNEDDS (NSS₄)

Transmission Electron Microscopy (TEM)

Transmission electron microscopy performed on the optimized S-SNEDDS formulation after 1000 fold dilution by distilled water. (Fig. (5)) demonstrated the ability of developed NBL S-SNEDDS to produce uniformly distributed, spherical oil droplets of nano size ranging from 160-175nm. This observation of TEM image is in line with the result observed in size analysis of droplets.

Differential Scanning Calorimetric Thermogram

The thermogram of pure NBL shows sharp endothermic peak at 235.4 °C corresponding to melting temperature indicating its crystalline nature. Gel 50/13 exhibited a sharp endothermic peak corresponding to its melting point at 51.5 °C. No considerable broadening of peaks was observed in physical mixture of NBL and Gel 50/13 (Fig. (6)) However a substantial decrease in peak intensity of the drug as well as that of the Gel 50/13 was evident suggesting a possible interaction between the NBL and Gel 50/13 which is not a chemical but physical interaction. The thermograms indicate that though the drug is not molecularly dispersed within the carrier, ruling out the possibility of formation of eutectic mixture, it is possible that the NBL is highly dispersed as very fine crystals among the amorphous amphiphilic Gel 50/13. The peak of NBL was dispersed in the solid SNEDDS suggesting that there may be decrease in the heat of fusion of this mixture as compared to the Gel 50/13 or NBL alone.

Apart from this, no polymorphic changes were observed in the optimized S-SNEDDS of NBL.

Fig. (5). TEM image of nanoemulsion obtained from NSS₄.

Powder X-Ray Diffraction Pattern

Fig. (7) presents the X-ray diffractograms of plain NBL, neat Gel 50/13, physical mixture of NBL and Gel 50/13 and NBL S-SNEDDS. NBL had characteristic sharp peaks at the diffraction angles of 16.4°, 18.5°, 21.4°, 22.5°, 25.7°, 30.1°, 31.9° and 32.4° showing a typical crystalline pattern. Gel 50/13 showed two intrinsic peaks at 19.4° and 23.2°. All of the major characteristic crystalline peaks for the pure NBL and neat Gel 50/13 were observed with reduced intensity in physical mixture of NBL with solid carrier Gel 50/13,
Novel SNEDDS of Nebivolol Hydrochloride


which might be due to the partial conversion to amorphous state of NBL. The characteristic intense peak of NBL was absent in S-SNEDDS indicating that the crystalline nature of NBL was completely changed to amorphous state, while the typical peaks of neat Gel 50/13 were seen in S-SNEDDS with reduced intensity. Like the DSC results in the S-SNEDDS formulations, NBL was present in a completely changed amorphous state or disordered crystalline phase in the lipid matrix. However, any different new peak was not present in that of S-SNEDDS formulation, suggesting the compatibility of NBL with Gel 50/13 and other SNEDDS excipients [11, 28].

Scanning Electron Microscopy

Scanning electron micrograph of plain NBL shows needle shaped crystals indicating the crystalline nature of the drug whereas Neat Gel 50/13 pellets show smooth surface (Fig. 8). The SEM images of mixture of NBL dissolved in Gel 50/13 showed few small crystalline particles of drug, indicating the change in surface morphology of drug particle due to entrapment in the Gel 50/13 and S-SNEDDS ensures the complete solubility of NBL since no crystals (NBL) was seen and it is a irregular mass produce of S-SNEDDS. This surface modification ensures the decrease in crystallinity and complete solubility of NBL in NBL S-SNEDDS.

In vitro Dissolution Study

The dissolution patterns of the three products viz. NBL S-SNEDDS (NSS₄), NBL and marketed tablet of NBL in three different dissolution media viz. 0.01 N HCl, SGF and SIF are depicted in Fig. (9).

It is evident from the observation that NBL S-SNEDDS showed a promising improvement in the in vitro dissolution profile compared to the pure NBL in all three dissolution media studied.NBL S-SNEDDS showed more than 82% NBL released in 20 min and nearly complete (more than 97%) NBL was released after 30 min of dissolution irrespective of the dissolution media, indicating that the release of NBL from S-SNEDDS was independent of the pH of the dissolution medium. On the other hand, plain NBL showed

Fig. (7). X-ray diffraction patterns of plain NBL (a), pure Gel 50/13 (b), NBL S-SNEDDS (c) and physical mixture of NBL with Gel 50/13 (d).
poor rate and extent of dissolution (less than 70% after 45 min) in 0.01N HCl and SGF, while only 25% of NBL was dissolved in SIF after 60 min because the plain NBL powder gets precipitated at this pH confirming the pH dependant dissolution behavior of NBL. In comparison to SIF rate of drug release from plain NBL was better in 0.01N HCl and SGF (about 60% drug release after 30 min). Since any oral dosage form would be first exposed to acidic conditions followed by alkaline pH in GI tract, the in vitro release of NBL from NBL S-SNEDDS was determined in SGF and in SIF [29].

For any poorly soluble drugs (BCS Class –II and IV) dissolution is the rate limiting step for its oral bioavailability, NBL is classified as Class-II drugs as per BCS classification system, and it is well known that its poor water solubility limits its dissolution and hence leads to low oral bioavailability (12%). Optimized S-SNEDDS presents the NBL in dissolved form and produced fine globules which disperse spontaneously whatever may be the pH of dissolution media. The rate and extent of NBL release from S-SNEDDS was found to be excellent and independent of pH which suggested that NBL S-SNEDDS will certainly be able to improve the oral bioavailability of NBL.

The rate of NBL dissolution from S-SNEDDS was significantly greater than the commercial tablet formulation of NBL. Marketed tablet preparation of NBL showed incomplete drug release (Not more than 90% of drug release after 60 min) in 0.01N HCl and SGF and only 27.11% of NBL was released in SIF after 60 min of dissolution study showing the similarity between NBL powder and marketed tablet preparation of NBL with respect to pH dependent dissolution. These results indicate that better dissolution behavior of self-emulsifying formulation compared to that of marketed NBL tablet.

**Ex vivo Permeability Study**

As shown by the everted sac diffusion data in Table 4, the permeability of NBL was found to be significantly high from S-SNEDDS formulation in comparison to that from pure NBL solution. It was observed that, only 19.87% of NBL could be transported through intestinal lumen from NBL solution, while, almost three fold increase in transportation of NBL was observed with S-SNEDDS formulation (57.48% of NBL was transported). This significant enhancement of permeability of NBL can be contributed by many parameters, the main one being the uniformly dispersed globules with nano size (less than 200nm). Moreover NBL is present in the dissolved state. The fine globule size increases the surface area facilitating the permeability of drug. S-SNEDDS of NBL was made up from excipients like Pecicol and Cr-RH 40, which are reported for their bioactive role in enhancement of bioavailability of poorly soluble drugs.
Fig. (9). In vitro dissolution profiles of NBL formulations in 0.01 N HCl (A), SGF (B) and SIF (C).

Table 4. Relative permeability of NBL from S-SNEDDS (nanoemulsion) and Plain NBL solution after 60 min.

<table>
<thead>
<tr>
<th>Test Sample</th>
<th>Collected Serosal fluid (mL)*</th>
<th>Conc. of NBL in Serosal fluid (µg/mL)*</th>
<th>Mucosal surface area (cm²)*</th>
<th>Relative Permeability (µg/cm²)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-SNEDDS (Nanoemulsion)</td>
<td>1.95±0.30</td>
<td>28.52±2.67</td>
<td>5.38±0.14</td>
<td>10.70±0.54</td>
</tr>
<tr>
<td>Plain NBL (Solution)</td>
<td>1.98±0.12</td>
<td>10.04±1.11</td>
<td>5.54±0.18</td>
<td>3.58±0.16</td>
</tr>
</tbody>
</table>

*Values expressed as mean ± SD (n=3).

Stability Assessment of NBL S-SNEDDS (NSS₄)

Over the period of 6 months it was observed that physical appearance of formulation did not show any sign of color change, secondly formulations remain as solid mass and consistency also did not change. S-SMEEDS of NBL were compatible with the shell of hard gelatin capsule and deformation of capsules was also not observed.

In addition the maintenance of the drug content (101.23% to 97.45%), solid state, similar dissolution efficiency (Q₂₀min in SGF was more than 94%) and very importantly the self-emulsification ability was also noted for over the period of 6 months demonstrating the reasonable stability of the formulation.

CONCLUSIONS

This study exhibits a novel approach in developing self-solidifying self-emulsifying drug delivery systems where a liquid self-emulsifying mixture may be incorporated into the
solid microstructure of stearoyl polyoxylglycerides (Gelucire® 50/13) which served as an emulsifying as well as a solidifying agent. The possibility of physical instability of the formulations due to the crystallization of the drug from the solid systems was minimized as the NBL remained dissolved in the lipid. The formulations may be filled into hard gelatin capsules in their molten state as they solidify as hard masses inside the capsules. However the solid formulations formed very fine emulsions upon the dispersion in aqueous media releasing NBL more rapidly independent of pH of dissolution media in comparison to plain drug powder and marketed tablet of NBL. Significant enhancement in intestinal permeability of NBL from S-SNEDDS formulation could also be achieved. Concerned with the most important parameter of a viable commercial product, Stability, SNEDDS of NBL have retained the desired quality parameters for a period of 6 months when stored in the stipulated conditions.

CONFLICT OF INTEREST
The authors confirm that this article content has no conflicts of interest.

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REFERENCES


