



Development and Evaluation of Self Micro Emulsifying Drug Delivery System (SMEDDS) for Nebivolol Hydrochloride

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Abstract: The present investigation aimed to prepare a self micro emulsifying drug delivery system (SMEDDS) for the dissolution enhancement of nebivolol hydrochloride. The main objective of this work was to develop, characterize, and evaluate a solid SMEDDS prepared by using the adsorption technique of a liquid SMEDDS to improve the solubility and dissolution rate of nebivolol hydrochloride. The excipients were chosen based on the high solubility of nebivolol hydrochloride, and their concentrations were optimized by constructing ternary phase diagrams. Thirty-two combinations were prepared using oil (Labrafac Lipophile WL 1349), surfactant (Kolliphor RH 40), and co-surfactant (Gelucire 44/14). Self-emulsification time, dilution studies, and thermodynamic stability studies were satisfactory. The *in vitro* nebivolol release profile showed a faster rate of dissolution compared with the pure nebivolol hydrochloride suspension and marketed formulation. The droplet size was in the range of 132.8±22.1 to 955.7±15.5 nm and zeta potential in the range -7.2±0.53 mV to -46.4±0.32 mV for the selected formulations. Formulation N17 (Labrafac Lipophile WL 1349 -10%w/w, Kolliphor RH40-72%w/w, Gelucire 44/14- 18%w/w) was considered as optimized formulation and showed drug release of 97.26 ± 1.16% in 0.1 N HCl in 120 minutes, the particle size of 132.8±22.1 nm and zeta potential of -46.4±0.32 mV. Thus, the present studies ratify that the bioavailability was improved by nebivolol SMEDDS formulation. The optimized liquid SMEDDS was further used for the preparation of Solid SMEDDS (S-SMEDDS) formulations by using adsorption carriers. The optimized Solid SMEDDS formulation exhibited 95.38 ± 0.76% *in vitro* drug releases, which was significantly higher than that of the drug solution. The optimized formulation of nebivolol-loaded S-SMEDDS exhibited complete *in vitro* drug release in 120 min as a compared pure drug solution. The present result confirmed the potential use of SMEDDS to improve the dissolution and oral bioavailability of poorly water-soluble nebivolol.

Keywords Nebivolol hydrochloride, SMEDDS, pseudo-ternary phase diagram, Labrafac Lipophile WL 1349, Kolliphor RH 40

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1. INTRODUCTION

Out of the many routes of administration available, the oral route remains the most popular dosage form among patients as it is easy to administer, carry around, formulation design flexibility, cost-effectiveness, causes minimal discomfort for many patients, and least sterility restrictions during manufacturing. Most of the newly discovered drugs are lipophilic in nature and have poor aqueous solubility, thereby posing problems in their formulation into delivery systems¹. The major challenge is the lipophilic drugs oral delivery owing to low aqueous solubility. Water insolubility is the major drawback for BCS class II drugs which lead to poor dissolution, and ultimately, low bioavailability of drugs is a challenge faced by the pharmaceutical industries^{2,3}. Different approaches have been attempted to increase the aqueous solubility of poorly soluble drugs. The most promising and new techniques to enhance dissolution and improve the bioavailability of poorly water-soluble drugs are Lipid microemulsion formulations, which particularly emphasize self-nano emulsifying (SNEDDS), self-micro emulsifying drug delivery systems (SMEDDS), and self-emulsifying drug delivery systems (SEDDS)⁴⁻⁶. Self-micro emulsifying oral drug delivery systems are growing popular in the delivery of poorly water-soluble BCS class II drugs^{7,8}. Self-micro emulsifying drug delivery systems (SMEDDS) as lipid-and surfactant-established formulations encompass a practical achievement in improving the oral bioavailability of poorly water-soluble drug compounds by maintaining the drug in a dissolved state, at the molecular level in small droplets of oil, throughout its transit through the GI tract⁹. SMEDDS are mixtures of oils, surfactants, and co-surfactant, and they are capable of forming thermodynamically stable oil-in-water (O/W) microemulsions upon moderate stirring provided by the stomach and the upper small intestine¹⁰. Lipids have an immense role in absorption and transportation via intestinal lymphatics. The lipids are likely to augment the lymphatic transport of a lipophilic drug substance leading to enhanced oral bioavailability⁸⁻¹¹. The drug, i.e., neбиволол, chosen in the present study is a BCS class II highly selective third-generation β_1 -receptor antagonist, an antihypertensive drug with poor water-solubility and high permeability (log P of 4.03) undergoes rapid first-pass metabolism caused by cytochrome P450 2D6 (CYP2D6) enzymes resulted in poor bioavailability (12%)¹². All these considerations have led to the development of solid oral SMEDDS. Several methods have been suggested to improve the solubility of neбиволол, Microemulsion technique¹³, Solid Dispersion¹⁴, cocrystals¹⁵, nanofibrous sheets¹⁶, solid lipid nanoparticles¹⁷. To above, numerous pharmaceutical approaches, including lipid-based formulations, have been developed to improve the dissolution rate and the absorption of poorly water-soluble drugs in the gastrointestinal (GI) tract by enhancing their solubility in vehicles. In the present investigation, SMEDDS were formulated, and their application in improving the oral bioavailability of a lipophilic antihypertensive drug, neбиволол, was also evaluated. The solubility behavior of neбиволол was tested in different vehicles, and an optimized SMEDDS containing neбиволол was formulated. A dissolution study was performed to evaluate the improved solubility and dissolution properties of neбиволол-loaded SMEDDS in comparison with pure neбиволол drug. As a liquid SMEDDS formulation, however, inherent defects, such as migration of the components, potential drug leakage, low stability during manufacturing, have limited its practical industrial application¹⁸. To overcome these difficulties, solid SMEDDS

(S-SMEDDS) formulations have been investigated as an alternative approach to improve the solubility of neбиволол drug.

2. MATERIALS AND METHODS

Nebivolol hydrochloride was a gift sample from Cadila Pharmaceuticals Ltd, Ankleshwar. Maisine 35-I, Gelucire 44/14, LabrafacLipophile WL 1349, Gelucire 48/16, PlurolOleique CC 497 were supplied by Gattefosse Pvt. Ltd., Mumbai. Kolliphor EL/ CremophoreEL, Kolliphor HS 15/ Solutol HS 15/ Poly oxyl15 hydroxy stearate, Kolliphor RH 40/ Cremophore RH 40, Kollisolv PEG 300/ Lutrol E 300/ Macrogol 300 were donated by BASF, Mumbai. Neusilin US2, Fujicalin were kindly supplied by Gangwal Chemicals Pvt. Ltd., Mumbai. Acrysol EL 135 was supplied by Corel Pharma, Mehsana Apricot oil, Hazelnut oil, Hemp Seed Oil, Wheat germ oil, Sesame oil, Cottonseed oil, Peanut oil, Soyabean oil, Coconut oil, Olive oil, Safflower oil, and walnut oil were purchased from AOS Products private Limited, Ghaziabad. Span 20, Span 80 were purchased from Merck Limited, Mumbai. Aerosil 200, Brij 35, Hydrochloric acid, PEG 200, PEG 400, Potassium dihydrogen orthophosphate, Propylene glycol, Sodium hydroxide pellets, Sunflower oil, Tween 20, Tween 80, Microcrystalline cellulose were purchased from SD. Fine- Chem., Mumbai. Triacetin, PEG 600 were purchased from Finar chemicals, Mumbai. All other analytical grade chemicals were used.

2.1 Methods

2.1.1 Solubility and screening

The solubility of neбиволол hydrochloride in each of various excipients, including oils, surfactants, and Co-surfactants, was determined by adding an excess amount of drug to 5mL of each vehicle ($n = 3$) in a test tube. The flask was shaken using an orbital shaker at $25 \pm 1^\circ\text{C}$ for 72 hr to achieve the solubility equilibrium. Then samples were removed and centrifuged at 5,000 rpm for 30 min to separate the insoluble neбиволол drug¹⁹. The concentration of dissolved Nebivolol hydrochloride was determined by UV spectrophotometer (DS 8000, Labindia, Mumbai, India) at 282 nm as reported in the analytical method²⁰.

2.1.2 Construction of the Pseudo-ternary phase diagram

The oil, surfactant, and co-surfactant selected from the solubility studies were used to construct the Pseudo-ternary phase diagram employing the aqueous titration method. Surfactant and Co-surfactant were mixed in different weight ratios i.e. 1:1, 1:2, 1:3, 2:1, 3:1, 4:1, 5:1 and 6:1. Oil and surfactant /co-surfactant mixture (S_{mix}) were mixed thoroughly in different weight ratios i.e., 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, and 1:9. The mixture of oil and S_{mix} at different weight ratios was titrated with water by drop-wise addition under gentle agitation. The resulting mixture was added under gentle agitation. The resulting mixtures were evaluated visually for transparency and flow properties. The endpoint of titration was the point where the mixture became turbid, or phase separation was observed. At this point, the amount of water, oil, surfactant, and co-surfactant added was noted and was used to construct the phase

diagram. Phase diagrams were constructed using chemix® software²¹.

2.1.3 Preparation of neбиволol hydrochloride loaded into liquid SMEDDS

Various formulations were prepared with a constant amount of 5mg(dose)neбиволol hydrochloride and varying ratios of oil, surfactant to co-surfactant. Surfactant and co-surfactant were blended in different weight ratios. To the above mixture, the required amount of oil phase was added and blended using a vortex mixer to obtain a good blend of Oil/S_{mix} mixture at a liquid state. 100 mg of above liquid concentrate, neбиволol hydrochloride 5 mg was added and mixed properly using a vortex mixer, and the formulated preparations were evaluated.

2.2 Liquid SMEDDS Characterization

2.2.1 Self emulsification and precipitation assessment

Evaluation of the self-emulsifying properties of SMEDDS formulations was performed using different compositions based on the speed of emulsification, clarity, and the apparent stability of the resultant emulsion. Visual assessment was performed by drop-wise addition of 1mL of the pre concentrated (SMEDDS) into 300mL distilled water and 0.1 N HCl solution. This was done in a glass beaker at room temperature, and the content was gently stirred magnetically at 100rpm (Remi equipment). Precipitation was evaluated by visual inspection of the resultant emulsion after 24 h. The formulation was then categorized as clear (Transparent or transparent with blushing), non-clear (turbid), stable (no precipitation at the end of 24hr), or unstable (showing precipitation within 24 hr)²².

2.3 Robustness to dilution

A dilution study was done to access the effect of dilution and pH dilution media on SMEDDS pre-concentrate by diluting SMEDDS to 50,100 and 1000 times with various dissolution media, distilled water, 0.1 N HCl, and phosphate buffer pH 6.4. The diluted micro-emulsions were stored for 24h and observed for any signs of phase separation or drug precipitation²³.

2.4 Thermodynamic stability

The objective of thermodynamic stability is to evaluate the phase separation and effect of temperature variation on SMEDDS formulations. Selected formulations were subjected to heating-cooling cycles. Six cycles between the refrigerator temperature of 4°C and 45°C temperature were performed with storage at each temperature for not less than 48 hr. Those formulations, which were stable at these temperature cycles, were centrifuged at 3500 rpm for 30 min and observed for phase separation, creaming, or cracking. The formulations which showed no phase separation were further exposed for three freeze-thaw cycles between -4°C and 25°C with storage at each temperature for not less than 48 h. The formulations were then observed for phase separation²⁴.

2.5 Content uniformity in L-SMEDDS

An amount of liquid SMEDDS equivalent to 5 mg of

neбиволol hydrochloride was carefully weighed and placed in a 100 mL volumetric flask containing 5 mL of methanol and mixed thoroughly to dissolve the drug. Then volume was made up to 100 mL with 0.1 N hydrochloric acid and mixed well with shaking and was sonicated for 10-15 min and filtered. Then this filtered solution was analyzed for neбиволol hydrochloride content using a UV-spectrophotometer at λ_{max} 282 nm²⁵.

2.6 In vitro neбиволol release studies

NebivololSMEDDS (equivalent to 5 mg neбиволol) were filled in size 0 hard gelatin capsules. The release of the drug from liquid SMEDDS and the pure drug was determined using USP type II dissolution apparatus. The dissolution medium consists of 900 mL of 0.1 N HCl maintained at 37±0.5°C and 120 min and replaced with an equal volume of fresh medium²⁶. The samples were filtered through Whatman filter paper and were analyzed using a UV spectrophotometer at 282 nm. All measurements were performed in triplicate.

2.7 Droplet size analysis

Droplet size and polydispersity index of microemulsion were determined using Zetasizer Nano ZS (Horiba Scientific SZ-100, Horiba, Kyoto, Japan). The samples were diluted with a ratio of 1: 100 (v/v) using distilled water and repeated in triplicate^{27,28}.

2.8 Zeta potential measurement

Zeta potential helps to predict the stability of the emulsion system. Zeta potential was measured by dynamic light scattering technique using particle size analyzer (Horiba Scientific SZ-100, Horiba, Kyoto, Japan). The samples were diluted with a ratio of 1:100 (v/v) with distilled water and repeated in triplicate^{27,28}.

2.9 Transmittance test

The stability of optimized microemulsion formulation is checked by measuring transmittance using a UV spectrophotometer, Transmittance of samples is measured at suitable wavelengths, and for each sample, three replicates assays were performed. This is done to see the impact of dilution on the prepared formulation. Turbidity and the transmittance of the sample were measured at 650 nm using double distilled water as blank²⁹.

2.10 Cloud point measurement

The optimized liquid self micro emulsifying drug delivery system was diluted with distilled water in the ratio of 1:250, placed in a water bath, and its temperature was increased gradually. Cloud point was measured as the temperature at which there was a sudden appearance of cloudiness visually³⁰.

2.11 Preparation of Solid SMEDDS

The optimized formulation was made solid using different solid carriers such as Neusilin US2, Fujicalin, Aerosil 200, and Microcrystalline cellulose (Avicel pH102) at ratios (1:1, 1:2). The SMEDDS formulation was added drop-wise over the solid adsorbent contained in a porcelain dish. After each addition, the mixture was homogenized using a glass rod to ensure the uniform distribution of the formulation. Resultant mass was passed through sieve no. 80 and stored in a desiccator until further use³¹.

Table 1: Compositions of various neбиволol hydrochloride solid SMEDDS formulations by using various solid carriers

Formulation Code	Solid carrier	Ratio (L - SMEDDS to carrier)	L-SMEDDS (gm)	Carrier (gm)
NA1	Aerosil	1:1	4	4
NA2	Aerosil	1:2	5	10
NN1	Neusilin	1:1	4	4
NN2	Neusilin	1:2	5	10
NF1	Fujicalin	1:1	4	4
NF2	Fujicalin	1:2	5	10
NM1	Microcrystalline cellulose(Avicel pH102)	1:1	4	4
NM2	Microcrystalline cellulose (Avicel pH102)	1:2	5	10

NA - Nebivolol hydrochloride - Aerosil formulation

NN - Nebivolol hydrochloride - Neusilin formulation

NF - Nebivolol hydrochloride - Fujicalin formulation

NM - Avicel pH 102 means microcrystalline cellulose. So it coded as Nebivolol - MCC, NM formulation

2.12 Evaluation of Solid SMEDDS

2.13 Flow properties

The micrometric properties such as angle of repose, Carr's index, and Hausner's ratio were estimated in triplicate for drug-using the standard procedure reported in pharmacopoeia and pharmacopoeial standards.³²

2.14 Drug content

S-SMEDDS containing 5 mg of equivalent neбиволol drug was dispersed in sufficient quantity (5 mL) of methanol and mixed thoroughly to dissolve the drug. Then volume was made up to 100 mL with 0.1 N hydrochloric acid. Samples were sonicated for 15 min and filtered. Filtered samples were analyzed at $\lambda_{\max}$ 282 nm.

2.15 In vitro drug release studies of solid SMEDDS

The *in vitro* dissolution study of solid SMEDDS and the plain drug was carried out by using USP Type II dissolution apparatus. The 5 mg dose equivalent solid SMEDDS were filled in '0' size capsules and placed in the flask of the dissolution apparatus. The dissolution medium consisted of 900 mL of 0.1 N hydrochloric acid maintained at 37 ± 0.5 °C and operated at 50 rpm. An aliquot of 5 mL was withdrawn at predetermined intervals of 10, 20, 30, 40, 50, 60, and 120 min and replaced with an equal volume of fresh medium. The samples were filtered through Whatman filter paper and were analyzed at $\lambda_{\max}$ 282 nm. All measurements were performed in triplicate.³³

2.16 Droplet size analysis and zeta potential measurement of reconstituted neбиволol hydrochloride microemulsion

The average droplet size and zeta potential were determined by photon correlation spectroscopy and laser Doppler velocimetry. Ten mg of S- SMEDDS was diluted with 25 mL of distilled water in a beaker and sonicated for 15 min. Samples were filtered and suitably diluted, and analyzed. Each sample was analyzed in triplicate.³⁴

2.17 Analysis of release Mechanisms of drugs

To study the release kinetics, data obtained from *in vitro*

dissolution study was fitted in various kinetic models: Zero-order as a cumulative percent of drug released versus time, first-order as log cumulative percentage of drug remaining versus time, and Higuchi's model as cumulative percent drug released versus square root of time, Hixoncrowel describes the release from systems when there is a change in a surface area and diameter of particles. To determine the mechanism of drug release, the data was fitted into Korsmeyer and Peppas equation as log cumulative percentage of drug released versus log time, and the exponent n was calculated from the slope of the straight line. For slab matrix, if exponent is 0.5, then diffusion mechanism is Fickian; if $0.5 < n < 1.0$, then it is anomalous transport. If n is 1.0, it is case II transport, and if $n > 1.0$, then it is super case II transport.³⁵

2.18 Compatibility study by FTIR

Chemical interaction between the drug and excipients was studied by the FTIR technique. FTIR spectra of the drug and optimized S-SMEDDS were recorded on FTIR spectroscopy (Shimadzu 8400, Japan) using the potassium bromide(KBr) pellet method. The scanning range was $4000-400$ cm⁻¹ at a resolution of 1 cm⁻¹.³⁶

2.19 Differential scanning calorimetry (DSC)

The physical state of neбиволol in S-SMEDDS was distinguished by, Differential scanning calorimetry. Indium standard was used to measure the DSC temperature and enthalpy scale. The powder samples were hermetically kept in the aluminum pan and heated at a constant rate of 10 °C/min, over a temperature range of 0 °C to 450 °C. The inert atmosphere was maintained by purging nitrogen at the flow rate of 60 mL/min.³⁷

3. RESULTS AND DISCUSSION

3.1 Solubility studies

Solubility studies were aimed to identify oil, surfactant, and co-surfactant that possess the good solubilizing capacity for neбиволol hydrochloride. The solubility of the drug was tested in different oil phases, and maximum solubility was found to be in Oleic acid (395.21 ± 15.2 mg/mL), and the oil phase was selected. The solubility of neбиволol hydrochloride in various oils is shown in Figure 1.

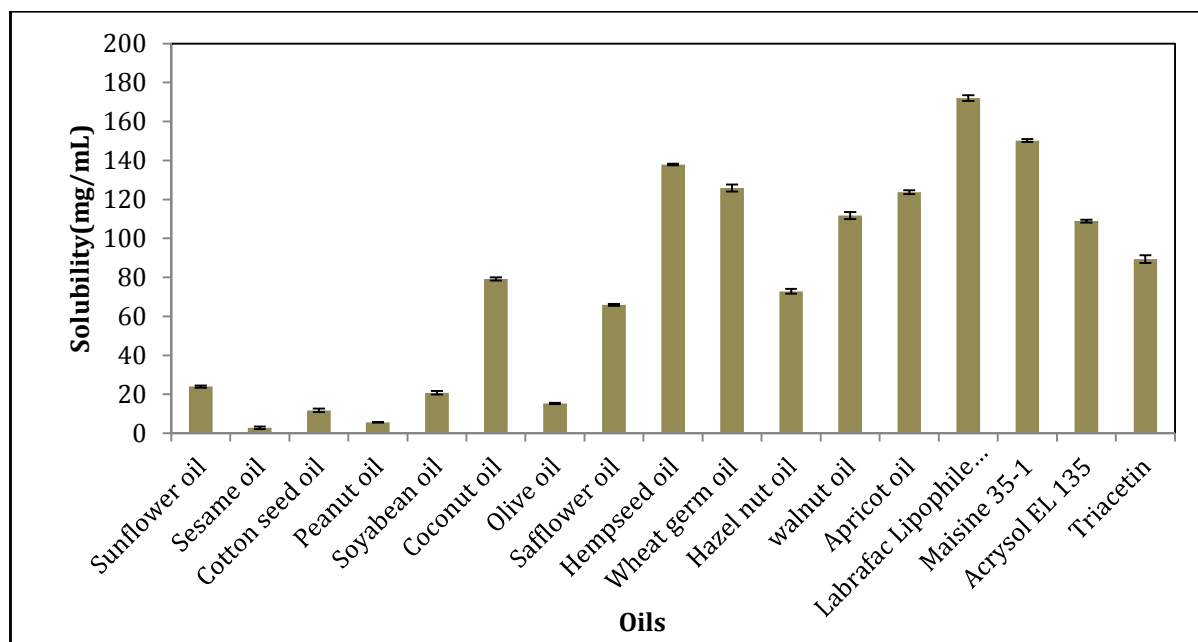


Fig 1: Solubility of nebivolol hydrochloride in different Oils

In this study, three nonionic surfactants (Kolliphor EL/ Cremophore EL, Kolliphor RH 40/ Cremophore RH 40, Brij 35 (block surfactant), Tween 80, Tween 20, Span 20, Span 80, Solutol HS 15) were selected. The solubility data profile was given as bar diagrams (Figure 2). Kolliphor RH 40/ Cremophore RH 40 showed the highest solubility of nebivolol hydrochloride as 157.83 ± 1.07 mg/mL, thus chosen as a surfactant for further studies. The next best surfactant is Gelucire 48/16, but it was not considered for further evaluation.

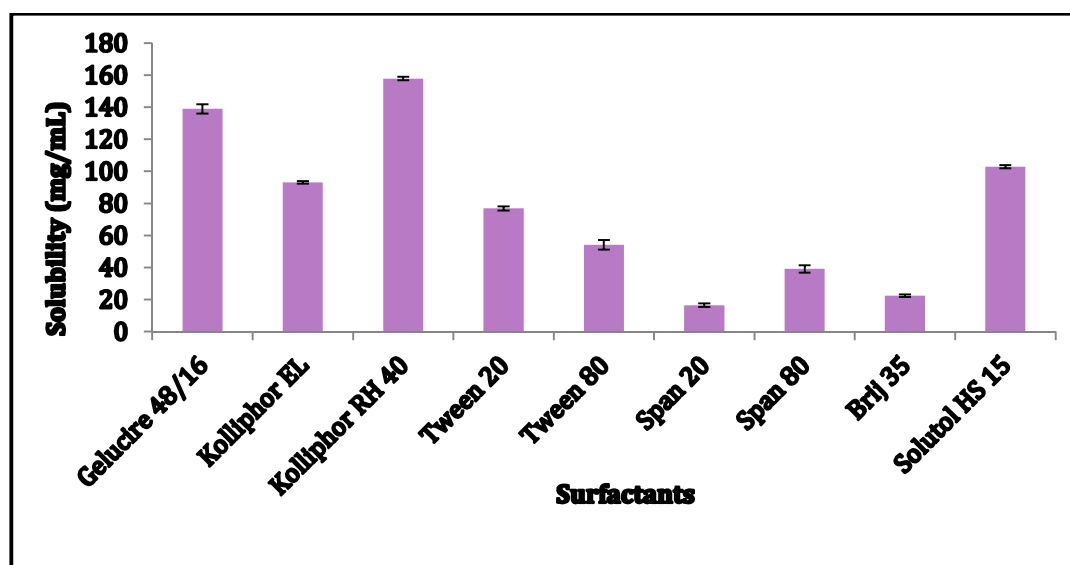


Fig 2: Solubility of nebivolol hydrochloride in different surfactants

Mixing of co-surfactant with nonionic surfactant facilitates dispersion of tiny globules and eventually absorption of lipophilic API from self-micro-emulsifying preparations³⁸. The incorporation of suitable co-surfactants lowers the tension between two phases and fluidizes the hydrocarbon region of the film, resulting in the enhancement in the spontaneity of emulsification, small in emulsion droplet size, and polydispersity³⁹. For nebivolol hydrochloride SMEDDS, four nonionic co-surfactants (PlurolOleique CC 497, PEG 200, 400, 600,

Propylene glycol, Gelucire 44/14, and Kollisolv PEG 300) were investigated. The solubility profile of nebivolol hydrochloride in co-surfactants was presented as bar diagrams in Figure 3. Higher solubility was found in Gelucire44/14, which was selected as a co-surfactant for SMEDDS formulations. As anticipated, low solubility was found in all other selected co-surfactants. The next best one is Kollisolv PEG 300.

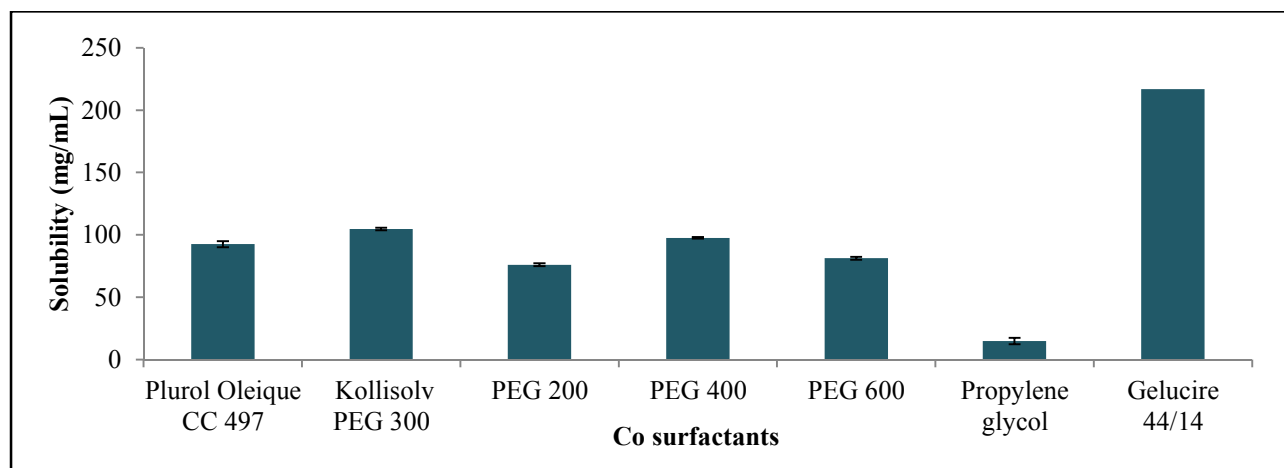


Fig 3: Solubility of nebulolol hydrochloride in different co-surfactants

3.2 Pseudo ternary phase diagram

Pseudo-ternary phase diagram were constructed to study the relationship between the phase behavior and the composition, which also help to determine the concentration range of components for the microemulsion formation⁴⁰. Labrafac Lipophile WL 1349, Kolliphor RH 40/ Cremophore RH 40, and Gelucire 44/14 were used to construct pseudo-ternary phase diagrams. Nine different combinations of oil to S_{mix} at different ratios (1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1) were used for the construction of Pseudo-ternary phase diagram. Pseudo ternary phase diagrams make it easy to find out the concentration range of components for SMEDDS and give an idea about the composition of a selected system and the nature of the resultant dispersion such as phase separation, coarse emulsion, and microemulsion and hence, assist in selecting optimum formulation. The *in vitro* performance of the resultant emulsions after titration with water was visually assessed using the grading system. The micro-emulsion phase was visually identified as the area where clear transparent and less viscous microemulsion. The ternary phase diagram was constructed using chemix® software. From 9 ternary phase diagrams constructed, one was reported in Figure 4, representing S_{mix} ratio 4:1, which showed the maximum microemulsion region. For oil:

S_{mix} ratios 9:1, 8:2, 7:3, 6:4 (except 4:1), 5:5 (up to 3:1), 4:6 (only 1:2, 1:3, 1:4, 2:1) less clear emulsion has produced. Microemulsion, clear with bluish appearance, has formed with an increased proportion of surfactant than oil, i.e., for oil to S_{mix} ratios 6:4 to 1:9. The microemulsion region containing the oil component is approximately 10-60% and the S_{mix} component 40-90%. Microemulsion area was increased with an increased proportion of S_{mix} than oil means increasing amounts of surfactant (Kolliphor RH 40) to co-surfactant (Gelucire 44/14). The blending of Kolliphor RH 40 and Gelucire 44/14 helped in improving the emulsification. Probably, the surfactant is capable of reducing o/w interfacial tension and enhanced interface fluidity when the co-surfactant is present. The used surfactants combination might have improved the fluidity and flexibility of the surfactant layer formed at the interface. The adsorption of S_{mix} at the interface provides a mechanical barrier to coalescence and forms a thermodynamically stable system by reducing the energy required to form a microemulsion. This approach has already been employed by Nasef AM et al.⁴¹. Thirty-two combinations were prepared using Labrafac Lipophile WL 1349, Kolliphor RH 40/ Cremophore RH 40, and Gelucire 44/14 combinations, and they produced micro-emulsion upon dilution.

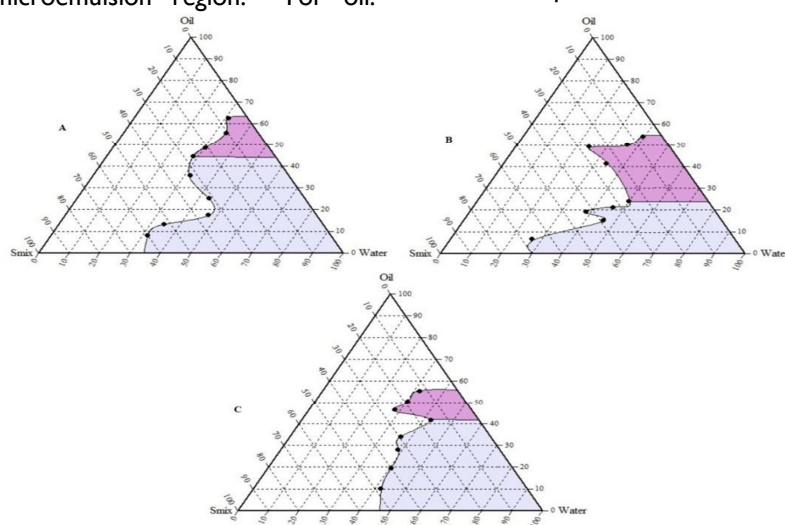


Fig 4: Pseudo ternary phase diagram for LabrafacLipophile WL 1349, Kolliphor RH 40 and Gelucire 44/14 combination for S_{mix} ratio A as 4:1, B as 5:1, C as 6:1plum colour shows emulsion region, and lavender colour shows the region of self-micro emulsification

As shown in Figure 4, upper section plum colour for the emulsion region and the lavender colour area (down section of graph) disclosed in the phase diagram represented the region of self-micro emulsification. Within this area, the SMEDDS could form fine o/w emulsion with only gentle agitation. Increasing the concentration of surfactant with respect to cosurfactant 4:1 to 6:1 S_{mix} ratios, the microemulsion region gradually increased. In spite of the high oil ratio, the micro emulsion region was improved. The order of S_{mix} ratios microemulsion region was found to be 4:1>5:1=6:1>3:1>2:1>1:1>1:2=1:3>1:4. Fixing the surfactant/co-surfactant ratio at 4:1 is the better choice when compared to 5:1, 6:1. Only 4:1, 5:1, 6:1 ternary phase diagrams were reported in Figure 4. As a larger microemulsion region is given more flexibility to find the optimal dosage composition, it is important to select the formulations which lead to stable microemulsion.

3.3 Preparation of neбиволol liquid SMEDDS

Different liquid neбиволolSMEDDS formulations (32) were prepared by selecting the concentration of oil and S_{mix} from pseudo ternary phase diagrams, Formulation codes for selected formulations as presented in Table 3. The liquid SMEDDS formulations were produced by the aqueous titration method. Various formulations were prepared with a constant amount of neбиволol is 5 mg (dose) and varying ratios of lipid, surfactant to co-surfactant. Surfactant and co-surfactant were blended in different weight ratios. To the above mixture, the required amount of oil phase was added and blended using a vortex mixer to obtain a good blend of oil/ S_{mix} mixture at a liquid state. Hundred mg of above liquid concentrate, neбиволol 5 mg was added and mixed properly using a vortex mixer.

Table 3: Formulation codes and percentage composition for selected liquid SMEDDS formulations containing neбиволol (5mg)

S_{mix} ratio	Oil : S_{mix} ratio	Formulation code	Composition (%w/w)		
			Oil (Labrafac Lipophile WL 1349)	Surfactant (Kolliphor RH 40)	Co-S (Gelucire 44/14)
4:1	1:9	N17	10	72	18
4:1	2:8	N18	20	64	16
4:1	3:7	N19	30	56	14
5:1	1:9	N23	10	75	15
5:1	2:8	N24	20	66.66	13.33
5:1	3:7	N25	30	58.32	11.66
6:1	1:9	N28	10	77.14	12.85
6:1	2:8	N29	20	68.57	11.42
6:1	3:7	N30	30	60	10

3.4 Formulation characterization of L-SMEDDS

3.5 Assessment of self-emulsification time

Self-emulsification time in distilled water, 0.1 N HCL less than 15 sec, which suggests a requirement of a very little amount of free energy for emulsification, thus the spontaneous formation of a microemulsion and lowered the interfacial tension and interfacial film curvature⁴². In generating the self emulsification time in distilled water is high compared to its time in 0.1 N Hydrochloric acid.

3.6 Assessment of Precipitation

SMEDDS formulation did not show any precipitation in 0.1 N HCl and distilled water which confirmed the ability of formulation of stable micro-emulsion.

3.7 Robustness to dilution

Robustness to dilution was performed with distilled water, 0.1 N hydrochloric acid, and phosphate buffer pH 6.4. Microemulsions did not show any physical change even after 24 hr of storage, indicating robustness. Also, dilution may affect the drug release profile, and the drug may get precipitated at higher dilutions. Hence, robustness to dilution was monitored by dilution of the SMEDDS at 50, 100, and 1000 times. The resulting emulsions were found to be in the acceptable microemulsion region. Even after 24 h, neither precipitation of the drug nor any phase separation was observed, showing the stability of the reconstituted emulsion.

3.8 Thermodynamic stability

The ability of the formulation to withstand different stress conditions was evaluated. No formulation showed any sign of precipitation or phase separation when subjected to different temperature conditions and centrifugation. It was concluded that SMEDDS formulations were stable thermally as well as under stressful conditions.

3.9 In vitro neбиволol hydrochloride release studies from SMEDDS formulations

Dissolution studies were performed for all 32 SMEDDS formulations and the pure drug in 0.1 hydrochloric acid. The results were compared with the pure drug. From the above results, nine formulations (N17, N18, N19, N23, N24, N25, N28, N29, N30) were selected as these formulations have shown the highest percentage (more than 85%) of cumulative drug release in 120 min. The cumulative percent neбиволol hydrochloride dissolution profile was reported in Figure 5. Drug release from SMEDDS formulations was found to be significantly higher compared to that of neбиволol hydrochloride alone. The SMEDDS formulation of neбиволol hydrochloride enhanced the dissolution behavior. A perusal to Figure 5 indicated an instantaneous increase in the neбиволol hydrochloride release from SMEDDS, similar to burst effect type, more than 50% in 10min. More than 85% of the drug was released within 120min, in the case of most of the SMEDDS formulations, while pure drug showed only 29.05% release. Therefore, spontaneous formation of micro-emulsion and a small droplet size allowed a faster rate of drug release. Hence higher absorption and higher

bioavailability were expected. It was also shown that an increase in surfactant concentration and a decrease in oil concentration in the formulation increases drug release.

Therefore, these 9 formulations (N17, N18, N19, N23, N24, N25, N28, N29, and N30) were considered as better formulations and subjected to further evaluation tests.

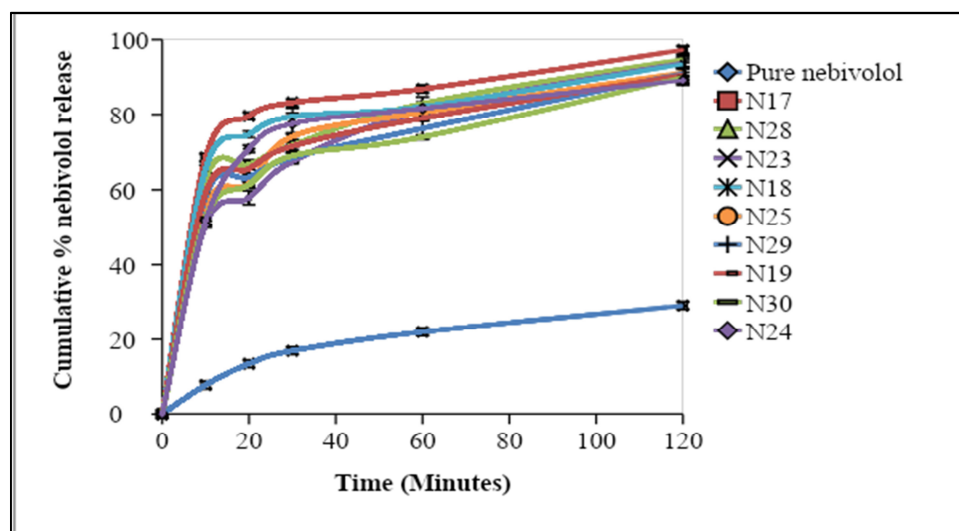


Fig 5: *In vitro* dissolution profile for selected neбиволol hydrochloride liquid SMEDDS formulations

3.10 Droplet size analysis

Droplet size distribution following self-emulsification is a critical factor to evaluate self-micro emulsifying systems *in vitro*. Droplet size distribution is an important factor in self-emulsification performance because it determines the rate and extent of drug release as well as the stability of the emulsion⁴³. The average globule size of all tested nine formulations at 1000 times dilution with distilled water was

found to be in the range 132.8 to 955.7 nm (Table-4). The mean particle size was obtained and recorded in Figure 6 for optimized formulation N17. Formulation N17 showed a smaller droplet size (132.8 nm) compared to other formulations as it contains a higher concentration of surfactant that promotes a faster emulsification process and result in finer droplet formation and had a polydispersity index of 0.184, indicating a unimodal size distribution (narrow particle size distribution).

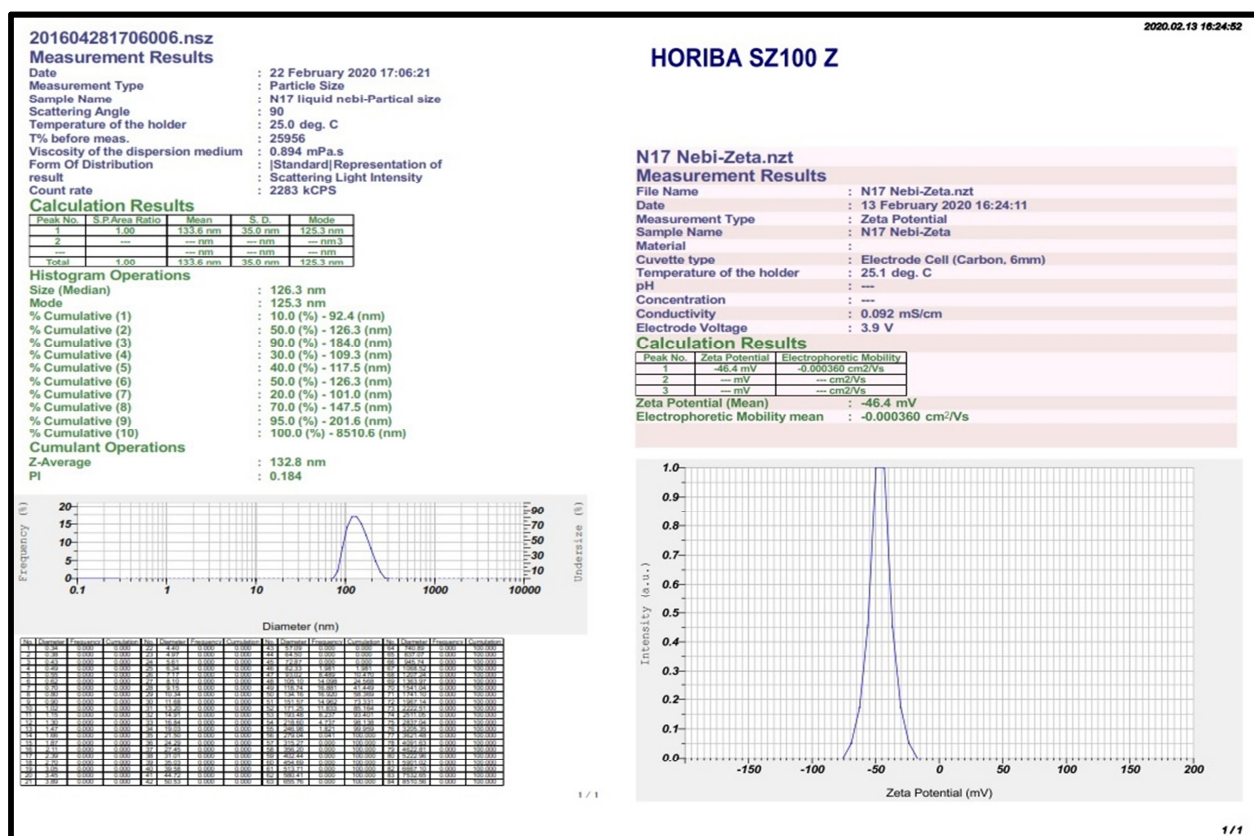


Fig 6: Spectra for particle size and zeta potential of formulation N17-liquid neбиволol SMEDDS formulation

3.11 Zeta potential determination

The magnitude of the zeta potential gives an indication of the potential stability of the colloidal system.⁴⁴ If the particles have low zeta potential values, then there is no force to prevent the particles from coming together, and there is dispersion instability. Figure 6

depicts the zeta potential measurement for optimized formulation N17. The zeta potential for 9 selected SMEDDS formulations was found to be in the range of -7.2 to -46.4 mV, which is presented in Table 4. A negative charge indicates the presence of fatty acids on the droplets. All formulations comply with the requirement of the zeta potential for stability.

Table 4: Comparative characterization data of selected liquid SMEDDS formulations of nebulivolol

Formulation code	Self emulsification			Particle size (nm)	Polydispersity index (PDI)	Zeta potential (mV)	Drug content	% release 120 mins	Drug in
	Distilled water	0.1 HCL	N						
N17	5.15 ± 1.93	4.00 ± 1.00	132.8±22.1	0.184±0.04	-46.4±0.32	99.82±0.26	97.26 ± 1.16		
N18	8.44 ± 1.21	8.18±0.58	551.3±42.6	0.612±0.03	-13.1±0.58	98.91 ±0.32	93.47 ± 1.22		
N19	7.47 ± 1.42	6.33 ±0.72	419.2±37.9	0.724±0.01	-29.7±0.71	98.15 ±0.90	90.60 ± 2.14		
N23	6.24 ± 1.34	5.33 ±0.71	602.5±18.2	1.025±0.06	-38.1±0.29	98.92± 0.98	93.83 ± 1.28		
N24	8.00 ± 1.87	7.45 ±0.48	713.8±26.3	0.823±0.07	-25.6±0.44	99.79 ±0.09	89.12 ± 1.15		
N25	9.35 ± 0.46	8.23 ±0.69	628.2±53.4	0.937±0.04	-12.6±0.65	98.28 ±0.18	91.27 ± 1.14		
N28	6.69 ± 1.66	6.35 ±0.45	541.1±41.8	0.539±0.05	-11.4±0.16	99.49 ±0.12	94.78 ± 0.86		
N29	7.66 ± 1.09	7.38 ±0.54	955.7±15.5	0.984±0.03	-13.5±0.80	99.89 ±0.10	91.02 ± 1.62		
N30	9.06 ± 0.95	8.89 ±0.35	819.2±56.7	0.585±0.02	-7.2±0.53	99.25 ±0.50	89.95 ± 1.36		

3.12 Transmittance test

The Transmittance of the best formulation of SMEDDS formulation (N17) is recorded. Optimized Formulation (N17) has a transmittance value of 97.29±1.52% (n=3), suggesting their clarity. This might be due to the smaller particle size, which increases the transparency of the emulsion. The percentage transmittance value near 100% (97.29%) indicates clear and transparent, with no turbid microemulsion formation.

3.13 Cloud point measurement

The cloud point for optimized nebulivolol hydrochloride liquid SMEDDS formulation was found to be 70.33±2.08°C, and it was observed that sudden emergence of cloudiness (turbidity).

3.14 Solid SMEDDS of nebulivolol hydrochloride

For the preparation of solid SMEDDS, the liquid SMEDDS formulation (N17) was mixed with carriers Aerosil 200, Neusilin US2, Fujicalin, and Microcrystalline cellulose (Avicel pH102) at various SMEDDS to carrier ratios 1:1, 1:2 %w/w. The resultant solid SMEDDS of uniform-sized powder obtained were kept in desiccators until further use³¹. These eight formulations were subjected to further analysis

3.15 Characterization of Solid self micro emulsifying powder

Solid SMEDDS were evaluated for powder flow properties, reconstitution properties and subjected for solid-state characterization.

3.16 Preformulation studies

The solid SMEDDS prepared in this work were evaluated for angle of repose, bulk density, tapered density, Carr's index, and Hausner's ratio. The results for bulk and tapped density of solid SMEDDS are shown in Table 5. They tapped densities were found to be higher than bulk densities. The angle of repose of NN1, NN2, NF1, NF2 is good compared to other formulations. These results are once again confirmed by Carr's index and Hausner's ratio. The tapped density and bulk density are also nearly the same for NF1, while other formulations exhibited wider differences and low values except for NN2. In other words, in Neusilin, Fujicalin must inherently have higher bulk densities. Among all the formulations reported in this work, NF1 gave better pre-formulation results compared to all formulations. The powder mass is free to flow and excellent to facilitate the encapsulation of medicament

Table 5: Bulk density, tapped density, and flow properties of solid SMEDDS of neбиволol hydrochloride

Formulation code	Bulk density	Tapped density	Angle repose	of Carr's index	Hausner's ratio	Influence of flow property
NA1	0.144±0.032	0.208±0.027	46.33±1.24	30.76±1.10	1.44±0.01	Poor
NA2	0.299±0.022	0.417±0.021	45.97±2.03	28.29±2.15	1.39±0.02	Poor
NN1	0.454±0.038	0.526±0.062	31.26±1.58	13.68±1.44	1.15±0.03	Good
NN2	0.542±0.026	0.634±0.018	34.55±2.19	14.51±1.48	1.16±0.01	Good
NF1	0.617±0.023	0.685±0.042	29.52±1.64	9.92±0.08	1.11±0.005	Excellent
NF2	0.388±0.037	0.449±0.018	31.18±2.95	13.58±1.27	1.15±0.02	Good
NM1	0.337±0.020	0.455±0.013	43.89±3.22	24.06±1.55	1.31±0.03	Passable
NM2	0.486±0.036	0.517±0.025	46.74±2.71	26.90±1.16	1.36±0.04	Poor

is the average of 3 determinations

NA- Nebivolol hydrochloride Aerosil formulations

NN- Nebivolol hydrochloride Neusilin US2 formulations

NF- Nebivolol hydrochloride Fujicalin formulations

NM- Nebivolol hydrochloride Microcrystalline cellulose (Avicel pH102) formulations

3.17 Drug Content

The neбиволol content in solid SMEDDS was reported in Table 6. Nebivolol content values varied from 95 to 98 % and were satisfactory.

Table 6: Drug content data for solid SMEDDS of neбиволol hydrochloride

Formulation code	Drug Content (%) AM* ± SD
NA1	95.94±0.36
NA2	95.78±0.42
NN1	96.45±0.78
NN2	97.33±0.67
NF1	98.16±0.83
NF2	97.73±0.55
NM1	95.11±0.93
NM2	95.82±0.48

*is the average of 3 determinations

3.18 In Vitro neбиволol hydrochloride solid SMEDDS release

The *In Vitro* neбиволol drug release from solid SMEDDS was conducted in 0.1N Hydrochloric acid solution. The percentage drug release Solid-SMEDDS was found in the range of 82.85±0.87% to 95.38±0.76% up to 120 min, respectively. When compared to formulations, the pure neбиволol showed poor release profile (29.05 ± 3.22) up to 120 min. Drug release from the SMEDDS formulation

(was found to be significantly higher as compared with that of plain neбиволol) (Figure 7). It could be suggested that the SMEDDS formulation resulted in the spontaneous formation of a microemulsion with a small droplet size, which permitted a faster rate of drug release into the aqueous phase, much faster than that of plain neбиволol. Thus, this greater availability of dissolved neбиволol from the SMEDDS formulation could lead to higher absorption and higher oral bioavailability.

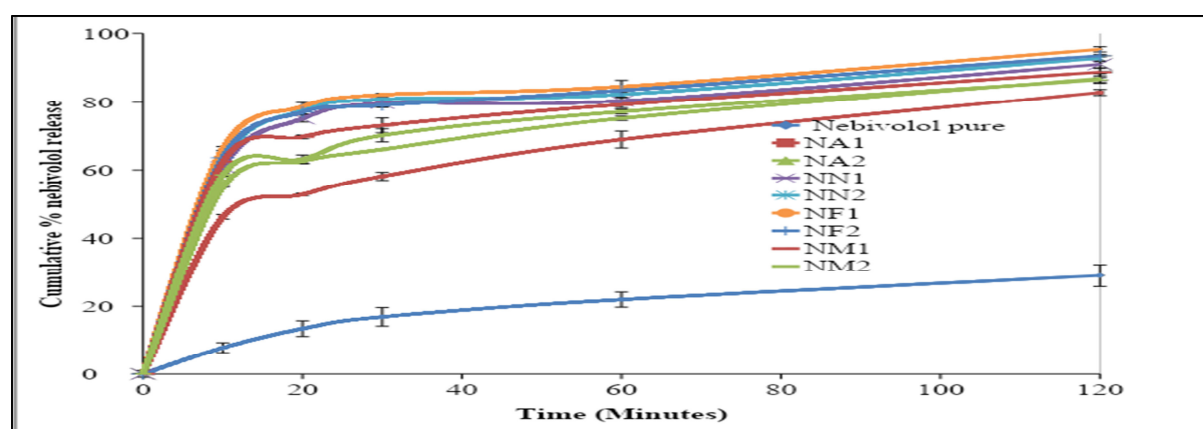


Fig 7: *In Vitro* neбиволol hydrochloride release of solid SMEDDS

3.19 Droplet size analysis and zeta potential measurement

Droplet size analysis revealed the effect of varying amounts of the solid carrier in the formulated SMEDDS (Table 7). Formulation NFI with a 1:1 (w/w) ratio of L - SMEDDS to

Fujicalin carrier showed the least mean droplet size, 144.1nm (polydispersity index, 0.438). The z-average diameter of the solid SMEDDS (144.1±3.66 nm; PDI, 0.438±0.02) was higher than that of liquid SMEDDS loaded with the drug (132.8±22.1 nm; PDI, 0.184±0.04). Both the z-average diameters of the liquid and solid SMEDDS were less than 200 nm.

Table 7: Droplet size (nm), polydispersity index, zeta potential (mV) neбиволol hydrochloride release of solid SMEDDS in 0.1 N Hydrochloric acid solution

Formulation code	Droplet size (nm)	Polydispersity index	Zeta potential (mV)
NA1	358.2±2.45	0.932±0.02	-13.5±0.95
NA2	579.6±1.65	0.989±0.03	-12.8±0.23
NN1	267.5±3.94	0.872±0.06	15.4±0.82
NN2	376.4±2.11	0.723±0.03	-27.6±1.25
NFI	144.1±3.66	0.438±0.02	-58.2±0.94
NF2	365.3±1.33	0.944±0.01	-13.0±2.58
NM1	670.7±0.82	0.826±0.01	-0.70±0.10
NM2	893.4±3.29	0.715±0.06	-0.40±0.09

3.20 Dissolution kinetics and Mechanisms of neбиволol hydrochloride solid SMEDDS optimized formulation NFI

The kinetics from neбиволol hydrochloride solid SMEDDS were analyzed to understand the order of drug dissolution. The data were processed for regression analysis using MS- Excel statistical functions. The plots of dissolution kinetics are recorded in Figure 8, depicting zero order, first order, Higuchi drug release,

KorsmeyerPeppas drug release mechanism, and Hixson Crowell cube root law. For the solid SMEDDS of neбиволol (NFI), the followed plots were reported in Figure 8. It followed the First – order, and the mechanism was Korsmeyer Peppas release mechanism. In Table 8, the n value (0.131) was less than 0.5, suggesting diffusion was Fickian with anomalous transport ($n < 0.5$). SMEDDS formulations promoted the spontaneous formation of microemulsion with a small droplet size, which permitted a higher rate of drug release than that of pure neбиволol.

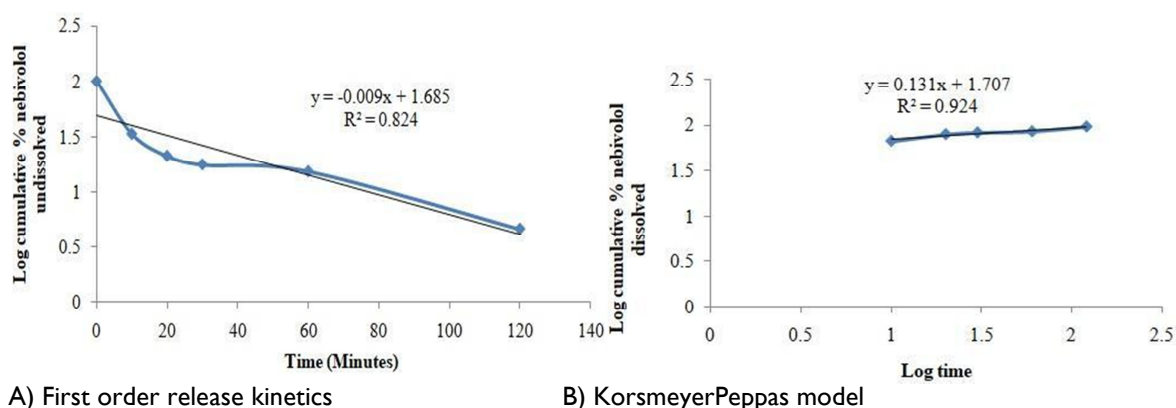


Fig 8: Dissolution Kinetics and Mechanisms of neбиволol hydrochloride Solid SMEDDS optimized formulation NFI

Table 8: Fitting of dissolution data of NFI Solid SMEDDS in 0.1 N Hydrochloric acid			
Kinetic order	Dissolution data regression		
	Equation	R ²	n value
Zero order	$y = 0.503x + 47.70$	0.416	
First order	$y = -0.009x + 1.685$	0.824	
Mechanism of drug release			
Higuchi's release model	$y = 7.752x + 26.75$	0.718	
Korsmeyer Peppas model	$y = 0.131x + 1.707$	0.924	0.131
Hixson Crowell cube root	$y = 0.018x + 1.000$	0.678	

3.21 Compatibility study by FTIR

FTIR spectra of neбиволol showed characteristic peaks of aromatic C-F stretching at 1226.19 cm⁻¹, Aromatic C=C stretching at 1641.48 cm⁻¹, Aromatic C-H stretching at 704.04 cm⁻¹, O-H stretching at 1433.16 cm⁻¹, N-H stretching at 3446.91 cm⁻¹, C-N stretch alkyl at

1064.39 cm⁻¹, Alkyl C-H stretch at 2875.96 cm⁻¹ shown in Figure 9. The FTIR of optimized solid SMEDDS also showed all these characteristic peaks with minor shifts shown in Figure 10. These results from FTIR spectral analysis indicated that there was no chemical interaction between drug and excipients used in the formulation.

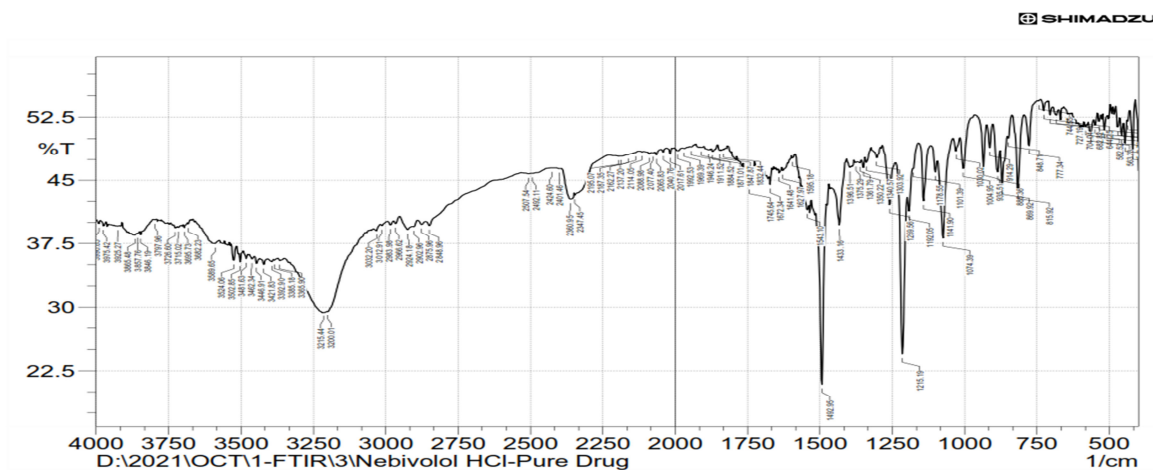


Fig 9: FTIR peak of nebigivolol hydrochloride pure drug

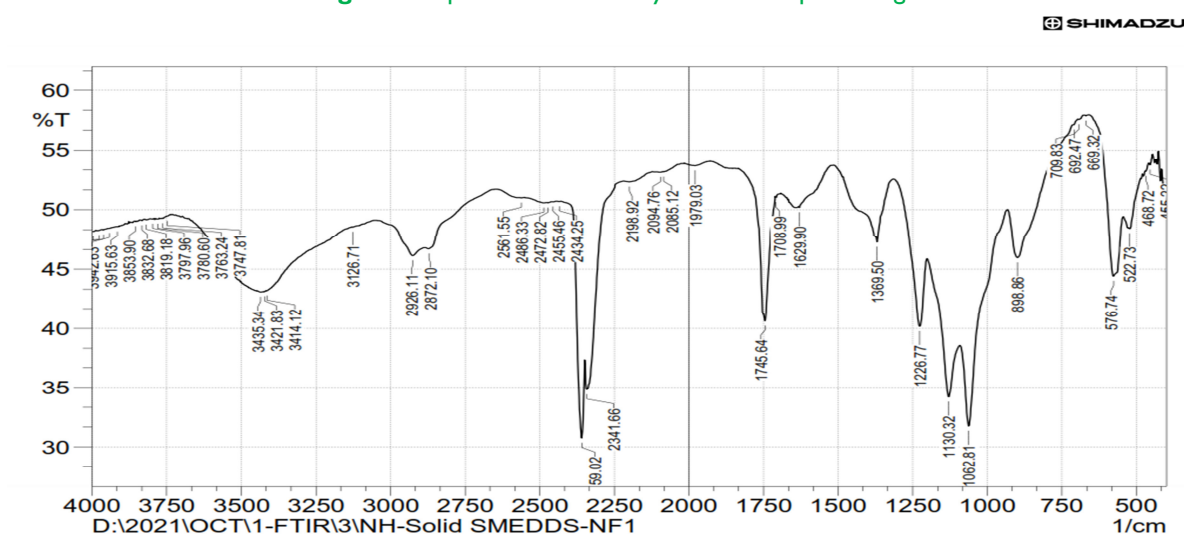


Fig 10: FTIR peak of optimized nebigivolol hydrochloride solid SMEDDS (NFI)

3.22 Differential scanning calorimetry (DSC)

DSC allows the determination of thermotropic phase transition behavior in a quantitative manner. The melting peak in DSC curves was recorded only in the case of analyzing pure nebigivolol, where a narrow peak with an onset

temperature of 228.9 °C was observed (Figure 11). There were also no endothermic effects in the DSC curve of solid nebigivolol-loaded SMEDDS (Figure 12). We, therefore, propose that nebigivolol remains molecularly dissolved in solid SMEDDS, which is composed of liquid SMEDDS entrapped in a solid carrier.

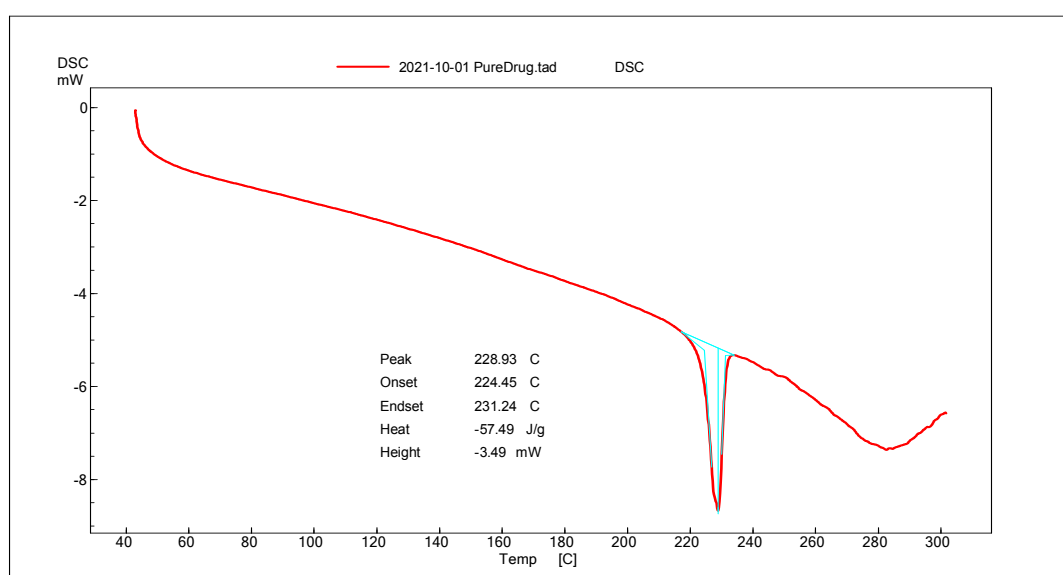


Fig 11: DSC thermograph of nebigivolol hydrochloride pure drug

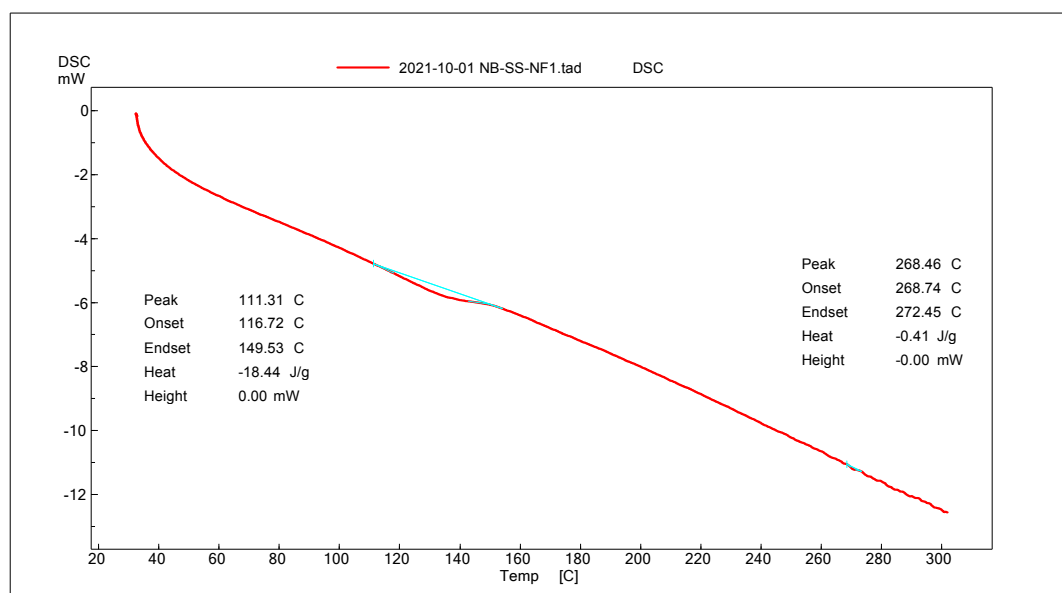


Fig12: DSC thermograph of optimized neбиволol hydrochloride solid SMEDDS (NF1)

4. CONCLUSION

In the current investigations, liquid SMEDDS and solid SMEDDS were prepared for antihypertensive drug neбиволol and evaluated for various parameters. Optimized liquid SMEDDS contains 10% Labrafac Lipophile WL 1349, 72% Kolliphor RH 40, and 18% Gelucire44/14, which showed spontaneous emulsification properties and good thermodynamic stability. The optimized liquid SMEDDS, NI7, was successfully transformed into a free-flowing powder using Fujicalin without affecting the self-micro emulsifying ability of the liquid SMEDDS. Liquid SMEDDS and solid SMEDDS showed a better *in vitro* drug release profile compared with pure API. Fujicalin produced a solid SMEDDS with microemulsion droplet sizes and improved the dissolution rate and the oral bioavailability of neбиволol due to the fast spontaneous emulsion formation and the decreased droplet size. The present study confirmed that the new self-micro emulsifying systems are a promising strategy for enhancing dissolution rate and thereby oral bioavailability of the neбиволol.

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5. AUTHOR CONTRIBUTION STATEMENT

Ramya Sri Sura had completed this work under the supervision of Subrahmanyam CVS and Shyam Sunder Rachamalla. All authors together contributed to this research work.

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7. CONFLICT OF INTEREST

The authors have no conflicts of interest.

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