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ORIGINAL ARTICLE

Design and Optimization of Self-Micro Emulsifying Drug Delivery Systems for Improved Solubility and Bioavailability of Nebivolol

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ABSTRACT
Nebivolol (NB) is a lipophilic molecule with low solubility in GI fluid, and high metabolism which leads to its low oral bioavailability 12%. The aim of the present investigation was to develop Self-micro emulsifying drug delivery systems (SMEDDS) to enhance the solubility and permeability of the drug. Solubility study, pseudo-ternary phase diagram, and 3^2 factorial design (Box-Behnken design-BBD) were used to select the components of the system and optimize the composition of liquid SMEDDS. Tween 20, Tween 80, Spans as surfactants, Polyethylene glycol (PEG, Propylene Glycol (PG) as surfactants, and Grape Seed Oil (GSD) as oil were all tested for their ability to promote self-micro emulsification. Based on data from the ternary phase diagram, in vitro drug release, droplet size, and zeta potential, formulation 4 (F4) was determined to be the most effective formulation. The improved formulation yielded a microemulsion with a droplet size of around 330 nm and a zeta potential of zero. Iteratively Differentiated Processing Nebivolol's molecular dissolution in the Solid SMEDDS was validated by calorimetry and powder X-ray diffraction. In vitro drug release tests for the F4 formulation indicated 78.86% and 99.05% drug release at 45 and 120 minutes, respectively. Studies in an ex-vivo setting indicated that the F4 formulation allowed 71.3% of the medicine to penetrate after 120 minutes, whereas the pure drug only allowed 30.75 % to do so. Based on these findings, it seems that SMEDDS may be used to improve the solubility and dissolution of chemicals that are already somewhat poorly soluble, such as Nevibolol.
Keywords: *Nebivolol, SMEDDS, oil, Surfactant, 3^2 factorial designs, optimization.*

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INTRODUCTION

Nebivolol (NB) is an oral, highly selective third generation β_1-receptor antagonist, having nitric oxide enhancing vasodilator effect, indicated for the treatment of hypertension [1,2]. Also, Nebivolol has reduced typical beta-blocker-related side effects such as fatigue, clinical depression, bradycardia, and impotence [3,4]. After oral administration of NB, the peak plasma concentration reaches within 0.5-2 h. Oral bioavailability of NB is 12% only because of first-pass hepatic metabolism caused by cytochrome P450 2D6 enzymes. It has a suitable log P of 4.03 and the recommended daily dose is 5 mg [5]. The drug is highly lipophilic belonging to the class BCS II, having low dissolution rate and bioavailability [6]. Various efforts have been made to develop effective delivery systems to improve water solubility and bioavailability of NB including preparation of liquid solid compact [8], solid dispersions [9], nanoparticulate delivery [10], oral nanoemulsion [11], orodispersible [12] and immediate release tablets [13].

One potential strategy to improve drug solubility and bioavailability is the use of a self-micro-emulsifying drug delivery system (SMEDDS). An isotropic combination of oil, surfactant, and cosurfactant that, following dilution with an aqueous medium in the GI tract and mild agitation, creates a fine oil-in-water (o/w) microemulsion, thus increasing the interfacial area and the drug's distribution [14]. Oil, surfactant, and cosurfactant are only a few of the ingredients that must be carefully chosen and used in the right amounts if an optimum SMEDDS formulation is to be created. Many methods from experimental design

have been used to the problem of formulating an effective treatment [15]. The ideal ratios of SMEDDS components have been determined empirically using one-factor-at-a-time methods. However, in addition to being approaches laborious, inefficient, and time-consuming, they also seldom give enough information to properly evaluate how each component affects the whole [16]. As a result, statistical optimization strategies [17] have been developed to evaluate the impact of mixture-related variables as well as the interaction among multicomponent independent variables.

MATERIAL AND METHODS

Materials

Nebivolol was purchased from Emcure Pharmaceutical Ltd. Pune.Polyethylene glycol (PEG)– 200,300, 600; Propylene Glycol (PG); Span – 20,60,80; Tween– 20,60,80 purchased from SD – Fine Chemicals Ltd, Mumbai, India. Pure Cotton Seed Oil, Pumpkin Seed Oil (PO), Corn Oil (CO), and Almond Oil (AO) were purchased from Shree Overseas Exports, Begumpur, New Delhi, India. Walnut Oil (WO), and Grape Seed Oil were purchased from the local market. All other chemicals used for the study were of analytical grades.

Preparation of L-SMEDDS

1. Selection of excipients and formulation of L-SMEDDS involves the following steps:
2. Solubility studies to selected excipients showing maximum drug solubility [18].
3. Emulsification efficiency of surfactants and co-surfactants to check their ability to emulsify selected oil [19].
4. Pseudo-ternary phase diagram was constructed to obtain the concentration range of components for the existing region of microemulsions [20].
5. Optimization and Evaluation of NB-loaded L-SMEDDS by 3^2 factorial design to observe the combined effect of the concentration of Grape Seed Oil (X1) as well as the concentration of Tween 20 (X2) on the Drug release (DR) at 45 mins (Y1), Drug release (DR) at 2hrs (Y2) for obtaining the optimized liquid SMEDDS [21]

Table 1: Variables and Constrains in 3^2 Factorial Experimental Design

Independent Variable	Level			Constrains
	-1	0	+1	
X_1: % of Grape Seed Oil	33.33	21.66	10	In the range
X_2: % of Tween 20	72	52.66	33.33	In the Range
Dependent Variables				
Y_1: DR at 45 mins				Minimize
Y_2: DR at 2hrs				Within the range

Preparation of Solid SMEDDS

The easiest method for changing L-SMEDD formulation into S-SMEDD formulation is the adsorption of the SMEDDS formulations on the surface of the inert solid carriers. After preparing the dosage equivalent of L-SMEDD, the formulation was transferred to a China dish where Aerosil was added gradually while being stirred vigorously. At long last, a free-flowing powder dosage equivalent was created [22,23].

CHARACTERIZATION OF SMEDDS

Fourier –Transform Infrared Spectroscopy (FTIR)

Pure medication, Aerosil, and the optimized formulation were all blended with IR grade KBR in a ratio of 1:100 before being compressed to a disc at a pressure of 15000 lb using a hydraulic press [24]. The discs were scanned using a Hitachi 295 spectrophotometer in a vacuum at a wavelength range of 4000-400cm^{-1}.

X-ray diffraction (XRD)

The geometry of an X-ray diffractometer has the sample rotating at an angle relative to the collimated X-ray beam, and the X-ray detector rotating at an angle relative to the sample in order to capture the diffracted X-rays [25]. Typically, powder pattern data is gathered at 2 from 5º to 70 º, angles that are specified in the X-ray scan, using equipment called a goniometer to maintain the angle and rotate the sample.

Scanning Electron Microscope (SEM)

Electrons colliding with sample atoms generate a wide range of signals that may be decoded to provide details about the surface topography and atomic composition of the sample. The post was affixed using a number of improved formulas [26]. After being sputter-coated with gold particles, this specimen was seen in an SEM (JSM-5610, JEOL, and Japan) at an acceleration voltage of 10 kV.

Particle size

When the characteristics of the emulsion do not change following infinite aqueous dilution, as is required by this approach, photon correlation spectroscopy (PCS) is an effective method for determining the droplet size of the emulsion [27].

Zeta potential

The zeta potential of the prepared formulation was determined by Malvern Zetasizer 3000HS. Prepared SMEDDS was diluted with double distilled water and the zeta potential was determined [28].

In Vitro Drug Release Profile

Drug release tests were performed using a USP dissolving apparatus-II at 100 rpm, 37 0.5°C, and with pure Nebivolol (2.5mg), Nebivolol loaded L-SMEDDS, and Nebivolol loaded S-SMEDDS. Separate 900ml containers of 6.8pH phosphate buffer contained pure Nebivolol (2.5mg) and an equal quantity of Nebivolol-loaded L-SMEDDS and S-SMEDDS formulation. Five milliliter aliquots were taken at regular intervals, and the buffer in the sink was refreshed by adding another five milliliters [29]. Absorbance at 252 nm was determined by measuring the samples in a UV double beam spectrophotometer with buffer as the blank. The drug release percentage was derived from the absorbance valves that were collected. And we can see how %CDR changes over time (in minutes).

Permeation studies

Wistar male rats weighing 180–200 g were utilized for the experiment. Until the moment of their sacrifice, the animals were kept in separate cages with temperature and humidity controls, and the rats had unrestricted access to water and food. For this experiment, ether inhalation was used to voluntarily kill the rats [30]. It was decided to cut open the abdomen, remove the intestine, and flush it with Krebs ringer solution to get rid of the mucus and the stuck-together food. Then, using a 7.4pH phosphate buffer, I cleaned the intestinal lining. SMEDDS of an improved formulation was injected into the intestine via one end that had been tied off. Then, 2.5 milligrams of pure Nebivolol were injected into the intestines. At 37°C, two 250-milliliter beakers were used to keep the intestines aerated and moist. Each beaker contains 100 ml of phosphate buffer (pH 7.4). Five-milliliter aliquots were taken at 5-, 10-, 15-, 30-, 45-, 60-, and 120-minute intervals, and the medium was replaced after each interval. Samples are analyzed in a UV double-beam spectrophotometer for absorbance at 252 nm.

RESULTS AND DISCUSSION

Solubility studies

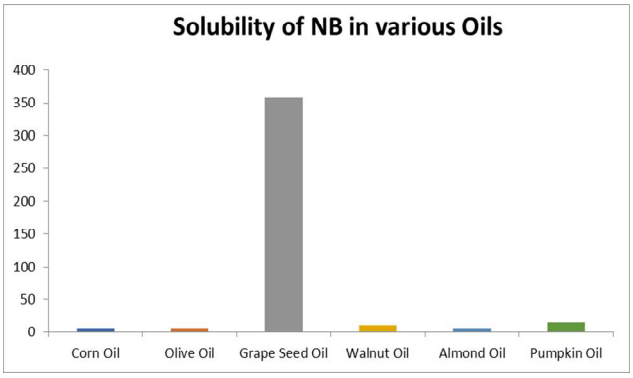

Fig.1: Solubility Profile of NB in oils

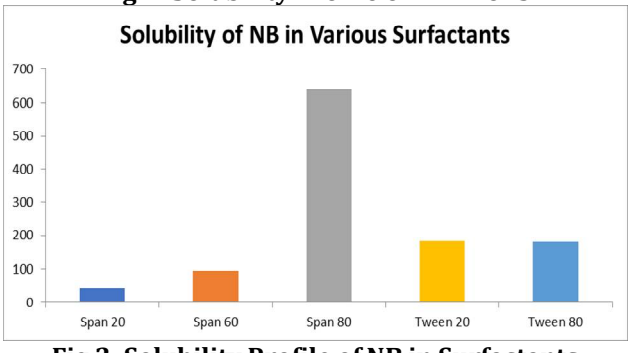

Fig.2: Solubility Profile of NB in Surfactants

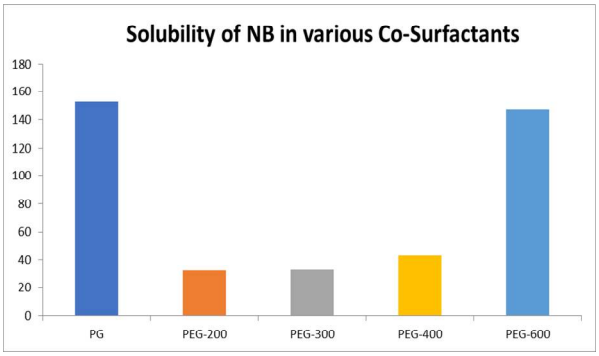

Fig. 3: Solubility Profile of Nebivolol in various Co-Surfactants

Ternary Phase Diagram

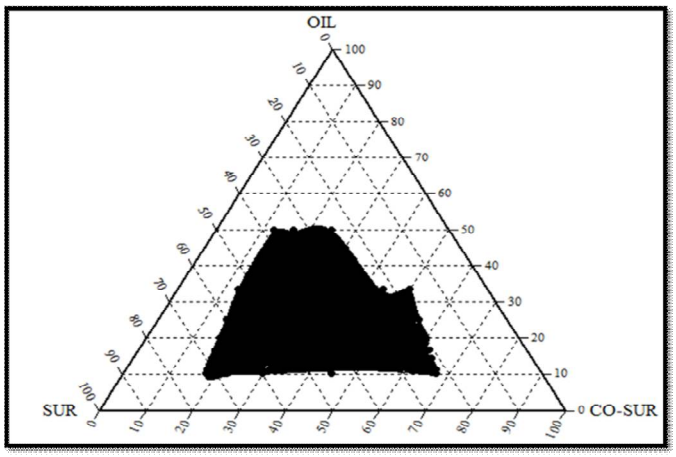

Fig. 4: Grape seed oil, tween 20, and propylene glycol in a SMEDDS ternary phase diagram. (The self-emulsification zone is shown in black.)

Experimental design

The responses of the 9 formulations prepared by 3^2 factorial design batches are shown in Table 3. All the data were computed by design expert software (Version 8.0.7.1). The best-fitted model for Y1 data was a quadratic, and the best-fitted model for Y2 data was a quadratic as well. The fitted regression equations relating the responses like Drug release at 45 min and Drug release at 2hr are shown in the following equations, respectively. The polynomial equations can also be used to draw conclusions considering the magnitude of the co-efficient and the mathematical sign it carries (i.e. either positive or negative). The positive sign indicated a direct effect whereas the negative sign indicated the inverse effect

Graphical presentation of the data helps to show the relationship between the responses and the independent variables. The information obtained from the graphs was similar to that obtained from mathematical equations by statistical analysis. (Figure 5,6).

Effect of independent variable on Y1(Drug release at 45 min)

According to the data, the values for Y1 varied from 67.72 to 78.86%. Dissolution release profile and other reactions fall within these ranges.

The polynomial equation is as follows:

$$Y_1 = 72.04 - 0.4217X_1 + 0.5633X_2 - 2.97X_1X_2 - 2.20X_1^2 + 4.29X_2^2$$

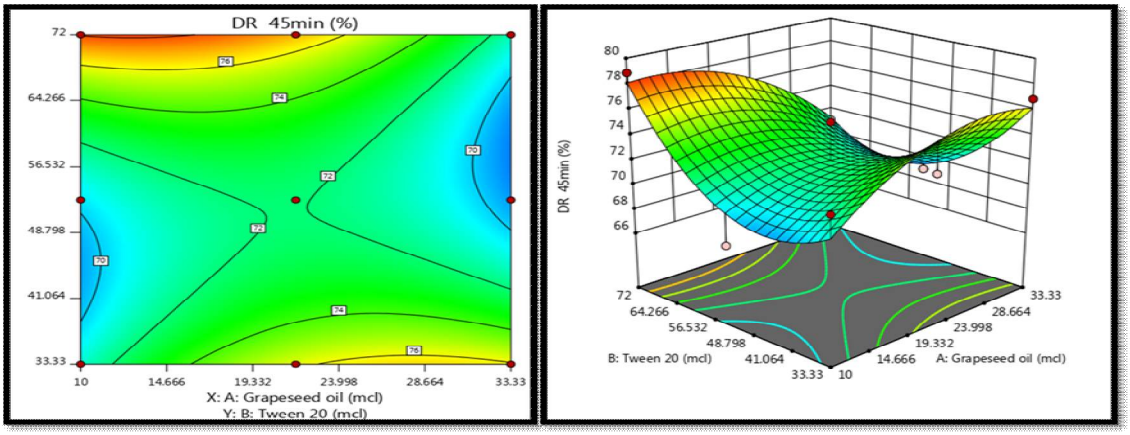

Fig. 5: Contour Plot (A), Response surface Plot (B) showing effect of (X_1) and (X_2) on response Y_1. Effect of independent variable on Y2(Drug release at 2hr)

The polynomial equation is as follows:

$$Y_2 = 97.36 - 0.6900X_1 - 0.2433X_2 - 0.3150X_1X_2 + 0.2533X_1^2 - 0.2307X_2^2$$

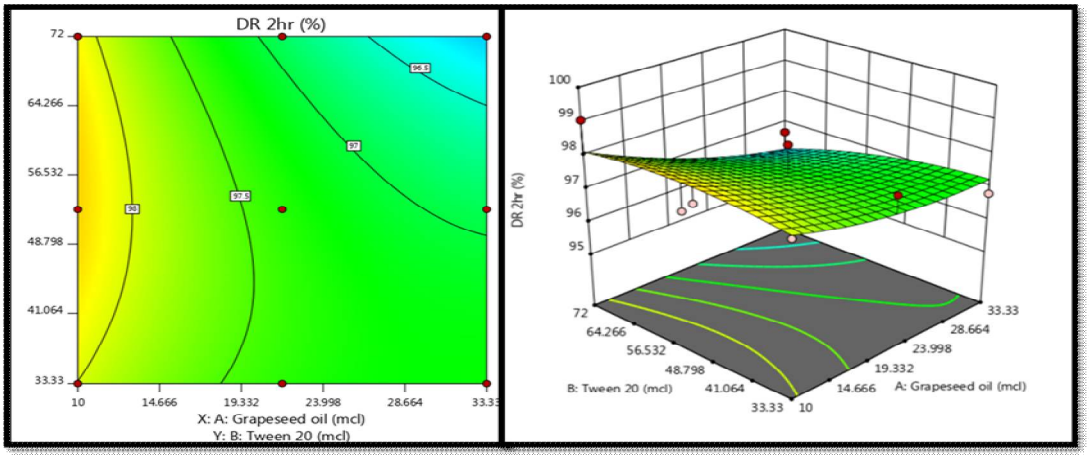

Fig. 6: Contour Plot (A), Response surface Plot (B) showing the effect of (X_1) and (X_2) on response Y_2.

Table 2: Observed responses in 3^2 Experimental design for S-SMEDDS formulations

Formula Code	Independent Variables		Dependent Variables	
	X_1 (µl)	X_2 (µl)	Y_1 (%)	Y_2 (%)
F1	-1	0	68.96	96.8
F2	-1	-1	76.38	95.4
F3	0	-1	75.03	98.3
F4	0	0	78.86	99.05
F5	-1	-1	67.72	97.48
F6	-1	0	76.83	96.8
F7	0	-1	71.04	96.69
F8	-1	0	72.78	97.9
F9	0	-1	73.29	97.9

The X1 coefficient was found to be 0.421, and the X2 coefficient to be 0.5633. For Y1, X1 has a positive impact whereas X2 has a negative effect, and vice versa for Y2. In contrast to X12, the interaction between X1 and X2 has a negative impact on Y1, whereas X12 has a positive effect on X1 and X2, and X22 has a positive effect on Y1. Counter plots and response surface plots, respectively, illustrated in Figures (5 and 6) are useful for analyzing the joint effects of two independent and one dependent variables. It is evident that grape seed oil plays an important role in drug release; this can be used in particular to control drug release in the stomach environment during the development of S-SMEDDS. The table below details the ingredients of the optimal formulation, which was chosen using the criterion of placing constraints on Y1 (minimize) and Y2 within the range. The ranges of variables where the optimal formulation may occur were predicted using an overall desirability function depending on all the evaluated formulation factors.

The anticipated and experimental values were found to be in very close agreement. Therefore, it follows that the universal mathematical equation can accurately predict Y1 and Y2.

Table 3: Point of Prediction and Confirmation

Response	Predicted Mean	Predicted Median	Std Dev
DR 45mins	72.037	72.037	2.960
DR 2hrs	97.357	97.357	1.342

Fourier –Transform Infrared Spectroscopy (FTIR)

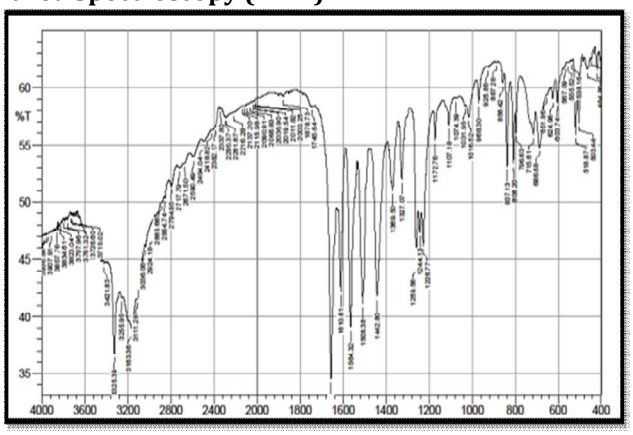

Fig.7: FTIR Spectra of Pure Nebivolol

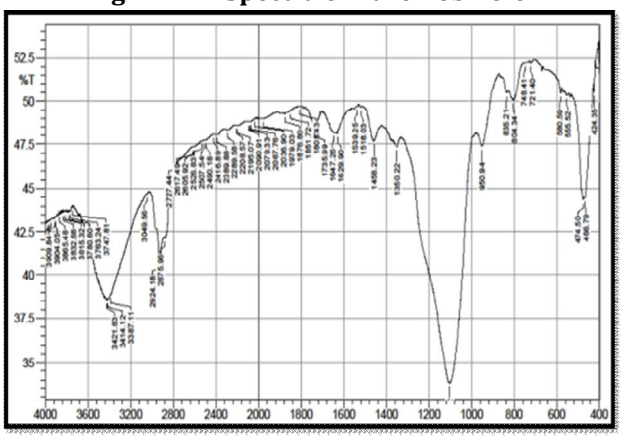

Fig.8: FTIR Spectra of Optimized Formulation

Table 4: FTIR data Interpretations

Functional Group	Bond nature	Characteristic Wave number range	Wave numbers in Pure drug	Wave numbers in optimized formulation
-OH	Stretching	3500-3200	3352	3355
-NH2	Stretching	3400-3250	3012	3151
C = N	Stretching	2260-2210	2243	2131
C = O	Stretching	1760-1665	2905	2875
-C-H	Stretching	3000-2850	2642	2468

This means that the drug's molecular structure was preserved and there was no chemical interaction between the medication and the carrier.

X-ray diffraction (XRD)

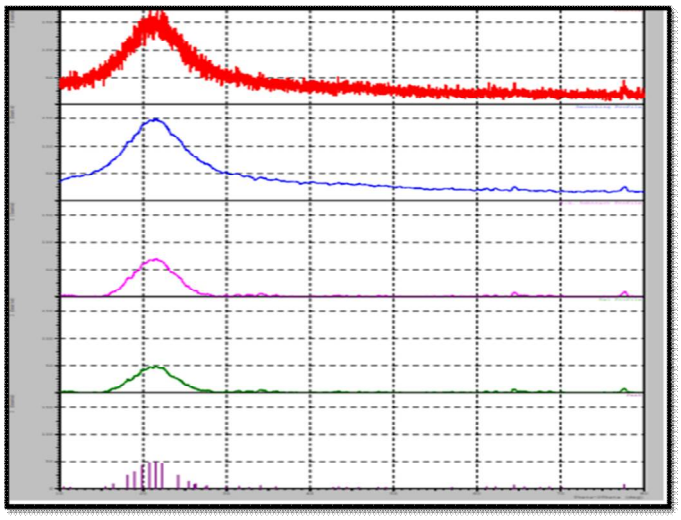

Fig. 9: XRD of Nebivolol (A) Group (B) Raw (C) Smooth

Scanning Electron Microscope (SEM)

Using a scanning electron microscope, the morphology and structure of Nebivolol-loaded SMEDDS were examined. In this case, a spherical form was used, and the formulation had a size distribution between 10 and 100 nm (Fig. 10).

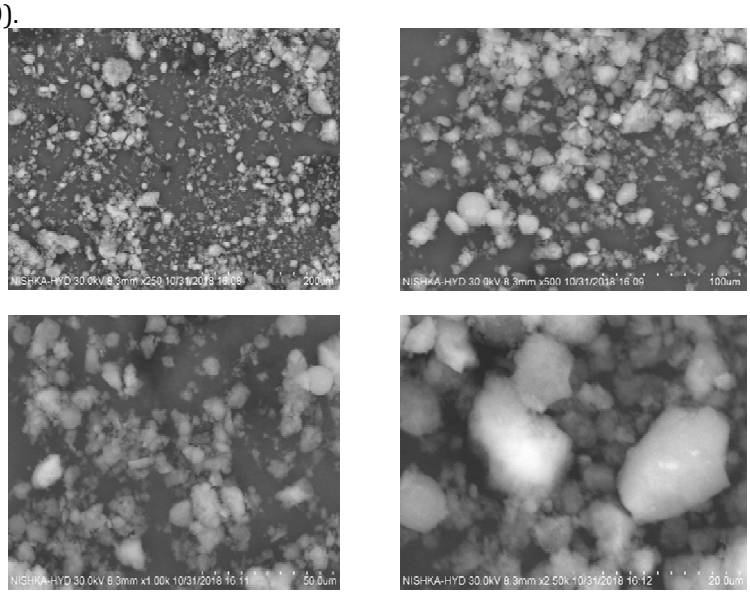

Fig.10: SEM of Optimized formulation of Nebivolol

S-SMEDDS Particle size of optimized formulation

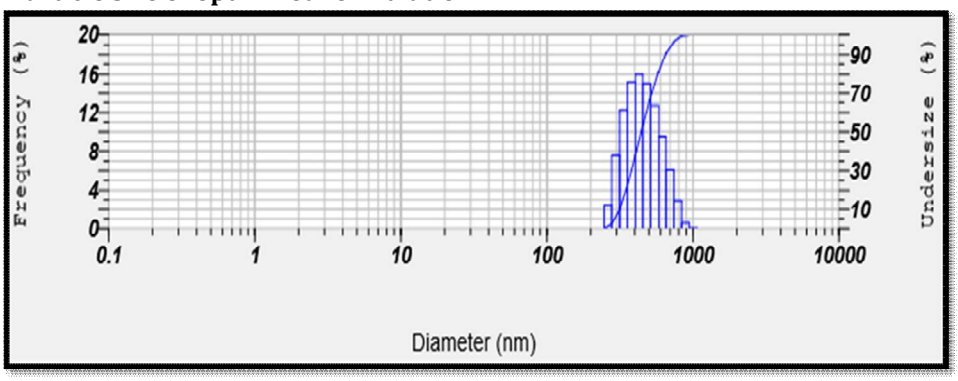

Fig.10: Particle Size distribution of Optimized formulation

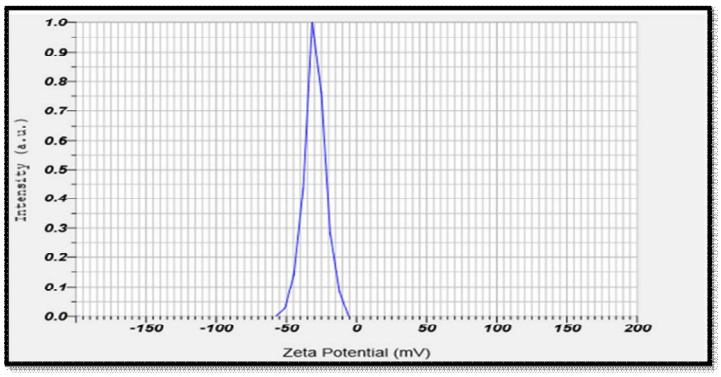

Fig.11: Zeta potential of Optimized formulation

Zeta potential of the optimized SMEDDS was found to be -30 mV, indicating the stable micro emulsion.

Dissolution release profile Data

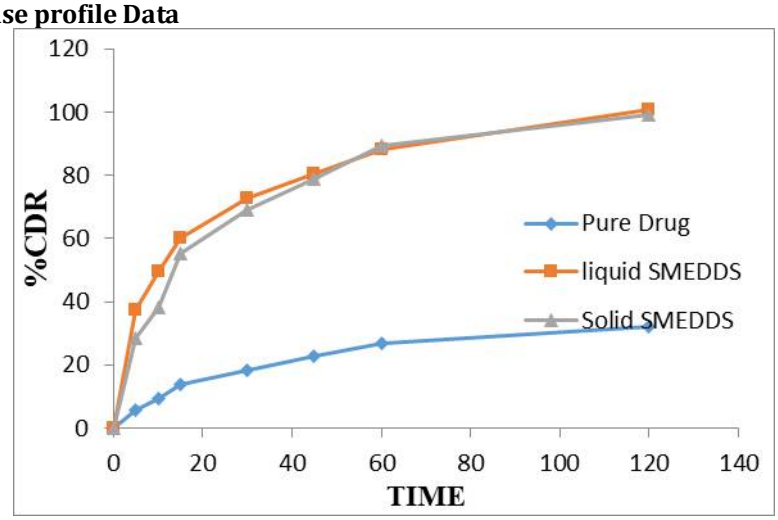

Fig.12: Plot between time Vs %CDR for dissolution

In vitro drug release tests for the F4 formation indicated a 78.86% and 99.05% drug release at 45 and 120 minutes, respectively. Studies in an ex-vivo setting indicated that the F4 formulation allowed 71.3% of the medicine to penetrate after 120 minutes, whereas the pure drug only allowed 30.75 % to do so. This clearly demonstrated the superior dissolution behaviour of the developed L-SMEDDS as compared to Plain drug and S-SMEDDS.

Permeation studies of pure drug and Optimized formulation

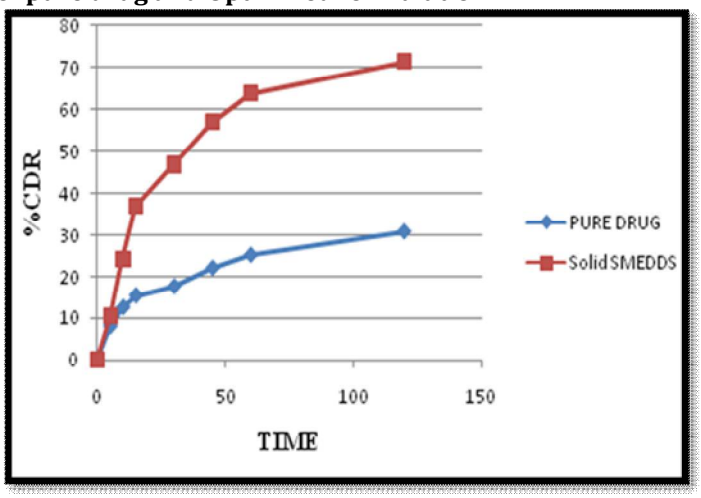

Fig.13: Plot between time Vs %CDR for permeation

Studies in an ex-vivo setting indicated that the F4 formulation allowed 71.3% of the medicine to penetrate after 120 minutes, whereas the pure drug only allowed 30.75 % to do so. The optimized formulation showed maximum drug release than pure drug. Thus, the amount of the drug diffused through the biological membrane was more when it was given in the form of SMEDDS formulations.

CONCLUSION

The objective of the present study was to enhance the solubility, dissolution and hence anti-Hypertensive activity of poorly soluble drug Nevibolol by formulating into self-emulsifying systems. Self-emulsifying formulations were prepared using Tween 20, PG, and Grape Seed Oil. Fourier transform infrared spectroscopy and differential scanning calorimetry studies were conducted to know the interaction between drug and excipients. Pseudo ternary phase diagrams were constructed using surfactant and cosurfactant in 1:1 to 1:4 and 2:1 to 4:1 to know the efficient self-emulsification region. The formulations were evaluated for their particle size, zeta potential, FTIR, XRD, in-vitro drug release and permeation studies.

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