RESEARCH ARTICLE

Oral Liquid Self-nanoemulsion of Nebivolol: Formulation and *In-Vitro* Characterization for Dissolution Rate Enhancement

Lubna A. Sabri,* Ahmed A. Hussein

[1]*Department of Pharmaceutics, College of Pharmacy, University of Baghdad, Baghdad, Iraq.*

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ABSTRACT

Nebivolol is a unique selective beta-blocker used for the treatment of several chronic cardiovascular diseases. It has poor solubility with low bioavailability (12%). Accordingly, this study improved nebivolol dissolution performance, and hence its oral bioavailability by preparation as self-nanoemulsion (SNE).

Method: Saturation solubility in various semisynthetic oils and emulsification efficiency were performed to select proper SNE vehicle combination. Twelve formulas were prepared by varying the proportion of selected mixture. Moreover, proper and adequate *in-vitro* characterization tests were conducted to select the best formula.

Results: The obtained results showed that imwitor 988, cremophor-EL, and propylene glycol (PG) mixture was provided satisfactory nanoemulsion region upon titration with water to be further loaded with lipophilic drugs. The prepared formulas with good stability under stressful conditions revealed a dramatically higher drug releasing rate than a plain drug suspension. The optimum nebivolol SNE could be attained at 10% imwitor:45% cremophor:45% PG w/w combination. This formula (A1) could rapidly form nanoemulsion under gentle agitation with 20.3nm ± 0.3 droplets size and polydispersibility index 0.196 ± 0.01, as confirmed by TEM. It also revealed three times enhancement in drug-releasing rate compared to pure nebivolol.

Conclusion: Thus, the developed formula can be utilized as a nanocarrier for oral delivery of nebivolol with good solubilization capacity, higher dissolution rate, and simple manufacturing requirements.

Keywords: Emulsification ability, Imwitor 988, Nebivolol, Self-nanoemulsion, Zeta potential. International Journal of Drug Delivery Technology (2021); DOI: 10.25258/ijddt.11.3.75

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INTRODUCTION

The oral route for drug delivery is still the most preferred, owing to easily self-administrated, lower cost, and more flexible in designing the dosage forms than the other route.[1] However, approximately 40% of the commercially available drugs for oral immediate-release are lipophilic. It means that the amount of drug reached the blood circulation is below the therapeutic level, resulting in a lack of pharmacological action. So, poor aqueous solubility remains one of the significant challenges to successful drug development for enhancement its bioavailability.[2]

Lipid-based drug delivery systems have historically attracted different researcher groups because of their high incorporation efficiency for lipophilic drugs, solving solubility problem.[3] Consequently, some colloidal lipid carriers have developed. They are well tolerated, biocompatible, bio-degradable with low cytotoxicity and physicochemical diversity.[4]

Recently, the self-emulsifying drug delivery system is one of the attractive lipid-based carriers for oral formulations due to their privilege merits that result in bioavailability enhancement.[5]

The self-nanoemulsifying drug delivery system (SNEDDS) refers to the anhydrous or preconcentrate emulsion. This system is typically composed of candidate drugs dissolved in a homogenous mixture of oil, surfactant, and co-surfactant, which can rapidly and spontaneously form oil-in-water nanoemulsion upon exposure to an aqueous media under mild agitation.[6] This system relies on the peristaltic movements and physiological fluids of the gastrointestinal tract (GIT) for *in-vivo* formation of a fine emulsion.[7] The resultant submicron droplets will maintain the drug in dissolved form and provide a large interfacial surface area for drug contact with the gut walls, and hence, improve absorption and produce a uniform, and reproducible blood concentration-time profile, particularly for drugs belong to Biopharmaceutical Drug Classification System (BCS) II and IV.[5] Additionally, most of the components utilized in SNEDDS as drug vehicles are bio-enhancer and promoter for intestinal lymphatic absorption.[8,9]

Nebivolol hydrochloride (NBH) is a third-generation cardioselective beta-blocker with vasodilatory action.[10] It is a unique antihypertensive and a prophylactic agent in coronary

**Author for Correspondence: lubnasabri14@gmail.com*

artery disease and heart failure. Moreover, it has strong antioxidant properties and anti-inflammatory.[11] NBH can be categorized according to BSC as a Class II drug. [12] Furthermore, the oral bioavailability of NBH is about 12% due to poor solubility and extensive hepatic metabolism and hence, limited its clinical efficacy.[13] Therefore, it could be a promising candidate for SNEDDS formulation exploiting the attractive properties of the components of such system.

AIM OF THE WORK

The present study aims to prepare liquid self-nanoemulsion (SNE) as a nanocarrier for nebivolol to enhance the dissolution rate by keeping the drug in a solubilized form within colloidal dispersion after oral administration. Furthermore, selecting an efficient self-nanoemulsifying vehicle of NBH with appropriate physical properties and higher dissolution rate will do through *in-vitro* evaluations and optimization study.

MATERIALS AND METHOD

Materials

The following materials were purchased from: NBH (Baoji Guokang Bio-Technology Co., China), imwitor®742, imwitor®948, imwitor®988, and miglyol®812N (IOI Oleochemical, GmbH, Germany), cremophor®EL, labrasol, propyleneglycol monolaurate and transcutol HP (International Laboratory, USA), tween 80 (CDH, India), triacetin (Hyperchem, Chain), propylene glycol, PEG200 and 400 (Evans Medical Ltd, Liverpool, England).

Method

Determination of Nebivolol Hydrochloride Saturation Solubility

The saturated solubility of NBH in various semi-synthetic oils, surfactants, and co-surfactants was measured by using shaking flask method.[14] Briefly, an excess amount of NBH was added to 5 ml of each excipient taken in a screw-capped test tube and mixed by vortex mixer (Labinco L46, Netherland). Formed suspension was then incubated at 25 ± 1°C for three days in an isothermal water bath shaker (GFL, Karl Kolb, Germany) to achieve equilibrium. After that time, the insoluble drug was removed by centrifuging at 3500 rpm for 20 minutes. The separated supernatant fraction was filtrated with a 0.45 µm Millipore syringe filter and adequately diluted with methanol. The drug concentration was measured spectrophotometrically (EMC-LAB, UV1100 model, Germany) at nebivolol λ_{max} using a pre-constructed calibration equation.

Emulsification Study for Surfactant and Co-surfactant Selection

The emulsification ability of different surfactants (labrasol, tween 80, and cremophor-EL) was investigated according to the method reported in the literature.[15] The selected oils (imwitor 948 and imwitor 988) were mixed with each surfactant separately in 1:1 weight ratio, vortexed to ensure homogenization of components. Then, the emulsification ability upon 100 fold dilution was determined by the several flask inversion for homogeneous dispersion. Besides that, the obtained dispersions were left undisturbed for two hours to measure its % transmittance at 650 nm by a spectrophotometer. Distilled water was used as blank.

Propylene glycol (PG) emulsifying property was also investigated by mixing with the selected surfactant and oily phase in (2:2:1) weight ratio. Then, the above mention procedure was repeated.

Pseudo-ternary Phase Diagrams Study

The solubility and emulsification data were considered, so a combination of imwitor 988 as oil phase, cremophor-EL and PG was selected after confirming their compatibility and miscibility with no phase separation was observed.

The water titration method was used to construct phase diagrams. Surfactant and co-surfactant expressed as S_{mix} were mixed with varying weight ratios (3:1, 2:1and 1:1 w/w). For each mixing ratio, nine different oil: Smix ratio mixtures were titrated by slowly adding dropwise distilled water to the transparent mixture under continuous gentle stirring. The point at which the system became turbid or viscous gel-like, titration was stopped. Then the %weight of oil, S_{mix}, and water in 100% w/w mixture was calculated to delineate the phase boundaries in the diagrams. Chemix software was used to plot phase diagrams.[16]

Preparation of Nebivolol Liquid Self-nanoemulsion

Four series of SNEDDS formulations selected from each S_{mix} triangle phase diagram were prepared with oil concentration of 10% to 40% increase by increment 10%, and the concentration of the surfactant/co-surfactant (S_{mix}) was calculated according to their ratios as shown in Table 1.

Imwitor 988, cremophor-EL and PG were premixed and warmed at 40°C on the water bath for 2 min to homogenized the blend. Subsequently, accurately weight NBH was added to the prepared SNEDDS mixture. The amount of nebivolol was

Table 1: Developed formulations of L-SEDDS using different weight percentages of oil, surfactants, co-surfactants, and constant amount of nebivolol.

Formula code	*S_{mix} ratio*	*Imwitor 988 %w/w*	*CremophorEL %w/w*	*PG % w/w*
A1	1:1	10	45	45
A2	1:1	20	40	40
A3	1:1	30	35	35
A4	1:1	40	30	30
B1	2:1	10	60	30
B2	2:1	20	53.3	26.7
B3	2:1	30	46.7	23.3
B4	2:1	40	40	20
D1	3:1	10	67.5	22.5
D2	3:1	20	60	20
D3	3:1	30	52.5	17.5
D4	3:1	40	45	15

kept constant (1% w/w) in all formulations. After that, NBH loaded the mixture was vortexed until complete dissolving of NBH and got a clear solution.

Physical Characterization of the Prepared NBH Liquid Self-nanoemulsion

Measurement of the Effective Droplet Size and Polydispersity Index (PDI)

The median droplet diameter and the distribution size width (PDI) of nanoemulsion formed upon 100 fold dilutions with 0.1N HCl were measured using dynamic light scattering technique (Brookhaven ZetaPlus apparatus, Holtsville, NY USA).

Self-emulsification Time and Dispersibility Test

Liquid-SNEDDS (500mg) was added to 0.1N HCl (100ml) with mild stirring by the magnetic bar at 37 °C, then visually observed. The time for complete dispersion of the pre-concentrate formulations and nanoemulsion formation was determined as an emulsification time.[17] For assessment of the *in-vitro* performance of the prepared SNEDDS, the grade of the obtained nanoemulsion was determined based on grade reported by literature.[18]

Physical Stability Studies

To assess the physical stability of the prepared liquid-SNEDDS formulas, they were subjected to accelerated stress condition (centrifugation) and different temperatures (thermodynamic study) to exclude unstable and biphasic formulations. The Centrifugation, Heating/cooling cycle and Freezing/thawing cycles tests were done according to the procedure described by Mundada and Sawant.[19]

Saturation Solubility Measurement of Liquid Nebivolol-loaded Self-nanoemulsion Formulations

The saturation solubility of NBH in nine successful formulations was performed according to the procedure previously mentioned to study NBH solubility in different vehicles.

Zeta Potential Determination

The electrokinetics potential of the formulas that passed the physical stability study was measured and converted to zeta potential.[20] The diluted dispersion of SNE was prepared in a similar way to size measurement.

Drug-releasing Study by Employing Dialysis Bag

The *in-vitro* NBH released test was performed using USP dissolution apparatus II (PharmaTest, Germany) at 37°C, and a rotating speed of 100 rpm, 500 mL of 0.1N HCl as dissolution media. The dialysis bag method was used to separate soluble drug in a molecular state from this entrapped in o/w nanoemulsion droplets or micelles formed upon SNEDDS dispersion in aqueous media. Before starting the experiment, dialysis membranes (molecular weight cutoff 8,000-14,000 Dalton) were soaked in freshly prepared 0.1N HCl for 12 hours at room temperature. The L-SNEDDS formulations equivalent to 5 mg of nebivolol were diluted ten times with release media, and pure NBH powder suspended in the same amount filled the dialysis bag. After that, the filled bag was fixed at the rotating paddle and immersed in releasing medium.[19,21]

Five milliliters sample was withdrawn from the dissolution jar and replaced with an equivalent volume of fresh dissolution medium at each predetermined time. The collected samples were analyzed for nebivolol concentration.

Selection of Optimum Liquid Nebivolol-loaded SNE Formula

The best formula of liquid NBH- loaded SNE formulas was chosen regarding the results collected from crucial characterization tests, include: droplet size distribution, emulsification time, saturation solubility, and *in-vitro* drug-releasing study.

Robustness of the Optimum Formula to Dilution and Phase Separation Studies

The prepared nanoemulsion pre-concentrates of he optimized formula (A1) were diluted to 50, 100, and 1000 fold with 0.1N HCl at 37°C to determine behaviour with varying fluid volume of the stomach. The obtained nanoemulsion was also monitored after 12 and 24 hours for checking its physical stability.

Viscosity Measurement for the Optimized Formula

The viscosity of the optimized formula was measured by NDJ-55 digital viscometer using spindle number 2 at 30 rpm speed at 25°C temperature.

Morphological Visualization by Transmission Electron Microscope (TEM)

The droplets shape of the SNE formula (A1) was investigated by visualization using TEM (Zeiss Libra 120, Germany). Before analysis, the formula was dispersed in distilled water (1:10 v/v) and negatively stained. After proper sample preparation procedure, the photographs were taken at suitable magnification power.[22]

Statistical Analysis

The data obtained were analyzed by one way-ANOVA to investigate significance using Microsoft excel 2010, at a significance level of 5%. Statistically, *p*-value equal to or less than 0.05 represented a significant difference.

RESULTS AND DISCUSSION

Determination of Nebivolol Hydrochloride Saturation Solubility

The solubility assessment of NBH in various selected SNE excipients was carried out to identify suitable vehicles with an excellent solubilizing capacity for NBH. The importance of maximal solubility of the drug is optimizing drug loading capacity, avoiding drug precipitation on dilution, and reducing the final volume of SNEDDS, making it suitable for oral administration.[23] The saturation solubility data in different vehicles were shown in Table 2.

It was obvious from (Table 2) that the results of NBH solubility in selected oils demonstrated no clear solubility rule that could correlate with oil type. That may be attributed

Table 2: The Saturation solubility of NBH in some semisynthetic oils, surfactants and co-surfactants

Oils	*Solubility (mg/mL) ± SD*	*Surfactants*	*Solubility (mg/mL)\ ± SD*	*Co-surfactants*	*Solubility (mg/mL) ± SD*
Triacetin	0.478 ± 0.08	Tween 20	5.78 ± 0.16	Propyleneglycol	27.78 ± 0.32
Miglyol812	0.41 ± 0.02	Tween 60	13.4 ± 0.11	PEG 200	13.81 ± 0.28
Imwitor742	5.88 ± 0.15	Tween 80	19.6 ± 0.05	PEG 400	8.6 ± 0.67
Imwitor988	12.52 ± 0.20	Cremaphor-EL	17.1 ± 0.13	Transcutol HP	11.9 ± 1.3
Imwitor948	11.75 ± 0.48	Labrasol	23.9 ± 0.04	Labrafil CS 1944	3.16 ± 0.2
PG-monolaurate	2.73 ± 0.04				

to the difference in chemical nature, molar volume, and HLB of the oils used. Drugs exhibited similar results with the log partition coefficient (3-4).[24]

The highest NBH solubility was shown in imwitor 988, followed by imwitor 948, with no significant difference (p>0.05) in the solubility between them. Therefore, both were chosen for further investigation of their miscibility with different high HLB surfactants to select the appropriate oil for the formulation of liquid-SNE.

It is worth mentioning that part of the crucial criteria that governed the choice of a suitable surfactant is its emulsification efficiency and affinity to oil rather than drug solubilizing capacity only.[25] Therefore, the tween 80, cremophor, and labrasol, which showed a satisfactory NBH solubility, were selected for further study.

Emulsification Study for Surfactant and Co-surfactant Selection

The efficiency of three selected surfactants with HLB value higher than 13 to emulsify the selected oils was depicted (Figure 1). It was found that imwitor 988 was easier emulsified than imwitor 948 as indicated by least number of flask inversion, and higher % transmittance values, because of its smaller molecular volume. It was reported as the length of hydrophobic alkyl chains increase, molecular volume increases.[26] Imwitor 948 mainly consists of mono- and di-ester of C18 oleic fatty acid with HLB value 3. While, Imwitor 988 comprises saturated glyceryl caprylate (C8)/caprate (C10) blending of mono-, di- and a trace quantity of triglycerides with HLB value equal to 6.[27]

Moreover, it can be seen that cremophor-EL and tween-80 exhibited better emulsification ability with imwitor 988 oil with

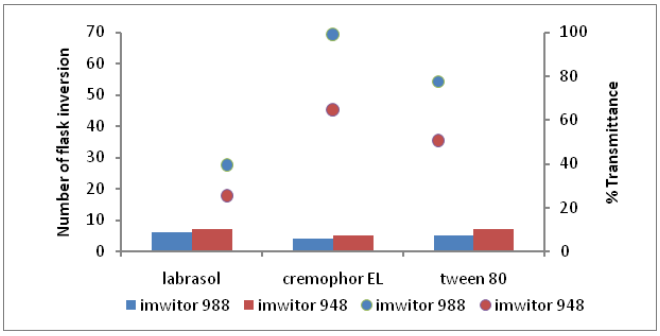


Figure 1: Various surfactants' efficiency to emulsify two selected oils represented by several flask inversion (bar) and % transmittance (circle).

% transmittance (98.8% ± 0.21 and 77.4% ± 0.14), respectively. Meanwhile, labrasol showed a transmittance value of 39.4 % ± 0.35. Despite higher labrasol solubilization ability, its poor emulsification efficiency was also reported by Tran *et al.* [28] when compared with chremophor-RH40 and tween 80 to emulsify capmul MCM and capryol 90 in the preparation of quercetin-containing SNEDDS.

Although the HLB values of surfactants used in this study are close, there was an appreciable difference in their emulsifying ability. Apart from HLB value, the observed results might be due to the difference in their structure. In other words, the hydrophobic tail lengths, number of the tail, and hydrophilic head size and shape impact nano-emulsification.[29] Such findings agreed with Basheer *et al.* [30] who found that the formation of micro/ nanoemulsion directly correlated with the number of carbon atoms, which present the fatty acid side chain of surfactant.

Furthermore, the addition of PG to imwitor 988 and cremophor mixture increased the % transmittance compared to surfactant alone (99.8 ± 0.06 %). Such results could be ascribed according to co-surfactants' role, particularly those with short-chain length, which assist the oil penetration into the hydrophobic part of the surfactant structure, leading to further lowering of the interfacial tension. The different curvatures required to form nanoemulsions can be taken by increasing the fluidity of the hydrocarbon region of the interfacial film by intercalation themselves between surfactant monomers.[31]

Pseudo-ternary Phase Diagrams Study

The phase diagrams of imwitor 988: cremophor-EL: PG mixture titrated with distilled water was illustrated in (Figure 2). A wider monophasic nanoemulsion region indicates a better self-nanoemulsifying activity and perfect intermolecular attraction among the oil phase, S_{mix} and water.[17]

Nanoemulsion spontaneously formed with gentle stirring during aqueous titration may attribute to adsorption and deposition of surfactant/co-surfactant on the interface of the oil globules and providing an elastic mechanical barrier that is stable and prevents aggregation, as explained by Nasr *et al.* formulated irbesartan SNE based on pseudo-ternary results.[32] From phase diagrams, it was noticed that little improvement in nanoemulsion shaded area achieved with increasing the S_{mix} to 2:1 and 3:1 ratio compared to 1:1 ratio. Thus, based on the nanoemulsion region of each phase diagram, different formulations were possible to prepare.

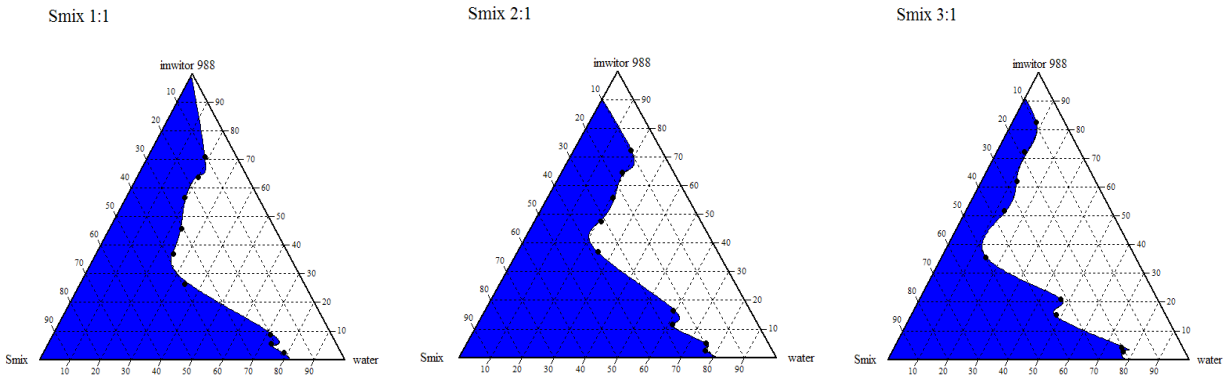


Figure 2: Pseudo-ternary phase diagrams of imwitor 988: S_{mix}: water at different S_{mix} (cremophor-EL: PG) ratios showing nanoemulsion (colored portion).

Preparation of Nebivolol Liquid Self-nanoemulsion

The twelve preconcentrate SNE formulas with a total weight of 500mg demonstrated a clear, homogeneous yellowish appearance with no separation or drug precipitation during storage before evaluation.

Physical Characterization of the Prepared NBH Liquid Self-nanoemulsion

Measurement of the Effective Droplet Size and Polydispersity Index (PDI)

The droplet size determination for SNE formulas is an imperative parameter to assess their performance aiming formulation optimization. In other words, the droplet size has strongly affected the rate of drug-releasing from formulation, absorption, and the stability of the nanoemulsion.[17]

It manifested from (Table 3) that all evaluated formulations showed a median droplet size ranging between (16.6–139 nm), indicating fulfilling the nanoemulsion criteria with a size of less than 200 nm. Moreover, the PDI values ranged between (0.02–0.22), which was less than 0.5. Such results indicated the uniformity of droplet size distribution of the formed dispersion and affirmed their homogeneity.[20]

The influence of SNE components proportions (oil %w/w and S_{mix}) on droplet size was studied. In general, at a fixed S_{mix} ratio, it was found that increasing oil% in the mixture resulted in a slight increase in the mean droplet size up to 40% w/w oil, which showed a significant increase ($p \leq 0.05$). The reason might attribute to decreased availability of surfactant/ co-surfactant for localization around the oil/water interface of a droplet in order to minimize interfacial tension and stabilize the system.[33]

Meanwhile, at a constant oil%, the larger droplets were obtained by decreasing the surfactant/ co-surfactant ratio, particularly at 40% oil, which might be due to the interfacial film expansion by the co-surfactant as explained by Sallam and Marin.[34] However, at less than 40% oil, a non-significant difference in size ($p < 0.05$) was observed among formulations.

The emulsification efficiency could be estimated by visual observation of time required for completely converting pre-concentrate formulations into fine dispersion under mild agitation to simulate dilution upon oral ingestion.

As observed in (Table 3), the time taken by all prepared formulas for emulsification ranged from 24 to 50 seconds. Furthermore, in less than one minute, all formulations produced nanoemulsion with clear (grade A) or tint blue (grade B) appearance. It was clear from obtained results that at fixed S_{mix}, increasing oil content increased the time required for emulsification, which was probably due to hindrance surface towards shearing. Conversely, increasing cremophor resulted lower in emulsification time and producing grade A nanoemulsion with small globule size because of the ability of the surfactant to reduce the interfacial tension between aqueous and oil phases facilitate oil dispersion in the aqueous phase by disruption of the interface between them. However, too high surfactant delay emulsification.[21]

Physical Stability Studies

After exposure to centrifugation and sudden temperature changes, most SNE formulations exhibited good stability and persisted against extreme conditions except A4, B4, and D4. They showed drug precipitation during the cooling-heating cycle, suggesting a particular concentration of SNE components was needed for the formation of thermodynamically stable SNE.[35]

Saturation Solubility Measurement of Liquid Nebivolol-loaded Self-nanoemulsion Formulations

The data of the solubilizing capacity of nine SNE formulations investigated for NBH were presented in (Table 3). The solubility ranged from 12.6 ± 0.58 to 18.1 ± 1.33 mg/mL, which means all formulations could solubilize NBH therapeutic dose. It was clear that the solubility of NBH in SNE formulations significantlyly improved ($p \geq 0.05$) with increased PG and cremophor contents, which was also confirmed by visual observation during preparation.

It was worth mentioning that in all prepared self-nanoemulsifying formulations, a fixed nebivolol concentration of 1% w/w (5 mg/500mg) was selected to be loaded which was lower than 80% of its saturated solubility. It found that they could emulsify readily with a low tendency of drug precipitation on aqueous dilution.

Table 3: Data of *in-vitro* characterization of twelve nebivolol-loaded SNEDDS formulations.

Formula code	*PS* ± SD in 0.1N HCl*	*PDI** ± SD in 0.1N HCl*	*Emulsification time ± SD (Sec)*	*Grade of dispersion*	*Saturation solubility (mg/mL)*
A 1	20.3 ± 0.3	0.196 ± 0.01	24 ± 1.02	A	18.1 ± 1.33
A 2	18.3 ± 0.2	0.14 ± 0.01	33 ± 2.58	A	15.8 ± 1.01
A 3	24.2 ± 0.2	0.08 ± 0.01	41 ± 1.3	A	15.2 ± 1.20
A 4	139.1 ± 0.9	0.16 ± 0.02	50 ± 2.06	B	—
B 1	16.6 ± 0.1	0.14 ± 0.01	28 ± 1.56	A	15.9 ± 1.42
B 2	18.5 ± 0.1	0.13 ± 0.01	31 ± 2.1	A	14.9 ± 1.39
B 3	18.8 ± 0.1	0.07 ± 0.02	37 ± 1.41	B	13.8 ± 1.3
B 4	120.1 ± 1.8	0.21 ± 0.01	44 ± 0.71	B	—
D 1	16.6 ± 0.2	0.09 ± 0.015	24 ± 3.27	A	14.0 ± 0.71
D 2	23.1 ± 0.2	0.05 ± 0.03	30 ± 0.22	A	13.3 ± 0.7
D 3	27.1 ± 0.1	0.02 ± 0.004	33 ± 0.17	B	12.6 ± 0.58
D 4	63.9 ± 0.8	0.22 ± 0.026	36 ± 1.07	B	—

PS*: Median droplets Size, PDI**: Polydispersity Index.

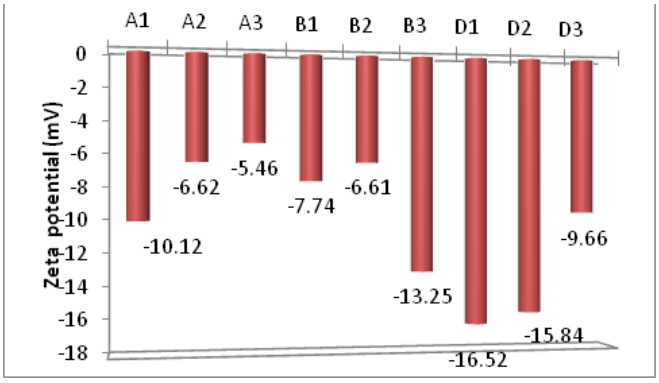


Figure 3: Zeta potential values for nebivolol self-nanoemulsion formulas.

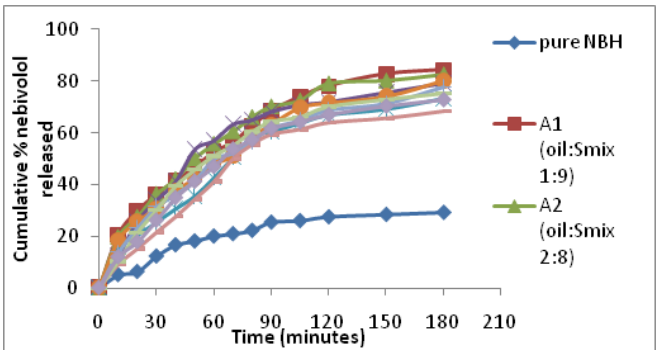


Figure 4: A comparative drug-releasing profile of nebivolol SNE formulations series with different S_{mix} (A-1:1), (B-2:1), (D-3:1) and plain nebivolol suspension.

Zeta Potential Determination

The values of the zeta potential of NBH-loaded SNE are illustrated in (Figure 3). The values were ranged from (-5.46 mV) to (-16.52 mV), which were lower than reported values for stability (beyond + 30 or -30 mV),[15] suggesting the absolute electrostatic repulsion was less important in maintaining the stability of this colloidal dispersion.

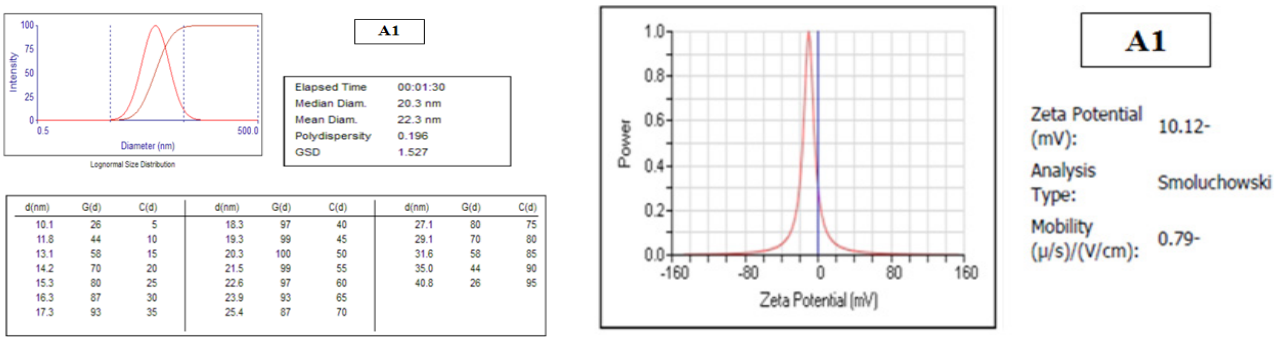
However, the dispersion would stabilize by forming a coat of surfactant around droplets surface, reducing interaction between them by steric repulsion.[22] A highly branched chemical structure of cremophor used as a surfactant imparted a steric hindrance and stability of the formulations. The obtained small negative value of zeta potential was originated from the ionization of free fatty acid present in the structure of SNE components (oil and surfactant).[26]

Drug-releasing Study In this context, it was stated that the drug first needs to dissolve appropriately in GIT fluid to be absorbed. Therefore, drug dissolution is an important step in determining the rate and extent of bioavailability, especially for a poor-water soluble drug. [36] Thus, the *in-vitro* drug-releasing were studied to compare the release of NBH from nine successful formulations and pure NBH powder.

Regardless of the difference in the percent of the components between the prepared formulations, all formulations showed a significantly ($p \leq 0.05$) higher releasing rate than plain drug as illustrated in (Figures 4). It could be explained that nebivolol SNEDDS had spontaneously formed a nanoemulsion with ultra-fine droplets size. Furthermore, a greater surface curvature permitted more solubilized NBH at the droplet interface, leading to a faster NBH releasing rate into dissolution media [20]. On the other hand, the plain NBH suspension revealed only 30% released at the end of 180 minutes due to its poor solubility.

Selection of Optimum Liquid Nebivolol-Loaded SNE Formula

After collecting the results from the evaluation tests of NBH SNE formulations, formula (A1) was selected as an optimum one. The formula (A1) showed good nanosize (20.3 ± 0.3 nm) with uniform droplet size distribution after diluted with 0.1N HCl indicated by low PDI value (0.196 ± 0.01) and adequate zeta potential as demonstrated in (Figure 5). Besides that, it had the lowest emulsification time (24 sec) with acceptable surfactant content. Moreover, high NBH loading was achieved in formula A1 (18.1 ± 1.33 mg/mL), which would ensure clarity and stability



d(nm)	G(d)	C(d)	d(nm)	G(d)	C(d)	d(nm)	G(d)	C(d)
10.1	26	5	18.3	97	40	27.1	80	75
11.8	44	10	19.3	99	45	29.1	70	80
13.1	58	15	20.3	100	50	31.6	58	85
14.2	70	20	21.5	99	55	35.0	44	90
15.3	80	25	22.6	97	60	40.8	26	95
16.3	87	30	23.9	93	65			
17.3	93	35	25.4	87	70			

Figure 5: The reports of droplet size distribution (left side chart) and zeta potential (right side) of the optimum nebivolol-loaded SNE formula A1.

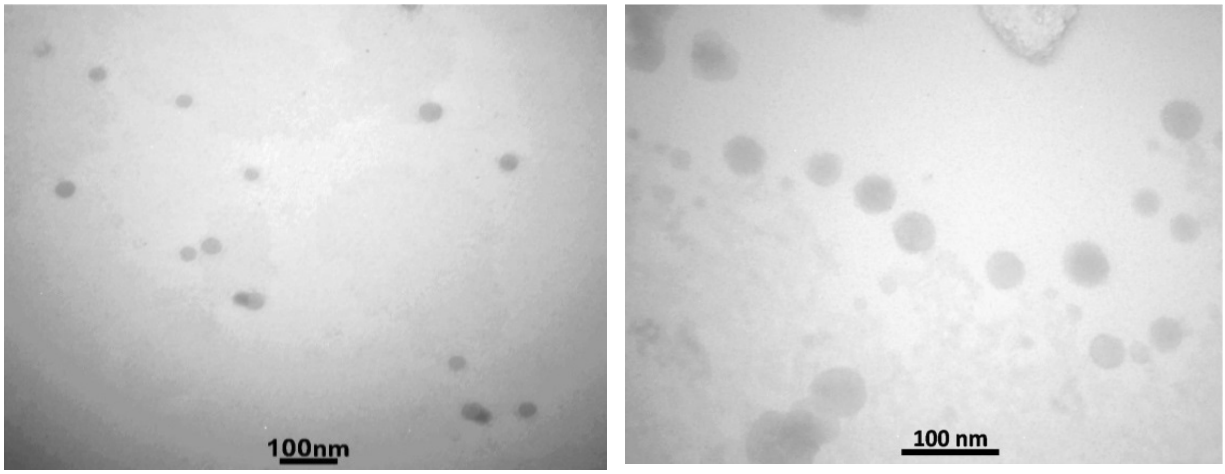


Figure 6: The TEM photograph of the optimized formula (A1) after dilution with distilled water.

upon dilution. Also, it revealed the highest release compared to other SNE formulations. Thus, formula A1 which consisted of 10% imwitor 988, 45% cremophor-EL, and 45% w/w PG, was selected as the optimum formula to achieve the study aim.

Robustness of the Optimum Formula to Dilution and Phase Separation Studies

The reconstituted formula A1exhibited good nanoemulsion stability that was affirmed by the maintenance of its clear appearance. No sign of turbidity or phase separation was observed in dilution media at various dilution folds after 12 hours of storing them at room temperature.

Viscosity Measurement for the Optimized Formula

The recorded viscosity of the optimized formula (A1) was found 246.95 ± 0.53 cps at 25°C. As a result, it was less than 10000 cps, it means that the prepared SNE could be packed in hard capsule shells by a conventional liquid filling machine.[19]

Morphological Visualization by Transmission Electron Microscope (TEM)

The photomicrograph of the optimum SNE formula A1 was revealed in (Figure 6). After dilution, the resulted dispersion confirmed nanoemulsion formation, which showed ultra-fine smooth surface spherical droplets distributed throughout the sample with no sign of aggregation.

CONCLUSION

The results of this research can conclude that nebivolol-loaded SNE formulations were successfully prepared using imwitor 988, cremophor-EL and PG by just applying simple mixing steps. They showed significant improvement in drug-releasing rate when compared with pure nebivolol powder. The optimum formula composed of 10% imwitor 988, 45% cremophor-EL, and 45% PG revealed sufficient drug loading, rapid emulsification in 0.1N HCl, and forming spherical nano-droplet. The optimized formula can fill in a soft or hard capsule for commercial oral administration.

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