Comparison between Conventional and Supersaturable Self-nanoemulsion Loaded with Nebivolol: Preparation and In-vitro/Ex-vivo Evaluation
Lubna A. Sabri*1 and Ahmed A. Hussein*

Abstract
Nebivolol (NBH) is a third-generation β1-blocker with high selectivity and vasodilator activity. Nevertheless, nebivolol exhibits low oral bioavailability, which may adversely affect its efficacy. Recently, supersaturable self-nanoemulsion (Su-SNE) is an advanced SNE approach that can address low bioavailability. The study aims to prepare nebivolol-loaded Su-SNE by reduction the amount of the prepared conventional SNE to half. Besides, an appropriate polymer type and concentration to prevent NBH precipitation upon oral administration have investigated. A conventional self-nanoemulsion (formula A) was prepared by dissolving NBH in 500 mg vehicle mixture of imwitor®988: cremophor-EL: propylene glycol. Then, eight Su-SNE formulas with the absence or presence of four different polymers were prepared and evaluated. In-vitro precipitation assay was performed to assess the precipitation inhibition capacity of polymers. The ex-vivo permeation through rat intestinal mucosa was also conducted for determination of permeability parameters. Results revealed that (Su-SNA formula SAS1) containing 5% soluplus could effectively retard the nebivolol precipitation. There was no statistical difference between formula A and SAS1; both maintained a higher apparent NBH concentration for approximately 240 min in 0.1N HCl. The permeation rate of conventional (formula A) and soluplus-based Su-SNE (formula SAS1) was significantly improved, and the permeation enhancement ratio was found 2.7 and 3.2, respectively, as compared with non-formulated NBH. Consequently, it is concluded that developing soluplus-based nebivolol SNE is a promising alternative approach. It can enhance nebivolol stability and permeability with half the amount of conventional SNE components.

Keyword: Imwitor®988, Permeation enhancement, Supersaturable self-nanoemulsion, soluplus.

Introduction
Nebivolol (NBH) is a third-generation β1-blocker with high selectivity. It also has vasodilator and oxidative stress reduction effect through promoting endothelium nitric oxide release (1). It has been prescribed for the management of hypertension and heart failure, particularly preferred for elderly patients and those with other co-morbid conditions like diabetes and peripheral vascular disease (2). Nevertheless, nebivolol is proposed based on the Biopharmaceutical Classification System as a Class II drug.

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Nebivolol is poorly soluble with extensive hepatic metabolism leading to variable and low oral bioavailability about 12%, which may adversely affect its efficacy (3). Therefore, different approaches have been adopted to improve solubility and hence, its oral bioavailability through formulating as nanofiber (4), β-cyclodextrin complex (5), solid dispersion (6) and co-crystal (3). Lipid-based drug delivery system (LBDDS) is another promising formulation approach. Today’s self-nano-emulsifying drug delivery system (SNEDDS) is considered as one of the most attractive LBDDS that could address the absorption issue in oral drug delivery (7).

SNEDDS refers to a preconcentrate or anhydrous nanoemulsion. It is typically composed of a drug candidate dissolved in a uniform, transparent mixture of oil(s), surfactant(s) and co-surfactant(s). Upon contacting with gastrointestinal fluids, SNEDDS can rapidly form in-situ a stable o/w nanoemulsion. Peristalsis aids nanoemulsion formation. The nano-size droplets provide a large surface area for drug releasing and absorption (8). However, SNEDDS application has limited by a high amount of surfactants which leads to side effects, such as mucosal irritation (9).

Meanwhile, reducing surfactant quantity may result in supersaturated state upon dilution. In other words, it causes insufficiently drug dispersing. Consequently, drug precipitation may occur and ultimately leading to a decrease in drug absorption. Therefore, the concept of “spring and parachute” is emerged. The term spring used to describe the generation of the supersaturation to increase the free drug concentration with high chemical potential gradient, which acts as a driving force for enhancing luminal absorption. Supersaturable self-nanoemulsion (Su-SNE) is one of the spring formulations, which produces supersaturation in GIT. On other hands, parachute refers to the preservation and prolongation of the supersaturation state without drug precipitation (10). It is well known that the two primary steps for drug precipitation are firstly nucleation and then crystal growth. These steps can be avoided by using various polymers or amphiphilic copolymers. So they are named as precipitation inhibitor excipients. They can thermodynamically or kinetically inhibit precipitation for an extended period, adequate for drug absorption (11).

So, this study aimed to prepare nebivolol supersaturable SNE to have physio-chemical characterisations similar to conventional SNE with reducing the total quantity of SNE vehicle. The impact of different type and concentration of polymers on drug precipitation was also studied to select optimised one. Moreover, the intestinal permeability parameters of the optimised polymer-based supersaturable formula, crude nebivolol suspension and conventional SNE were investigated for comparison.

Materials and Methods

Materials

Inwitor® 988 was kindly gifted by (IOI Oleochemical, GmbH, Germany). The other following materials were purchased from; nebivolol hydrochloride (Baoji Guokang Bio-Technology Co., China), cremophor®EL (International Laboratory, USA), propylene glycol (Evans Medical Ltd, Liverpool, England), HPMC-K100, PVP-K30 (Hyperchem, Chain), soluplus (BASF, Germany) and PEG6000 (HiMedia Laboratories Pvt Ltd, India). All other reagents utilised were of analytical grade.

Methods

Preparation of Conventional and Supersaturated Nebivolol loaded Self-nanoemulsion

The data obtained from a preliminary study had used to select efficient SNE vehicle for NBH. Among various tested oils, surfactants and co-surfactants, the mixture of inwitor-988: cremophor-EL: propylene glycol (PG) had excellent miscibility. It also exhibited a satisfying monophasic region in the pseudo ternary phase diagram. It found that a combination of 10% w/w inwitor, 45% w/w cremophor and 45% w/w PG had proper SNE properties, physical stability and nebivolol-loading capacity of 18.1 ± 1.33 mg/ml. So, it coded as (formula A) and considered as conventional SNE. While supersaturable self-nanoemulsion coded as (formula SA) was developed by reducing the amount of conventional SNE vehicle to half. Both formulas A and SA were prepared by adding nebivolol to a homogenous vehicle. The mixture was vortexed and then, sonicated until a clear liquid obtained. On other hands, hydroxypropyl cellulose (HPMC K100), polyethylene glycol (PEG 6000), polyvinylpyrrolidone (PVP K30) and soluplus were selected. The effect of different selected polymer on formula SA performance was studied. So, each polymer powder was mixed with the formula (SA) and then, vigorously vortexed to obtain a clear liquid or uniform suspension (12).

Table 1 illustrates the contents of the prepared SNE formulations. All prepared formulations were kept at room temperature until further evaluation.
<table>
<thead>
<tr>
<th>Formulation Composition (mg)</th>
<th>A</th>
<th>SA</th>
<th>SAH</th>
<th>SAG</th>
<th>SAP</th>
<th>SAS1</th>
<th>SAS2</th>
<th>SAS3</th>
<th>SAS4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nebivolol *</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Cremophor-EL</td>
<td>225</td>
<td>112.5</td>
<td>112.5</td>
<td>112.5</td>
<td>112.5</td>
<td>112.5</td>
<td>112.5</td>
<td>112.5</td>
<td>112.5</td>
</tr>
<tr>
<td>Propylene glycol</td>
<td>225</td>
<td>112.5</td>
<td>112.5</td>
<td>112.5</td>
<td>112.5</td>
<td>112.5</td>
<td>112.5</td>
<td>112.5</td>
<td>112.5</td>
</tr>
<tr>
<td>HPMC-K100</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>12.5</td>
</tr>
<tr>
<td>PEG 6000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>12.5</td>
</tr>
<tr>
<td>PVP K30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>12.5</td>
</tr>
<tr>
<td>Soluplus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>12.5</td>
</tr>
<tr>
<td>Total weight (mg)</td>
<td>505.5</td>
<td>255.5</td>
<td>268</td>
<td>268</td>
<td>268</td>
<td>268</td>
<td>258</td>
<td>262</td>
<td>280.5</td>
</tr>
</tbody>
</table>

Nebivolol* 5mg equivalent to 5.5 mg nebivolol hydrochloride added to each formula.

**Characterization of Supersaturable-SNE Formulations**

**Droplet Size Distribution and Polydispersibility Index Measurements**

Each of the prepared formulations was dispersed in distilled water with (1:100) dilution and agitated by a magnetic stirrer for 5 minutes. The mean droplets size of the nanoemulsion resulted were determined using laser particle size analyser (Brookhaven ZetaPlus, Holtsville, USA).

**Determination of Self-nanoemulsifying Time for Supersaturable Formulations**

The self-emulsification time was assessed by adding each formula, that represents a single dose of nebivolol to 100 ml of 0.1N HCl in a glass beaker on a magnetic stirrer, which stirred gently at 50 rotations per minutes (rpm). The time needed for complete homogenisation of dispersion was evaluated visually by the formation of clear or slight bluish liquid appearance (13).

**In-vitro precipitation Test Under Non-sink Condition**

The in-vitro precipitation test was carried out in order to evaluate the impact of the different type and concentration of polymers on maintaining a supersaturation state as a function of time (14). Four doses of the conventional and supersaturable-SNE were added to 100 ml of 0.1N HCl to ensure supersaturated state in tested media. The stirring speed was adjusted at 100 (rpm), and the temperature was 37 °C. Samples of 3 ml were withdrawn from the test medium at 10, 15, 30, 45, 60, 120, 180 and 240 min without volume replenishment. Then, the withdrawn samples were filtrated with a filter syringe of 0.22 μm (15). The filtrated samples were suitably diluted. Their nebivolol content was determined using UV-spectrometry (UV1100 model, EMC-LAB, Germany). The apparent nebivolol concentration-time profile was finally constructed.

**Morphological Visualization by Transmission Electron Microscope (TEM)**

Transmission electron microscope (Zeiss Libra 120 PLUS Carl Zeiss NTS, Germany) was used to investigate the morphology of the SNE formulas (A and SAS). Before analysis, each formula was diluted with distilled water (1:10 v/v). Then, a few drops of the prepared dispersion were applied to a carbon-coated copper grid to form a thin liquid film, and the excess of dispersion was blotted with the aid of filter paper. The film was negatively stained with phosphotungstic acid (2% w/v) solution for 5 min and left to dry under room temperature (16). The stained sample was viewed, and the photographs were taken at suitable magnification power.

**Ex-vivo Permeability Study**

Non-everted rat gut sac method, as described by Ruan et al. (17) with mild modification, was carried out to study ex-vivo permeation of the prepared NBH loaded-SNE (formula A) and supersaturable-SEN (formula SAS1) to be compared with pure NBH powder. Five male Sprague-Dawley rats, their weight approximately 180–220 g, were supplied by the animal house in the College of Pharmacy-University of Baghdad. The Search Ethics Committee
approved the procedure followed in this experiment. All animals had received humane care, which complied with the Guideline for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No 85–23, revised 1996). The rats were only permitted to drink water with no food given overnight before conducting the study. Each rat was sacrificed by firstly anaesthetised by inhalation of diethyl ether before cervical dislocation for the experiment. The entire small intestine was detached after making midline abdominal incision of 4–5 cm, and then an equal length of the jejunum segments was cut. Immediately, the segments were cleaned with ice-cold normal saline solution (sodium chloride 0.9% w/v) utilising a syringe equipped with a blunt needle. After tying one end of each segment with silk thread, the intestinal sac was filled with one dose of NBH-loaded of the selected formulations (A, SAS1 and pure NBH) that diluted to 1 ml with phosphate buffer. Then, the sac was carefully ligated to the paddle of the dissolution apparatus II (PharmaTest, Germany) after tightly closing of both sac sides. Each intestinal sac was immersed in a 500 ml of the permeation media (phosphate buffer saline pH 7.4) at 37°C in a dissolution apparatus which operated at 100 rpm and continuously gassed with oxygen (approximately~ 20 bubbles/minute).18 At predetermined time intervals, a sample of 5 ml was collected and directly replenished with the same volume of fresh medium. The collected samples were filtered through 0.22 μm Millipore syringe filter and assayed for its nebivolol content. In this study, the cross-sectional area of the intestinal sac (S) was equal to 11.775 cm², which calculated by applying (equation 1). Taking the length of the sac (h) was 15 cm and assuming that it has a cylindrical shape with an inner radius (r) was 0.125 cm.

\[ S = 2\pi r h \]  

(equation 1)

The apparent permeability coefficients (P_{app}) were calculated using (equations 2):

\[ P_{app} = \frac{d Q/dt}{S \cdot C_o} \]  

(equation 2)

Where \(d Q/dt/S\) is the drug flux into the acceptor solution. The steady-state rate (flux) can be achieved by plotting the cumulative amount of drug permeated across the intestinal membrane versus time. By applying linear regression analysis of the data. The slope of the linear part of the graph would identify, which represents the flux. While \(C_o\) is the initial drug concentration in the mucosal side.19 The permeation enhancement by formulation was calculated by dividing the permeation rate at steady state (flux) of the selected formulas on plain NBH suspension. The cumulative NBH diffused (Q_{20min}) into acceptor jar after 120 minutes was also calculated. The extrapolation of the linear steady-state line to the time axis represented the lag time.

**Compatibility study**

The drug-excipient compatibility was determined by Fourier Transform Infrared (FTIR) spectroscopy (Shimadzu, Japan) to identify pure powder and detect any possibility of interaction or complexation between drug and excipients. The FTIR spectra of the pure NBH and Su-SNE formula (SAS1) were determined by placing directly without any previous sample preparation onto the crystal and scanned over the range between 4000 - 400 cm⁻¹ wavenumber at a resolution of 8 cm.

**Statistical analysis**

The experiments results were given as a mean of triplicate samples ± standard deviation (SD). By employing one-way analysis of variance (ANOVA) followed by Post Hoc analysis can detect the differences between the data of interest. The result was significantly considered different when the probability level (p) was less than 0.05 using Microsoft Excel 2007 and IBM Statistical Package for the Social Sciences (SPSS), version 23.

**Results and Discussion**

**Preparation of conventional and supersaturated nebivolol loaded self-nanoemulsion**

In order to achieve our aim, the amount of each SNE components of the optimum formula (A) was reduced to half. Thus, the saturated formula (SA) may be prone to the risk of drug precipitation after dilution with an aqueous solution. Therefore, different hydrophilic polymers as a precipitation inhibitor were added to formula (SA) to protect the supersaturated state and stabilise the colloidal dispersion.

The prepared formulations appeared as clear mixture except for HPMC K100, and PVP K30-loaded formulas. They appeared as opaque homogeneous suspension, which may be attributed to the inability of HPMC and PVP to dissolve in the SNE components. However, they may be useful once added to the aqueous medium to suppress precipitation.

**Characterization of Supersaturable-SNE Formulations**

**Droplet Size Distribution and Polydispersibility Index Measurements**

The average droplet size of the prepared formulations was listed in (table 2). As shown from the table that the average droplet size of formula (SA) and (A) was the same. Since both formulas (A) and (SA) composed of low drug proportion compared to vehicle proportion (1:100) and (1:50), respectively. As a result, they maintained their self-nano-emulsifying capacity after dispersion into aqueous media. The same result was also observed by Ke et al.20 Furthermore, supersaturable formulations containing PEG-6000 or PVP-K30 also exhibited an average droplet size that approximated similar to
conventional SNE formula. While, both HPMC and soluplus-incorporated SNE had average size significantly (p < 0.05) higher than formula (SA). That could happen when the droplet surface coated by the polymer. Also, it found that an increased amount of incorporated soluplus caused a slight increase in average size. Such results indicated that the added polymers were either adsorb and/or incorporate onto the surface of the droplets (21). On another hand, all formulations had low PDI of < 0.5, represented a homogeneity of the size distribution that could be further confirmed by TEM.

Determination of self-nanoemulsifying time for supersaturatable formulations
The emulsification efficiency of the supersaturatable SNE formulations was shown in (table 2). It had appeared that time taken to form a clear or slight bluish dispersion upon dilution was less than two minutes for all prepared formulations. It was worth noting that the addition of a small amount of different polymers to the formula (SA) did not significantly change in the emulsification time, that ranged between 34 and 73 seconds. In the same concern, Lee D R et al. found that supersaturatable formulas containing Kollidon VA64 or PVP were instantly emulsified upon contact with the aqueous medium (12). However, the employment of 5% HPMC K100 (formula SAH) or 10% soluplus (in formula SAS4) as precipitation inhibitor would increase the emulsification time to 73 and 65 seconds, respectively. That might due to increased viscosity of the system, and thus delay spreading of the formulas in the aqueous medium (11).

<table>
<thead>
<tr>
<th>Formula code</th>
<th>Droplet size (nm) ± SD</th>
<th>PDI ± SD</th>
<th>Emulsification time (sec) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>19.6 ± 0.4</td>
<td>0.213 ± 0.025</td>
<td>24 ± 1.02</td>
</tr>
<tr>
<td>SA</td>
<td>20.04 ± 2.03</td>
<td>0.228 ± 0.013</td>
<td>37 ± 2.5</td>
</tr>
<tr>
<td>SAH (5%)</td>
<td>278.91 ± 0.36</td>
<td>0.319 ± 0.015</td>
<td>73 ± 1.5</td>
</tr>
<tr>
<td>SAG (5%)</td>
<td>18.65 ± 0.78</td>
<td>0.169 ± 0.058</td>
<td>34 ± 3.1</td>
</tr>
<tr>
<td>SAP (5%)</td>
<td>23.47 ± 2.57</td>
<td>0.343 ± 0.024</td>
<td>52 ± 2.5</td>
</tr>
<tr>
<td>SAS1 (5%)</td>
<td>55.28 ± 1.12</td>
<td>0.294 ± 0.005</td>
<td>36 ± 1.2</td>
</tr>
<tr>
<td>SAS2 (1%)</td>
<td>46.14 ± 1.4</td>
<td>0.348 ± 0.005</td>
<td>53 ± 1.5</td>
</tr>
<tr>
<td>SAS3 (2.5)</td>
<td>57.62 ± 4.7</td>
<td>0.344 ± 0.01</td>
<td>35 ± 1.5</td>
</tr>
<tr>
<td>SAS4 (10%)</td>
<td>61.4 ± 1.28</td>
<td>0.251 ± 0.013</td>
<td>65 ± 3.5</td>
</tr>
</tbody>
</table>

In-vitro precipitation Test Under Non-sink Condition
The non-sink condition helps to achieve the required degree of supersaturation and hence, ensure the susceptibility of the drug to precipitate when subject to aqueous media (14).
The in-vitro precipitation evaluation for formulations in the present and absence of precipitation inhibitor revealed in (figure 1). It can be seen from the apparent NBH concentration-time curves that the formula (SA) showed decrease concentration of NBH to about 66.5 % at 2 hours compared to conventional SNE formula (A), which maintained NBH concentration above 90% along the experimental period. That indicated the loss of solubilising capacity of SNE vehicle and hence, resulted in NBH precipitation to nearly its saturation solubility after 6 hours when diluted with 0.1N HCl. In contrast, the addition of hydrophilic polymer to supersaturatable formula (SA) showed a significant difference (p < 0.05) in apparent NBH concentration-time profile. The results of delay precipitation came in agreement with that reported for other drugs by researchers who explained them based on precipitation inhibitor’s chemical and physical nature (11, 22).

Figure 1. The apparent concentration-time profile of nebivolol-loaded conventional and polymer-based supersaturatable SNE formulations in 0.1N HCl.
Statistically, it was found that the addition of PEG, PVP or soluplus revealed a significant difference (p<0.05) from formula SA, which do not contain any polymer. Meanwhile, HPMC addition showed no significant difference (p > 0.05) from formula SA. Among different polymers, soluplus 5% (formula SAS1) showed a comparable apparent concentration to the conventional SNE, formula A profile with no statistically significant difference (p > 0.05) between them. Such results could explain that the interaction at the crystal surface can slow nucleation and crystal growth leading to hampering precipitation kinetics. Furthermore, soluplus may prevent aggregation and/or destruction of the drug-loaded oil droplet in a gastric fluid as reported by Song et al. through incorporation ability of a hydrophobic portion of soluplus into the drug-loaded droplets and formation of a more condensed structure. Besides that, its polyethyleneglycol group would sterically maintain colloidal dispersion (23). On the other hand, a different amount (1, 2.5, 5 and 10% w/w) of soluplus-based supesaturable formulations was prepared to assess the effect of the amount of soluplus on supersaturated condition. Results exhibited in (figure 2) shown that the presence of 5% soluplus appeared sufficiently useful for delay crystallisation. Subsequently, the formula (SAS1) was selected as an optimum Su-SNE for further characterisation.

Figure 2. Apparent concentration-time profile of supersaturable nebivolol-loaded SNA with a different amount of soluplus in 0.1N HCl.

Morphological visualization by Transmission Electron Microscope (TEM)

Photographs depicted in (figure 3) revealed that conventional SNE (formula A), and supersaturable-SNE that containing 5% soluplus polymer (SAS1) consisted of regular spherical, smooth surface globules with the size below 100 nm. The nanodroplets appeared as uniform dark, while the surroundings were found to be bright. There was no aggregation or precipitation. Microscopic observation showed that both tested systems exhibited similar rounded shape morphology, suggesting that suitable self-nano-emulsifying ingredients can effectively generate NBH-loaded nanoemulsion.

Figure 2. Transmission electron micrographs of conventional SNE (formula A; left side) and supersaturable-SNE containing soluplus (formula SAS; right side).
Ex-vivo permeability study

The amount of nebivolol permeated through intestinal mucosa was demonstrated in (figure 4). The steady-state flux was calculated from the slope of penetration profiles; the permeation rate and cumulative amount permeated from conventional NBH-loaded SNE and soluplus-based supersaturable SNE were found to be significantly higher (p < 0.05) than plain NBH suspension. The diffusion parameters were summarized in (table 3).

Table 3. The Diffusion Parameters of Permeability study for Nebivolol from Plain Suspension, Conventional and Supersaturable SNE.

<table>
<thead>
<tr>
<th>Formula code</th>
<th>Cumulative amount diffused at 120 min ((Q_{120 \text{ min}} \mu\text{g}))</th>
<th>Flux ((dQ/dt.S)) ((\mu\text{g/min.cm}^2))</th>
<th>Permeability coefficient ((P_{app}10^{-5} \text{ cm/min}))</th>
<th>Lag Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure NBH</td>
<td>965.6 ± 4.8</td>
<td>0.71 ± 0.02</td>
<td>14.31 ± 0.31</td>
<td>6.5</td>
</tr>
<tr>
<td>A</td>
<td>2895.9 ± 5.6</td>
<td>1.98 ± 0.01</td>
<td>39.6 ± 0.14</td>
<td>2.5</td>
</tr>
<tr>
<td>SAS</td>
<td>3210.14± 15.4</td>
<td>2.37 ± 0.07</td>
<td>47.5 ± 0.04</td>
<td>2.3</td>
</tr>
</tbody>
</table>

From the above (table 3), it deduced that the conventional and supersaturable SNE showed permeation enhancement ratio of 2.7 and 3.2, respectively, as compared with non-formulated NBH. Moreover, it was found that after 120 min, about 57.92± 0.11 % and 64.2 ± 0.31 % of the initial amount of nebivolol were permeated from formula A and SAS1, respectively, compared to only 19.31± 0.1 % from NBH suspension. It is noteworthy that the supersaturable formula (SAS) provided high luminal concentrations of the drug in molecular form, thus resulting in a higher flux across the intestinal membrane. However, there was no statistical difference in permeation rate with \((p > 0.05)\) between formulas A and SAS1.

The obtained data came in agreement with previous studies, concerned with the impact of self-nanoemulsion formulation on intestinal permeation enhancing (24). They ascribed a significant permeation improvement by self-nanoemulsion formulations could be attributed to presenting of the drug in a wholly solubilised form at its absorption sites. Additionally, the nanometric size of droplets of resultant emulsion provides a large interfacial surface area for penetration. Besides that, the employment of permeability enhancer components in the SNE formulation might improve the intestinal permeation by disturbing the intestinal membrane integrity and increasing its fluidity (25).

In this study, cremophor-EL and PG were used as a surfactant and co-surfactant, respectively. Both are known for their permeation enhancing properties (26, 27). Also, soluplus has reported for its action as permeation enhancer of insoluble drug and inhibitor of the efflux pump function (28).

Compatibility study

NBH displays characteristic peaks at 3390, 3190.26, 1072.42 cm\(^{-1}\) corresponding to O-H stretching, N-H stretching and C-N stretching of a secondary amine, respectively as illustrated in (figure 5). The other principal intense peaks are related to N-H bending at 1489.05 cm\(^{-1}\), aromatic C-H in-plane bending at 1211.3 cm\(^{-1}\) and alcoholic C-O stretching observed at 1101 cm\(^{-1}\). Other peaks are 1620.21 and 1543.05 cm\(^{-1}\) for aromatic C=C stretching. Finally, peak at 1141.86 cm\(^{-1}\) is related to C-O-C stretching of cyclic ether (3, 5). Meanwhile, the FTIR spectrum of Su-SNE formula (SAS1) showed fewer peaks of the drug. The peaks were overlapping in the fingerprint region, which indicated trapping of NBH inside the SNE components. Moreover, there was a broad peak of O-H at wavenumber ranges (3600–3200 cm\(^{-1}\)) which hidden NBH characteristic peaks at 3390 and 3190.26 cm\(^{-1}\). That can be ascribed by hydrogen bond formation between NBH and SNE components, that reflected NBH solubilisation in the formula.

Also, the principal peaks of NBH at 1624.06 and 1543 cm\(^{-1}\) for aromatic C=C, as well as, 1211.3 cm\(^{-1}\) maintained in their known position with a slight shift of N-H bending peak to 1492.9 cm. Moreover, no new peaks were noticed, which indicated that FTIR spectroscopy validated the compatibility between NBH and oil, surfactant, co-surfactant excipients of formula SAS1.

Figure 4. The cumulative amount of NBH permeated through rat intestinal sac from NBH-loaded conventional, supersaturable SNE and plain suspension.
Figure 5. The FTIR spectrum of (a) pure nebivolol powder, and (b) the soluplus-based supersaturable self-nanoemulsion (SAS1) formula.

Conclusion
From this study, it is deduced that successful supersaturable self-nanoemulsion (SuSNE) could be prepared by adding amphiphilic polymer, 5% w/w soluplus. The optimum Su-SNE (formula SAS1) composed of nebivolol therapeutic dose, which dissolved in 262.5mg mixture of imwitor 988, cremophore EL, propylene glycol and soluplus. It emulsified rapidly upon dilution within 36±1.2 sec to yield nano-size emulsion as confirmed by TEM. The formula SAS1 could maintain nebivolol supersaturation without precipitation in 0.1N HCl. That indicated by high apparent nebivolol concentration more than 90% over 240 min, which was similar to conventional self-nanoemulsion. Moreover, 5% soluplus-based supersaturable self-nanoemulsion enhanced nebivolol intestinal permeability by 3.2 folds compared to pure powder. So the nebivolol supersaturable self-nanoemulsion can be considered as an alternative approach to conventional self-nanoemulsion.

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