DOI: https://doi.org/10.31351/vol32issSuppl.pp164-176

Design, Optimization and Characterization of Self-Nanoemulsifying Drug Delivery Systems of Bilastine [#]

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[#] 2nd Scientific Conference for Postgraduate Students Researches.
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Abstract

Self-nanoemulsifying drug delivery systems are innovative methods that have a potential to resolve a variety of drug formulation issues, including solubility, stability and bioavailability. Bilastine is a potent and high selective H1-antihistamine. The aim of this study is to develop bilastine as an oral self-nanoemulsion to enhance its permeability and possibility of lymphatic transport. Based on the solubility investigations of bilastine in some oils, surfactants and cosurfactants, fifteen formulas of liquid self-nanoemulsion drug delivery systems (SNEDDS) were formulated utilizing oleic acid, tween 60 and transcutol as oil, surfactant and co-surfactant respectively. Pseudoternary phase diagrams were used to evaluate the component phase behavior and the area of the nanoemulsion. The prepared formulas were evaluated for particle size, polydispersity index, zeta-potential, self-emulsification time, drug content, and robustness to dilution. When compared to the pure drug powder, the produced SNEDDS formulations showed enhanced drug release. This study showed that a formula 8 with a 20% oleic acid, 40% tween 60, and 40% transcutol composition exhibited lower particle size (71.976 ± 0.23 nm) and higher zeta-potential (-20.32) with acceptable drug content (95 %± 0.42) compared to other formulas and better in-vitro drug release characteristics than pure bilastine powder. All of these criteria favor the development of self-nano emulsifying drug delivery systems as a potential approach to enhance the bioavailability of drugs like bilastine that are poorly soluble.

Keywords: Antihistaminic drug, Bilastine, SNEDDS, Transcutol, Tween 60

تصميم وتحسين وتوصيف أنظمة توصيل الأدوية ذاتية الاستحلاب بالنانو من البيلاستين [#]

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الخلاصة

تعد أنظمة توصيل الأدوية ذاتية الاستحلاب من الأساليب المبتكرة التي لديها القدرة على حل مجموعة متنوعة من مشكلات صياغة الأدوية ، بما في ذلك القابلية للذوبان واالثباتية والتوافر الحيوي. يعتبر البيلاستين من مضادات الهستامين اش١ القوية والانتقائية للغاية. الهدف من هذه الدراسة هو اعداد البيلاستين كمستحلب نانوي عن طريق الفم لتحسين نفاذه من الصيغ المعدة وإمكانية النقل اللمفاوي. تم تحضير خمسة عشر صيغة من أنظمة توصيل الأدوية ذاتية الاستحلاب باستخدام حمض الأوليك كطور زيتي و توين ٦٠ كمادة خافضة للتوتر السطحي وترانسكيوتول كخافض مساعد للتوتر السطحي بناءً على دراسة ذوبان البيلاستين في هذه المكونات. تم تقييم منطقة مستحلب النانو وسلوك طور المكون باستخدام مخططات ثلاثية الحالة. تم تقييم الصيغ لعوامل أخرى أيضًا ، بما في ذلك حجم الجسيمات ، مؤشر التشتت المتعدد، زيتا بوتينشيال ، وقت الاستحلاب الذاتي ، محتوى الدواء ، وقوة التخفيف. أظهرت نتائج هذه الدراسة أن الصيغة ٨ التي تحتوي على ٢٠٪ حمض الأوليك ، و ٤٠٪ توين ٦٠ ، و ٤٠٪ ترانس كيوتول أظهرت حجم جزيئات أقل (٧١,٩٧٦) وزيتابوتينشيال أعلى (-٢٠,٣٢) مع محتوى دوائي مقبول (٩٥٪) مقارنة بالصيغ الأخرى وخصائص أفضل لإطلاق الدواء في المختبر نسبة الى مسحوق البلاستين النقي. يتم دعم اعتماد أنظمة توصيل الأدوية ذاتية الاستحلاب بالنانو بواسطة كل هذه العوامل كإستراتيجية محتملة لتعزيز التوافر الحيوي للادوية ضعيفة الذوبان مثل البيلاستين.

الكلمات المفتاحية: دواء مضاد للهستامين، بيلاستين، ترانسكيوتول، توبين ٦٠ ، نظام دوائي ذاتي الاستحلاب.

Introduction

Bilastine (BL) is a new, well tolerated second generation H1-antihistamine. It is indicated for the treatment of chronic spontaneous urticaria and seasonal rhino conjunctivitis [(1)]. BL is also effective in all nasal symptoms including obstruction and in allergic conjunctivitis [(2)]. It belongs to BCS class II [(3)]. Its chemical formula is $C_{28}H_{37}N_3O_3$ and its Mwt is 463.61 Dalton, Log P is 2.41[(4)]. Its bioavailability is about 60% [(5)]. Self-nanoemulsifying drug delivery systems(SNEDDS) are lipid-based formulations that, when in contact with the aqueous medium of gastrointestina secretions while being lightly stirred by peristaltic activity, generate a small oil-in-water nanoemulsion [(6)]. The drug is presented in solubilized state in this spontaneous emulsion, and the droplets small size creates a significant amount of interfacial area on the surface for absorbing drug [(7)]. These factors together lead to enhance bioavailability.

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Received: 28/4 /2023
Accepted: 22/ 6 /2023

The aim of the study is to enhance BL dissolution rate, its permeability from prepared formulas and possibility of lymphatic transport resulting in a higher oral bioavailability of the medicine compared to pure drug powder.

Materials and Methods

Materials

Bilastine was bought from Hyper Che m LT D Co (China), oleic acid oil (pharmaceutical grade) was purchased from Hunan ER-KANG Pharmaceutical Co Ltd, China. Olive oil, avogado oil, castor oil and lavender oil were purchased from Emad domestic company, Iraq. Imwitor 742 and Miglyol 812 N were obtained from IOI Oleochemical, GmbH, Germany. Labrafil® CS 1944 was obtained from Gattefosse, Mumbai, India. Tween 20, 60 and 80 were obtained from SCRC, China, Xi'an Sonwu biotech co.,Ltd, China and Riedel-De-Haen, Germany respectively. Span 20 was obtained from S.D. Fine-Chemical limited, India. Labrasol® ALF was obtained from International Laboratory, USA. Transcutol® HP was purchased from Energy chemical company, China. Propylene glycol was obtained from Evans Medical Ltd, Liverpool, England. PEG 200 and PEG 400 were obtained from Fluka Chemi AG, Switzerland. The additional compounds were of analytical grade and were used exactly as they were given.

Methods

Differential colorimetric scanning analysis

Around two milligrams of pure BL were weighed, sealed in a special aluminu m pan, and put into the Differential Colorimetric Scanning **(**DSC) equipment to complete the process. Using nitrogen as a blank gas, the sample was heated to a maximum temperature of 3 00 °C at a rate of 1 0 °C / min.

Solubility studies

An excess amount of BL was added to 2 ml of different vehicle (oils, surfactants, and co-surfactants). All samples were placed in a water bath shaker for 72 h at 25°C ± 2ºC to achieve equilibrium. The undissolved drug was then separated by centrifugation at 3500 revolutions per minute for twenty minutes. The supernatant was separated, filtered using a 0.45 millipore syringe filter, and then sufficiently diluted with ethanol. Subsequently, using a previously created calibration equation, the BL concentration in diluted samples was determined spectrophotometrically at BL λ max (283 nm) [8].

Selection of surfactant and co-surfactant

The choice of surfactant was based on its emulsifying properties. The number of inversions necessary to emulsify the oil phase in water was examined to assess the emulsification capability of surfactants. Selected oil was combined in 1:1 weight ratio with different surfactants, heated between 45 and 50 °C and then vortexed for two minutes for homogenization. The mixture was then diluted in a 1:100 ratios with distilled water, and the number of inversions necessary to emulsify the oil in aqueous medium was then noted. The resulting mixture was allowed to stand for 2 hours, and a UV-vis spectrophotometer was used to measure the percent transmittance at 650 nm while using deionized water as a control [9].

On the other hand, the choice of co-surfactant was made based on how well it would improve the surfactant's capacity for emulsification. In order to achieve this, a chosen surfactant was combined in a 1:1 ratio by weight with several co-surfactants, resulting in a mixture known as "S_{mix}". Oil was added to the S_{mix} in a 1:4 weight ratio after the mixtures were heated at 45–50 °C to homogenize the contents. The process mentioned above was then repeated [10].

Pseudo-ternary phase diagrams study

The combination of the surfactant and co-surfactant, S_{mix}, was blended at various weight ratios (4:1, 3:1, 2:1, 1:1, 1:2, and 1:3). Oil was added to each of these ratios and blended using a vortex mixer to create a mixture containing nine different oils: S_{mix} ratios (1:9 to 9:1 w/w range) [11]. Thereafter, transparent and homogeneous oil mixtures were titrated by adding deionized water gradually and drop by drop while continuously mixing with a mild magnetic stirrer. The mixture was visually examined for phase transparency and flowability after each addition of water. Titration was terminated when the solution was turbid or gel-like in consistency. To identify the phase boundaries in each, the percentage weights of the oil, S_{mix}, and water in the 100% w/w combination were computed. Phase diagrams were drawn using the OriginLab program. With one apex representing the water, one representing the oil, and one representing S_{mix} at a fixed weight ratio, the shaded area was believed to be a visually clear area in a triangle plot [12].

Formulation of bilastine-loaded SNEDDS

Fifteen formulas were formulated according to the triangle phase diagram. Five different S_{mix} (1:3, 1:2, 1:1, 2:1, 3:1) were selected. To prepare ten grams of SNEDDS components mixture, the oil concentration was ranged from 15 % to 30 % for each selected S_{mix}. While the concentration of tween 60 and transcutol was calculated according to their ratio (S_{mix}) as shown in table (1). The effects of oil concentration and S_{mix} on the particle size distribution and emulsification time were studied.

To ensure blending homogeneity, oleic acid oil, tween 60, and transcutol were pre-mixed and warmed at 40 ^{0}C in the water bath for 3 min. Accurately weighed BL was subsequently mixed with the prepared SNE components blend. The concentration of BL remained constant (1% w/w) in all prepared formulas. BL-loaded formulas were

stirred at 500 rpm for 10 min then 1500 rpm for 20min. [13]. Finally, the prepared liquid was sonicated by prob Sonicator using 30% amplitude, the pulse was 2 sec on and 2 sec off for 5 min to equilibrate.

Characterization and evaluation of Bilastine-loaded SNEDDS

Globule size and polydispersity index (PDI) Measurement

Each stable formula was diluted with deionized water 100 times while being stirred with a magnetic stirrer at 37 °C to create fine nanoemulsion. The resulting particle size of each nanoemulsion was examined using the dynamic light scattering method utilizing a particle size analyzer device (Malvern zetasizer, USA), and the PDI was subsequently determined [14].

Determination of zeta potential

The formulations had their electrophoretic mobility evaluated and translated to zeta potential (ZP) by (Malvern zetasizer, USA), with in-built software. ZP value gives information about the repulsive forces between the droplets. The aqueous dispersion of the prepared SNE formulas was prepared in the same manner as in globule size measurement [15,16].

Self-emulsification time and dispersibility test

Liquid-SNE formulas (500mg) was mixed with 100 mL of 0.1N HCl under mild agitation by magnetic stirrer and examined by visual observation. An emulsification time was calculated based on how long it took for the prepared formulations to completely disperse and generate nanoemulsions [17].

The resulting solutions were visually evaluated according to the following grading system:

Grade A has a clear appearance that rapidly forms within 1 min.

Grade B appears as less clear (i.e., bluish translucent appearance) and forms within 1 min.

Grade C shows white-bluish, similar to milk in appearance, which forms in less than 2 min.

Grade D reveals grey emulsion with a slightly oily appearance, which needs more than 2 min for emulsifying.

Grade E exhibits inadequate emulsification represented by large oil globules appear on the surface [6].

Robustness to dilution and phase separation tests

The formulations had been diluted to 50, 100 and 1000 times with 0.1N HCl and deionized water in separated flasks, in order to predicate to in-vivo dilution behavior. Diluted systems were shaken with the aid of a magnetic stirrer to ensure complete homogeneity and done at 37 ^{0}C and 100 rpm to simulate body temperature [1].

After that, the nanoemulsions were allowed to stand for two hours. Their optical clarity was determined by term percent transmittance at 650 nm. The diluted SNE were also checked for any sign of drug precipitation or separation after 24 h to determine physical stability [18].

Thermodynamic stability studies

For further assessment of physical stability as well as exclusion unstable formulations, the prepared liquid-SNE formulations were put under stress conditions including centrifugation and thermodynamic study. The SNE formulations were diluted 100 times with deionized water and subjected for 30 min to 3500 rpm centrifugation and then, checked appearance or any phase separation [19]. The stabilized formulations were then subjected to six cycles of heating and cooling at two distinct temperatures (4°C and 45°C). Formulas would remain at each temperature for at least 48 hours. Stable SNE formulas were further undergone freezing and thawing at (-20 ºC and 25ºC). They were carried out for three cycles; each one was not less than 48 hr. Assessing stability would be based on BL precipitation or phase separation and creaming [19]. The formulas, which successfully passed above mentioned physical stability study, were chosen for further evaluation.

Drug content

Each Successfully stable formula was dissolved in 100 ml ethanol and thoroughly mixed. Drug absorbance was measured using a UV-visible spectrophotometer after the proper filtration and dilution [16].

In-vitro drug release study

The USP dissolving equipment, Type II, was used for the in-vitro drug released test, which was carried out at 37 °C with a 100-rpm rotation speed, moreover, the dissolving medium was 300 mL of 0.1N HCl. The dialysis bag was employed to gather the amount of truly free BL without interference of unreleased drug from nanoemulsion [20]. Before the *in-vitro* release study began, dialysis bags (Mwt cut off 8,000–14,000 Daltons) were soaked for 24 hours at room temperature in freshly prepared 0.1N HCl [21]. BL powder (5mg) and Each liquid SNE formulation (0.505 g) was diluted ten times with releasing medium. Then, they were filled in 10 x 3.5 cm dialysis membrane. Both ends of the bag were tightly ligated to prevent any leakage, and fixed to the rotating paddle and immersed in releasing medium [22].

At previous predetermined intervals, a sample volume (5 mL) was taken. Each collected sample was replenished by an equivalent volume of 0.1N HCl to keep a sink condition. The collected samples were subjected to the analysis for drug concentration using UV-spectrophotometer at its maximum wave length.

For quantitative kinetic analysis of BL release profiles, DDsolver software was applied to investigate the best data fit according to zero order,

first order, Higuchi, Hixson-Crowell and Korsmeyer-Peppas model [23].

Ex-vivo intestinal permeability study

These experiments were approved by the Search Ethics Committee that performed in accordance to the latest WMA Declaration of Helsinki – Ethical Principles for Medical Research [24] and with respect to guidance for using laboratory animals as published by the US National Academy of Science [25].

Five male wistar rats weighing between 200-250 g had been supplied by the animal house at College of Pharmacy/University of Baghdad. One day before the experiment, the rats were only permitted to drink water and were not provided any food. Each rat was humanely sacrificed by being first given a diethyl ether inhalation as anesthetic. The cervical vertebrae were dislocated after the conformation of pain reflex disappearance. After a 4-5 cm midline abdominal incision, the entire small intestine was detached, leaving about 15 cm of duodenum and cut the jejunum into same length segments. The jejunum segments were cleaned with ice-cold normal saline solution using 10ml syringe and cannula. One end of each segment was tied with silk thread. Then the intestinal sacs were filled with various BL-loaded formulae and pure BL powder as a control, all of which were dispersed in 1 mL of phosphate buffer. The other ends of segments were then tied with silk thread and ligated to the paddle of dissolution apparatus. The volume of dissolution medium (phosphate buffer saline) was 300ml. the speed of paddle was fixed at 100 rpm and the temperature at 37ºC. 5 mL sample was removed at a predetermined period and immediately replaced with the equal volume of fresh medium. The samples were collected, filtered, and their BL content was measured.

The cross-sectional area of each intestinal sac (S) was 7.86 C^2 which was calculated by applying equation

$S = 2 \pi r h$ ---------- equation 1

Assuming that the intestinal segments were cylindrical in shape. The length of the sac was 10 cm (h) and the radius (r) was 0.125 cm. The apparent permeability coefficients (P_{app}) were calculated using equation 2.

$P_{app} = (dQ/dt) / (S * C_o)$ ---------- equation 2

Where (dQ/dt) / S is the drug flux into the dissolution medium. Plotting the quantity accumulated as a result of drug permeation through the intestinal membrane vs time was used to calculate the steady-state rate (flux). The slope of the linear part of the graph represents the flux. C_o represents the initial drug concentration at the mucosal side [26]. Permeation enhancement by formulation was obtained through dividing the permeation rate at the steady-state (flux) of the selected formulas on flux of pure BL powder. After 120 minutes, the cumulative BL diffused into acceptor jar was also calculated. The lag time was depicted by extrapolating the linear steady-state line to the time axis.

Statistic evaluation

The average of the three duplicate samples and standard deviation (SD) were used to represent the experiment's results. The similarity factor (f2) equation was used to statistically evaluate the in-vitro dissolution study results. The f2 test scores were scaled from 0 to 100. When the value of f2 is ≥50, two dissolution profiles are regarded as similar. When there are more than three or four available dissolution time points, this method is preferable for comparing the dissolution profile [27].

Results and Discussion

Differential scanning calorimetry

The DSC was used to ascertain the drug's crystalline state and to offer precise details regarding the physicochemical status of BL. In addition, assess BL's thermal and thermotropic behavior. Pure BL's DSC thermogram (figure 1) reveals abrupt endothermic behavior at 203.4 ^{0}C, which is in consistent with the melting point measurements given in the references [28].

Solubility studies

SNEDDS can be administered orally by avoiding drug precipitation during dilution and minimizing the final volume of SNEDDS. Optimizing drug loading capacity is the first benefit of a drug's maximum solubility. Second, the stability of the whole system will improve during storage [29].

In this work, oleic acid as the oil phase, tween 60 as the surfactant, and transcutol as the cosurfactant revealed the maximum solubility of BL. The solubility studies are shown in figure 2.

Emulsification study for surfactant and co-surfactant selection

For oil, surfactant and co-surfactant mixture that used for SNE formulation, it was essential to disperse efficaciously within seconds under a gentle stirring condition to form spontaneous nanoemulsion. Thus, the measurement of the number of inverting needed for dispersion was performed to judge the ability of various surfactant/co-surfactants to emulsify selected oils. Moreover, percent transmittance measurement after two hours of the dispersion quantitatively represents optical clarity, stability and homogeneity. The clarity of formed dispersion was standardized by determining percent transmittance which should not be less than 80%. The optical clarity of fine dispersion is inversely proportional to the intensity of light scattered [30,31]. Table (2) depicted the number of flask inversions and percent transmittance of oleic acid and surfactants combination. From the table 2, it is clear that the surfactants with close HLB elicit similar results. The

difference in number of flask inversion and percent transmittance may be related to the HLB value and to the difference in surfactants structure. In other words, the hydrophilic head size and shape, the number of tails, and the lengths of the tails all affect the formation of nanoemulsions [32,33]. As the results of emulsification of tween 60 and 80 was too close so tween 60 was selected for its higher percent transmittance and better solubility to bilastine.

The emulsification efficiency of various cosurfactants with tween 60 and oleic acid is shown in table 3. It was evident from table 3 that the addition of co-surfactant to oil-surfactant mixture led to an increase in the percent transmittance compared to surfactant alone (table 2). From these findings, Although Transcutol® HP, propylene glycol and PEG 200 appeared a similar activity as co-surfactant, transcutol was selected due to the higher percent transmittance and to the higher solubilizing potential for BL, and hence improving drug loading capacity.

Such results may be explained by the contribution of co-surfactants, particularly those with short chains, which let oil penetrate toward the hydrophobic tail of the surfactant molecule, so lowering interfacial tension much farther. By enhancing the fluidity of the hydrocarbon component of the surface film by intercalating themselves between surfactant monomers, the various curvatures necessary to create nanoemulsions can be achieved [34].

Pseudoternary phase diagram

Relationship between SNE composition and phase behavior could be clarified by building an outline of pseudoternary phase diagram in absence of BL. The diagram can identify the monophasic region as well as determine the proper concentration range of oil, surfactant and cosurfactant for developing stable BL-loaded SNE formulations [35].

The nanoemulsion areas were identified using the pseudoternary phase diagram, and the proper concentrations of three components that could lead to the creation of SNEDDS were established (oleic acid, tween 60 and transcutol). Several ratios of tween 60 and transcutol as S_{mix} were used, namely, 4:1, 3:1, 2:1, 1:1, 1:2, 2:1, 3:1 to construct pseudoternary phase diagrams. In figure 3, 4:1 phase diagram showed narrowest shaded area, so this ratio was excluded from the study.

The pseudoternary phase diagrams for each group were created separately for each S_{mix} ratio. During aqueous titration, a nanoemulsion spontaneously formed with mild magnetic stirring. It might be caused by surfactant &/or co-surfactant concentration and adsorption at the oil globule interface. The formation of a robust and elastic mechanical barrier that inhibits aggregation, as explained by Nasr et al. [9] who used pseudoternary data to develop irbesartan SNE.

Preparation of Bilastine-Loaded SNEDDS

In this study, fifteen formulas with a total weight of 505.5mg were prepared by varying S_{mix} and oleic acid oil percent. The prepared anhydrous fifteen formulas demonstrated clear, homogeneous pale yellowish appearance. F1, F4, F7, F10 and F13 showed drug precipitation upon storage for 48 h before evaluation, which can be related to higher drug loading above the saturated solubility of the drug, so they were excluded from evaluation.

Characterization of The Prepared Bilastine-Loaded SNEDDS

Measurement of The Globules Size, Polydispersity Index (PDI) and Zeta Potential

The size of the globules has a significant impact on the rate and extent of drug release from the formulation, absorption, and the stability of the nanoemulsion. Therefore, the measurement of the globule size of nanoemulsion is a vital parameter to assess its performance aiming formulation optimization [36].

Furthermore, the quality of the homogenous dispersion is usually described by PDI that means the size distribution width. In other words, it means the ratio of SD to average particle size. A low PDI value describes a limited range of droplet sizes, which indicated consistency in the size distribution, homogeneity and mono-dispersion of the formulated self-nanoemulsion as well as long term stability [37].

Table (4) summarized the experimental results of average globules size and PDI measurement for nanoemulsion in distilled water and 0.1N HCl to simulate in-vivo behavior of prepared self-nanoemulsion. Table (4) manifested that the mean particle size ranged from 71.97 to 162.06 nm, and 75.04 to 199.2 nm in distilled water and 0.1N HCl, respectively. All evaluated formulations showed average globule size < 200 nm, which fulfil criteria of nanoemulsion. In addition to that, the PDI values for all formulations ranged between 0.199 and 0.358, that was below 0.5. The outcomes showed uniformity of oil globules dispersion and affirmed their homogeneity.

Zeta potential value can be used to detect charge magnitude at droplet surface, which will provide an idea about repulsion among similarly charged adjacent droplets. Thus, ZP could relate to colloidal dispersion stability through resisting aggregation and coalescence in GIT environment or during storage time [38]. Hernández and Goymann reported that ZP values above 8-9 mV are required for nanoparticle stability [39]. In general, formulations having ZP value higher than + 30 mV or lower than - 30 mV are regarded as absolutely electrically stable [38].

The values of ZP are presented in table 4. The values were ranged from -12.36 to -22.32 i.e., within acceptable range. Zeta has the potential to

prevent droplets attracting each other, which result in precipitation. Also, the formation of repulsive forces prevents enlarged droplet sizes [40]. The ionization of free fatty acids and glycol present in the structure of SNE components (oil and surfactant) may be the source of the obtained negative values of zeta potential [41].

Table 4 shows that there was no clear relation found between the measured zeta potential and the concentration of oil or Smix. This may be related to the nature of zeta value that was not a simple function of a certain factor but may involve some other complex interactive factors.

Figures 4 and 5 reveals the results of particle size, PDI and zeta potential for SNEDDS formulations.

Self-emulsification time and dispersibility test

The effectiveness of the prepared systems to produce homogenous nanoemulsion when subjected to dilution upon oral ingestion is described using the emulsification study as a key criterion. So, the effectiveness could be evaluated by eye observation of the time needed for total transformation of the prepared formulations into fine dispersion upon mild agitation [42].

The emulsification time of the prepared formula varied from 34 sec to 152 sec as shown in table 5.

Robustness to dilution and phase separation studies

Upon oral ingestion of dosage form, SNEDDS would experience dilution over time by GIT fluids. The prepared formulas were therefore evaluated for resistance to various dilution folds and tested their ability to withstand infinite dilution that simulates *in-vivo* conditions.

Except for F3, F12, F14, and F15, which when diluted with 0.1N HCl revealed phase separation as shown in table 6, the majority of the reconstituted formulations displayed good nanoemulsion stability as evidenced by maintenance of their appearance with no sign of drug precipitation or phase separation in any dilution media after 24 hours of storage at room temperature.

Thermodynamic stability study

To further ensure the stability of the prepared formulas against creaming, coalescence, flocculation, sedimentation and phase separation, they were exposed to stress conditions since nano and microemulsions differ thermodynamically and kinetically from conventional emulsions. Therefore, the prepared formulas were visually assessed after exposing to centrifugation and sudden changing in temperature that include heating-cooling cycles and freezing- thawing cycles [43].

Table 7 illustrates the data of thermodynamic stability study. Four formulas showed un stability (F11, F12, F13 and F15). All formulas passed the centrifugation test because the nanoemulsion was kinetically stable, while the temperature changes caused instability in some formulations as the nanoemulsion was sometimes thermodynamically unstable [44]. Table 7 revealed that certain concentration of oleic acid, tween 60 and transcutol was necessary for formation of thermodynamically stable SNE.

Drug content

Drug content was ranged between 85% to 101% as revealed in table 7. These results are within acceptable range, as stated by United States Pharmacopoeia requirement of drug content [45].

Dialysis membrane method for in vitro bilastine release

Conventional dissolution tests are useful for determining just how SNEDDS disperse in dissolution media, but they are insufficient for simulating in-vivo dissolution and assessing the actual drug release profile since they're unable to discriminate between the portions of drug that are dissolved and those that are entrapped in O/W nanoemulsion droplets that formed upon SNE dispersion in an aqueous medium [6].

The dialysis bag approach was employed to do this, allowing a more precise assessment of drug release from the SNEDDS formulations by only allowing the dissolved drug to permeate. In this study, a dialysis membrane with an 8000–14000 Da molecular weight cutoff was employed to guarantee that the particles exposed to the dissolution media had a large surface area [46]. All tested formulations were diluted with 0.1N HCl to avoid their stickiness to the membrane [47].

In 0.1 N HCl, the in-vitro release characteristics of the produced formulae and the pure medication were evaluated. Regardless how differently the prepared formulations' component ratios varied, all formulations demonstrated higher drug release than BL pure powder, as seen in figure 4. In comparison to the pure drug, all of the prepared liquid SNEDDS formulations exhibited different release patterns (f_2 50). The rapid self-emulsification properties of SNEDDS and their ability to create nanoemulsions with small droplet sizes upon dilution could contribute for the apparent improvement in the in-vitro drug release profiles [48].

Ex-vivo intestinal permeability study

The BL cumulative amount permeated through the jejunum sac was demonstrated in figure (5). The calculated steady-state flux, the permeability coefficient and cumulative amount permeated at 120 min from various BL-loaded SNE were considerably higher than pure BL suspension ($p < 0.05$). The diffusion parameters were summarized in table (8).

From the above table (8), it can be inferred that as compared to unformulated BL suspension, formulae 2 and 5 exhibited permeation enhancement ratios of 1.8 while formulae 3 and 8 showed 2.2 permeation enhancement. Also, it was revealed that after 120 min, only 60 ± 0.1% of the initial quantity

of BL permeated from plain suspension, whereas all formulae released BL completely after 120 min for F2,3, 5 and at 90min for F8 with significant difference ($p < 0.05$).

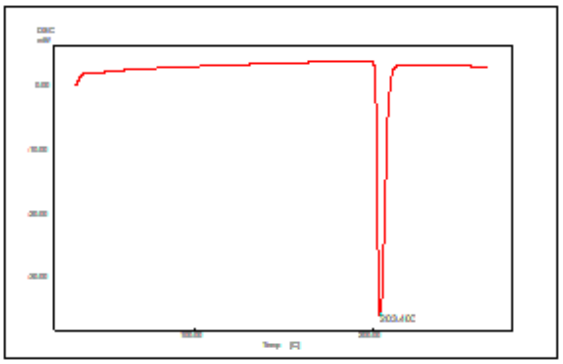
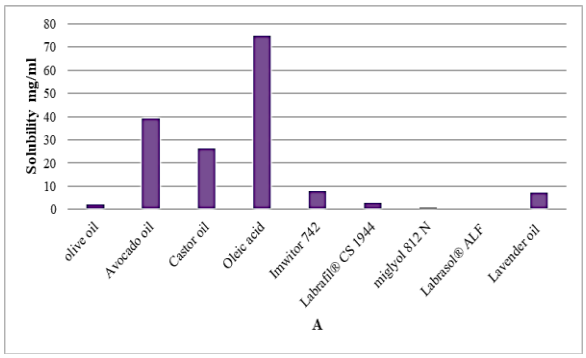
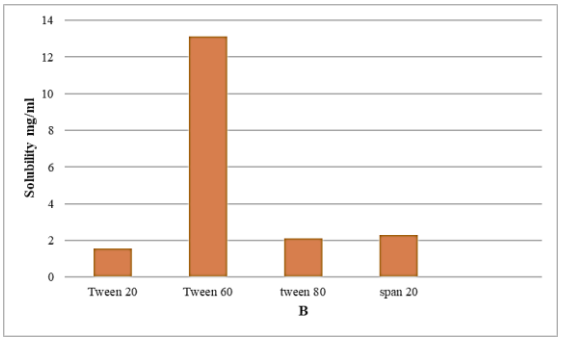


Figure 1. DSC thermogram of bilastine





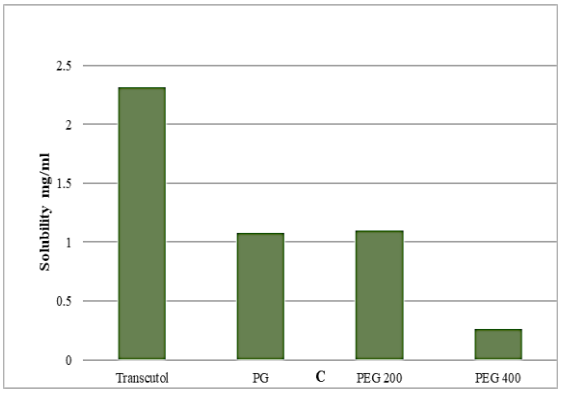


Figure 2. Studies on The Solubility of Bilastine in: A (different oils), B (surfactants), and C (cosurfactants).

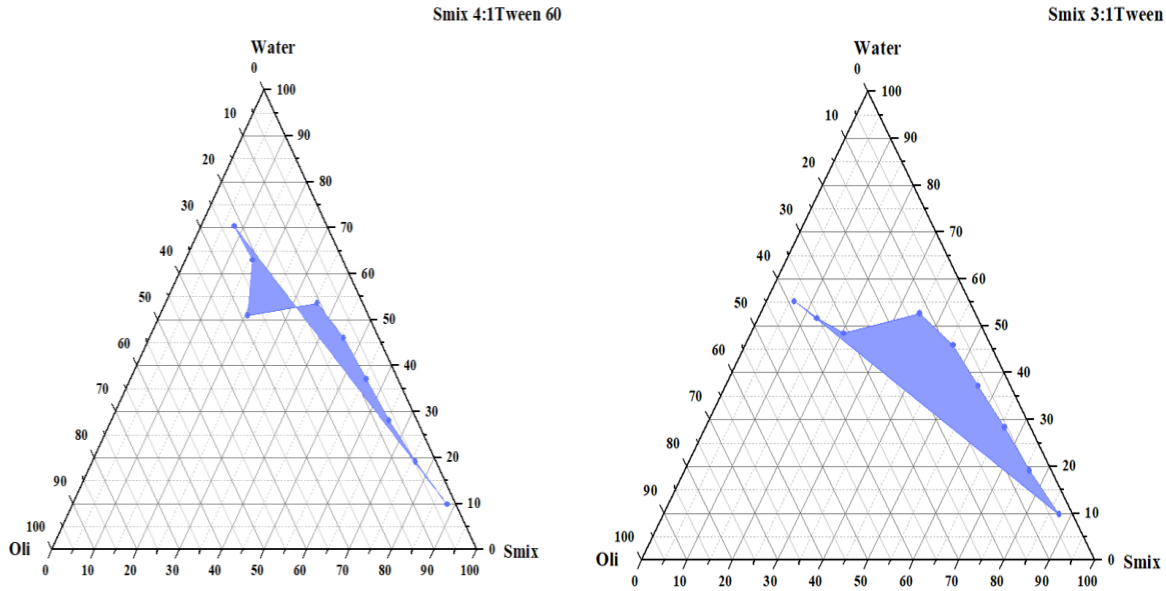
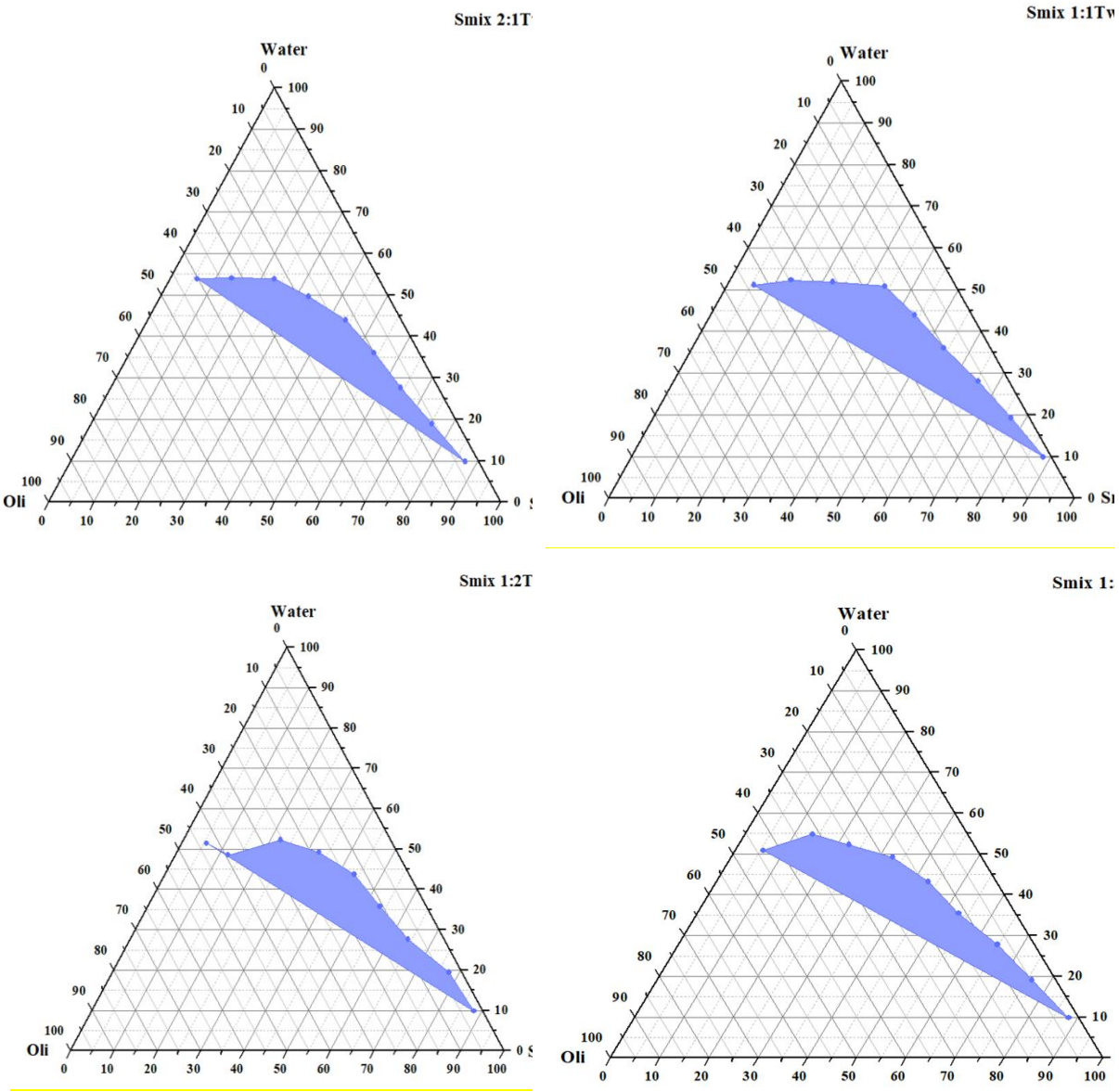


Figure 3. Pseudo-Ternary Phase Diagrams of Oleic Acid: Tween 60: Transcutol at Different S_{mix} Ratios Showing Nanoemulsion (colored portion).



Continued figure 3.

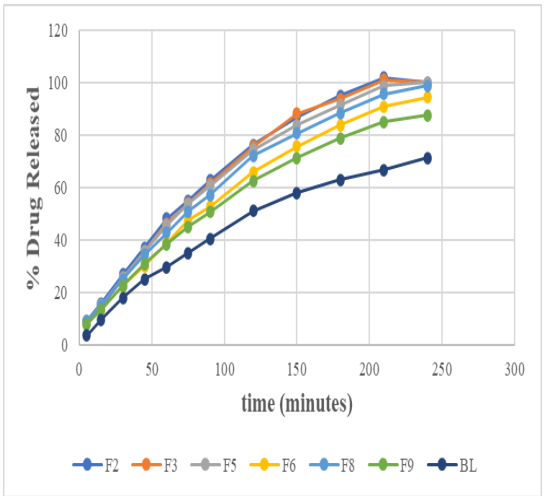


Figure 4. *in-vitro* release profile of bilastine-loaded SNEDDS compared with pure bilastine.

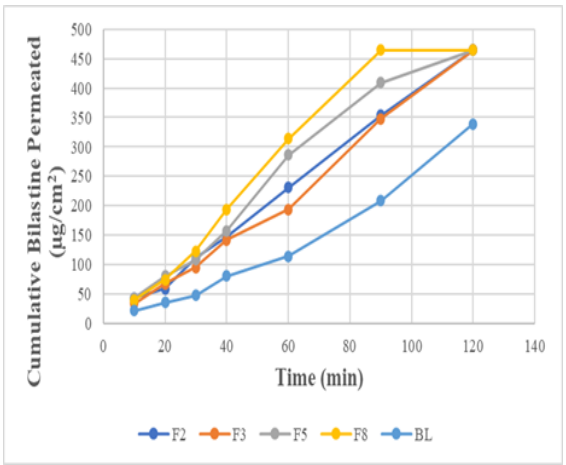


Figure 5. The Cumulative Amount of Bilastine Permeated Through Rats Jejunum Sac from Various Bilastine Loaded SNE and Plane Bilastine Suspension.

Table 1. Composition of liquid SNE formulation with constant amount of bilastine.

Formula code	S_{mix} ratio	Oil:S_{mix} ratio	Bilastine (g)	Oleic acid oil (g)	Tween 60 (g)	Transcutol (g)
F1	1:3	1.5:8.5	0.1	1.5	2.125	6.375
F2	1:3	2:8	0.1	2	2	6
F3	1:3	3:7	0.1	3	1.75	5.25
F4	1:2	1.5:8.5	0.1	1.5	2.833	5.666
F5	1:2	2:8	0.1	2	2.666	5.333
F6	1:2	3:7	0.1	3	2.333	4.666
F7	1:1	1.5:8.5	0.1	1.5	4.25	4.25
F8	1:1	2:8	0.1	2	4	4
F9	1:1	3:7	0.1	3	3.5	3.5
F10	2:1	1.5:8.5	0.1	1.5	5.666	2.833
F11	2:1	2:8	0.1	2	5.333	2.666
F12	2:1	3:7	0.1	3	4.666	2.333
F13	3:1	1.5:8.5	0.1	1.5	6.375	2.125
F14	3:1	2:8	0.1	2	6	2
F15	3:1	3:7	0.1	3	5.25	1.75

Table 2. Emulsification efficiency of various surfactants with oleic acid at ratio 1:1 w/w

Surfactant	Surfactant HLB value	Oleic acid	
		Flask inversions	%T ± SD
Tween 60	14.9	8	51.7 ± 0.34
Tween 80	15	8	57.4 ± 0.88
Span 20	9	12	25.8 ± 0.59

Table 3. Emulsification Studies with Various Co-surfactant with Tween 60 as Surfactant and Oleic acid as oil at Ratio 1:2:2 w/w.

Cosurfactant	Tween 60 (%Transmittance)
Transcutol® HP	96.9 ± 0.75
Propylene Glycol	94.1 ± 0.68
PEG 200	95.6 ± 2.41

Table 4. The Effective globule Size and PDI of Bilastine SNE Dispersed in DW and 0.1N HCl and Zeta Potential.

Formula code	PS* ± SD in DW	PDI** ± SD in DW	PS* ± SD in HCl	PDI** ± SD in HCl	Zeta potential
F2	98.82 ± 1.7	0.2716 ± 0.04	82.86 ± 7.67	0.2426 ± 0.033	-19.67
F3	104.8 ± 2.33	0.226367 ± 0.02	118.73 ± 1.97	0.2333 ± 0.021	-18.23
F5	89.22 ± 3.57	0.2718667 ± 0.05	78.45 ± 3.829	0.2739 ± 0.013	-19.37
F6	91.836 ± 6.05	0.223567 ± 0.007	112.3 ± 8.991	0.2274 ± 0.01	-19.17
F8	71.976 ± 0.23	0.287933 ± 0.002	75.806 ± 2.03	0.2728 ± 0.02	-20.32
F9	105.666 ± 2.49	0.268 ± 0.011	85.286 ± 8.399	0.2512 ± 0.017	-18.36
F11	99.596 ± 8.17	0.252 ± 0.01	94.676 ± 4.641	0.2526 ± 0.015	-16.97
F12	110.133 ± 7.45	0.2301 ± 0.04	103.563 ± 5.848	0.2343 ± 0.031	-13.96
F14	130.866 ± 7.46	0.1994 ± 0.04	130.2 ± 4.368	0.2178 ± 0.04	-12.36
F15	162.0667 ± 24.91	0.3588 ± 0.07	155.36 ± 31.07	0.3007 ± 0.096	-16.97

PS *: median droplet size in nano-meter. PDI**: polydispersity index. DW: distilled water.

Table 5.The Dispersibility grade and Self-nanoemulsification time of liquid bilastine-loaded self-nanoemulsion

Formula Code	Emulsification time (seconds) ± SD	Grade
F 2	36 ± 0.58	A
F 3	42 ± 1.52	A
F 5	37 ± 0.58	A
F 6	58 ± 1.53	B
F 8	34 ± 2.48	A
F 9	49 ± 3	B
F 11	51 ± 1.53	A
F 12	72 ± 2.51	B
F 14	83 ± 1.53	C
F 15	152 ±4.5	C

Table 6. Data of Dispersion Stability and Percent Transmittance (%T) at Different Fold Dilution in Both Deionized Water and 0.1N HCl.

Formula Code	%T at 50X in DW	%T at 100X in DW	%T at 1000X in DW	%T at 50X in HCL	%T at 100X in HCL	%T at 1000X in HCL	Dispersion stability in 0.1N HCl after 24 hr
F 2	91.5	99.2	99.3	90.2	97.6	98.7	Stable
F 3	78	88.8	97.6	85.2	90.5	91.3	Unstable
F 5	93.5	95.2	98.7	94.6	95.1	99.5	Stable
F 6	69.8	87.4	95.5	72.3	89.4	96.8	Stable
F 8	99.1	99.7	99.8	99.5	99.6	99.7	Stable
F 9	89.5	97.8	99.6	92.4	95.3	98.7	Stable
F 11	86.2	89.5	95.6	88.7	92.8	95.3	Stable
F 12	65.7	79.9	89.9	54.2	87.4	91.5	Unstable
F 14	40.2	91.4	95.6	46.9	84.7	94.1	Unstable
F 15	22.9	82.3	92.6	35.6	79.5	93.7	Unstable

Table7.The results of thermodynamic stability study for bilastine loaded self-nanoemulsion.

Formula code	Centrifugation test	Heating - cooling cycles test 45ºC to 4ºC	Freezing – thawing cycles test -20ºC to- 25oC	stability	% Drug content ± SD
F2	√	√	√	stable	96.2 ± 0.27
F3	√	√	√	stable	98 ± 0.3
F5	√	√	√	stable	101 ± 0.37
F6	√	√	√	stable	100 ± 0.01
F8	√	√	√	stable	95 ± 0.42
F9	√	√	√	stable	100 ± 0.01
F11	√	√	X	unstable	94.5 ± 0.26
F12	√	√	X	unstable	85 ± 0.35
F14	√	√	X	unstable	91.2 ± 0.21
F15	√	X	-	unstable	94.8 ± 0.28

Table 8. The Diffusion Parameters of Permeability Study of Bilastine from Various Bilastine –Loaded SNEDDS and Plane Bilastine Suspension.

Formula code	Cumulative amount diffused at 120 min ($Q_{120\ min}$-µg)	Flux (dQ/dt.S) (µg/min.Cm^2)	Permeability Coefficient (P_{app}*10^{-4}-Cm/min)	Lag time (min)
F2	1409.2 ± 5.1	4.1	11.2	3
F3	1344.3 ± 5.4	5	13.7	20
F5	1547.9 ± 6.8	4.1	11.2	5
F8	1674.2 ± 7.3	5.03	13.8	2
BL	690.5 ± 4.1	2.3	6.2	31

Conclusion

According to the study's findings, bilastine-loaded SNEDDS containing 20% oleic acid, 40% tween 60, and 40% transcutol demonstrated good thermodynamic stability and globule sizes in the Nano metric range. The increased *in-vitro* drug release profiles and *ex-vivo* study of all formulations when compared to pure bilastine powder demonstrate enhancing properties of the SNEDDS components and provide the potential of greater absorption and bioavailability.

Acknowledgment

The authors are extremely grateful to the College of the Pharmacy/University of Baghdad for providing the necessary facilities to carry out this work.

Conflicts of Interest

Declared none.

Funding

No external funding.

Author Contribution

The authors confirm the contribution to the paper as follows: study conception and design: Ishraq and Shaimaa; formulation, analysis and interpretation of results and draft manuscript preparation: Ishraq; supervision and review: Shaimaa. Both authors reviewed the results and approved the final version of the manuscript.

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