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Research Article

Development and Assessment of a Self-Nanoemulsifying Drug Delivery System Containing Bilastine: An Effective Strategy to Improve Dissolution

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Abstract

Background: Innovative approaches, including self-nanoemulsifying drug delivery systems, have the potential to solve a variety of drug formulation challenges, such as solubility, stability, and bioavailability. The drug candidate in this research is the strong and extremely selective H1-antihistamine, bilastine, that belongs to BCS II with low solubility and high permeability. ***Objective***: This research aims to formulate an oral self-nanoemulsion of bilastine to improve dissolution. ***Methods***: A total of fifteen liquid self-nanoemulsion drug delivery systems (SNEDDS) formulas were developed using oleic acid, cremophor, and Transcutol as the appropriate oils, stabilizers, and co-stabilizers based on the solubility studies of bilastine in various oils, stabilizers, and co-stabilizers. We used pseudoternary phase diagrams to look at the behavior of the component phases and the area of the nanoemulsion. The physicochemical properties of the formulas, in addition to thermodynamic stability, droplet size, polydispersity index, zeta potential, self-emulsification time, drug content, and durability of the developed formulations, were assessed. ***Results***: This study demonstrated that formula 5, with a 20% oleic acid, 27% cremophor, and 53% Transcutol composition, had lower globular size, acceptable drug content, better *in-vitro* drug release, and *ex-vivo* permeability characteristics than pure bilastine powder. ***Conclusions***: The mentioned factors all point to the preparation of self-nanoemulsifying drug delivery systems as a potential means for improving the dissolution of poorly soluble drugs like bilastine.

Keywords: Bilastine, Drug delivery system, Dissolution, Self nano-emulsion.

تطوير وتقييم نظام توصيل الأدوية ذاتي الاستحلاب النانوي المحتوي على البيلاستين: استراتيجية فعالة لتحسين الذوبان

الخلاصة

الخلفية: الأساليب المبتكرة، بما في ذلك أنظمة توصيل الأدوية ذاتية الاستحلاب النانوي، لديها القدرة على حل مجموعة متنوعة من تحديات صياغة الأدوية، مثل الذوبان والاستقرار والتوافر البيولوجي. الدواء المرشح في هذا البحث هو مضاد الهيستامين H1 القوي والانتقائي للغاية، البيلاستين، الذي ينتمي إلى BCS II مع قابلية ذوبان منخفضة ونفاذية عالية. **الهدف**: يهدف هذا البحث إلى صياغة مستحلب نانوي ذاتي عن طريق الفم من البيلاستين لتحسين الذوبان. **الطرائق**: تم تطوير ما مجموعه خمسة عشر تركيبة لأنظمة توصيل الأدوية ذاتية الاستحباب السائل (SNEDDS) باستخدام حمض الأوليك والكريموفور والترانسكوتول كزيوت ومثبتات ومثبتات مشتركة مناسبة بناء على دراسات قابلية الذوبان للبيلاستين في مختلف الزيوت والمثبتات والمثبتات المشتركة. استخدمنا مخططات الطور الزائف للنظر في سلوك المراحل المكونة ومساحة المستحلب النانوي. تم تقييم الخصائص الفيزيائية والكيميائية للصيغ، بالإضافة إلى الاستقرار الديناميكي الحراري، وحجم القطرات، ومؤشر التشتت المتعدد، وإمكانات زيتا، ووقت الاستحلاب الذاتي، ومحتوى الدواء، ومتانة التركيبات المطورة. **النتائج**: أظهرت هذه الدراسة أن الصيغة 5، التي تحتوي على 20٪ حمض الأوليك، و 27٪ كريموفو، و 53٪ من تركيبة Transcutol، لها حجم كروي أقل، ومحتوى دوائي مقبول ، وإطلاق أفضل للدواء في المختبر، وخصائص نفاذية خارج الجسم الحي من مسحوق البيلاستين النقي. **الاستنتاجات**: تشير جميع العوامل المذكورة إلى إعداد أنظمة توصيل الأدوية ذاتية الاستحلاب النانوي كوسيلة محتملة لتحسين إذابة الأدوية ضعيفة الذوبان مثل البيلاستين.

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INTRODUCTION

In the field of pharmaceutical product development, a challenge of achieving the needed concentration of drug at the intended target sites is often encountered due to poor aqueous solubility, which is a limitation for many drug substances with high therapeutic efficacy. Oral administration is the preferred route of drug delivery, as self-administration is convenient, safe, lacking acrimony, has a high patient compliance rate, inexpensive, and can accommodate a variety of drug types [1]. Bilastine (BLS), with the chemical name 2-[4-

(2-{4[1-(2-ethoxyethyl)-1 H-1, 3 benzodiazol-2yl] piperidine-1-yl} ethyl) phenyl] 2-methylpropenoic acid is used for managing allergic rhinoconjunctivitis caused by various allergens such as pollen, mold, dust mites, animal dander, and so on. It is considered safer than other antihistamines due to its lack of sedative and cardiotoxic effects. Bilastine bioavailability is relatively low (61%). The reduced and variable bioavailability of bilastine may be attributed to several factors, including incomplete erratic absorption and P-gp-mediated efflux transport in the intestine [2-4]. Nanoparticles, liposomes, self-nanoemulsifying drug delivery systems (SNEDDS), and other new carrier systems have been created in recent years to improve the bioavailability and biological activities of many hydrophobic compounds. These carrier systems can protect the compounds from degradation and improve their solubility and stability, resulting in better efficacy and pharmacokinetic profiles [5]. SNEDDS has shown significant potential in improving the solubility, absorption, and bioavailability of lipophilic drugs. SNEDDS are typically made up of lipids, stabilizers, and co-stabilizers, which, when exposed to the GIT motility environment, form mixed micelles and nanodroplets of emulsified lipids. These nano-micelles are promising candidates for the delivery of medications that are not very soluble in water because they enable a relatively higher drug uptake by the GIT mucosa when compared to conventional solutions of drugs [6,7]. The study's goal is to improve the dissolution of bilastine in an oily dispersion in an effort to improve its eventual bioavailability.

METHODS

Materials

Hyper. Chem. LTD Co. (China) provided the bilastine powder, and Hunan ER-KANG Pharmaceutical Co. Ltd. (China) supplied the naturally occurring oleic acid oil (pharmaceutical grade). We bought oils from the Iraqi native company Emad, including argan oil, sweet almond oil, cinnamon oil, ginger oil, lavender oil, and thyme oil. Labrafac PG was obtained from GATTEFOSSE, France. The supplier of tricetine oil was Central Drug House (CDH®), India. Tweens 20 and 80 were purchased from Riedel-De-Haen in Germany and Xi'an Sonwu Biotech Co., Ltd. in China, respectively. Span 20 was obtained from S.D. Fine-Chemical Limited in India. The Chinese company Shanghai Machlin Co., Ltd. sold Cremophor® EL to us. International Laboratory in the United States provided the Labrasol® ALF. We bought Transcutol® HP from the Energy Chemical Company in China. The supplier of propylene glycol was Central Drug House (CDH®), India. PEG 200 and PEG 400 were bought from Fluka Chemi AG in Switzerland. All other substances used in the present work were of analytical grade.

Measurement of melting point

According to USP guidelines, the melting point of BLS powder was assessed using the capillary tube method by an electrical melting point instrument. The tube was placed into the melting point equipment, covered from one end with the BLS powder, and the temperature was gradually raised. The melting point of the powder was determined to be the temperature at which it liquefied [8].

Solubility studies

In tubes with 2 mL of each of the oils, stabilizers, and co-stabilizers, BLS was added in excess. To reach equilibrium, the samples were shaken in a water bath for 72 hours at a temperature of 25 °C ±2 °C. The undissolved drug was subsequently separated using centrifugation for 20 minutes at 3500 revolutions per minute. A 0.45-millipore syringe filter was used to remove the supernatant, and it was then appropriately diluted with ethanol. Then, spectrophotometrically, the BLS concentration in diluted samples was measured at BLS λ max using a previously created calibration equation [9].

Choosing a stabilizer and a co-stabilizer

Our choice of stabilizer was based on its proven ability to help with emulsification, which makes it possible to create a stable and uniform mixture. To evaluate the emulsification capacity of stabilizers, the number of inversions required to emulsify the oil phase in water was looked at. The chosen oil was mixed in a 1:1 weight ratio with various stabilizers, heated to between 45 and 50 °C, and then homogenized using a vortex for two minutes. The mixture was then diluted with distilled water in a ratio of 1:100, and the number of inversions required to emulsify the oil in the medium was recorded. The stability of the formulation was evaluated by measuring the percentage transmittance at a wavelength of 650 nm using a UV-Vis spectrophotometer, with deionized water as a control, following a 2-hour standing period. [10]. The co-stabilizer was selected based on how well it would enhance the stabilizer's ability to emulsify. To accomplish this, a selected stabilizer and several co-stabilizers were mixed in a mixture known as "S_{mix}" at a weight ratio of 1:1. After the mixtures were heated to a homogeneous state at 45–50 °C, oil was added to the S_{mix} in a 1:4 weight ratio. The previous procedure was then carried out using different oils [11,12].

Study of pseudo-ternary phase diagrams

Different weight ratios (4:1, 3:1, 2:1, 1:1, 1:2, and 1:3) were used to blend the stabilizer and co-stabilizer combination, known as S_{mix}. Each of these ratios had oil added to it, and a vortex mixer was used to create a mixture containing nine different oils: S_{mix} ratios (between 1:1 and 9:1 w/w) [13]. After that, deionized water was slowly added to clear and uniform oil

mixtures while they were being mixed continuously with a gentle magnetic stirrer and closely watched to see how clear the phases were and how well the mixture flowed. The titration was stopped when the solution turned turbid or took on the consistency of a gel. The percentage weights of water, oil, and S_{mix} in the 100% w/w combination were calculated to determine the phase boundaries in each. Using the Origin Lab program, phase diagrams were created. The shaded region in a triangle plot was thought to be a visually clear region, with one apex representing the water, one representing the oil, and one representing S_{mix} at a fixed weight ratio [14].

Development of SNEDDS loaded with bilastine

The triangle phase diagram served as a framework for the production of fifteen formulas. A total of five S_{mix} (1:3, 1:2, 1:1, 2:1, and 3:1) were chosen. For each selected S_{mix}, an oil concentration range of 15% to 30% was used to prepare ten grams of self-nanoemulsion (SNE) component mixture. As shown in Table 1, the concentration of Cremophor and Transcutol was calculated using the corresponding ratios (S_{mix}).

Table 1: Formulation of a liquid SNE with a fixed amount of bilastine

Formula code	S_{mix} ratio	Oil:S_{mix} ratio	Bilastine (g)	Oleic acid (g)	Cremophore (g)	Transcutol (g)
1	1:3	1.5:8.5	0.1	1.5	2.125	6.375
2	1:3	2:8	0.1	2	2	6
3	1:3	3:7	0.1	3	1.75	5.25
4	1:2	1.5:8.5	0.1	1.5	2.833	5.666
5	1:2	2:8	0.1	2	2.666	5.333
6	1:2	3:7	0.1	3	2.333	4.666
7	1:1	1.5:8.5	0.1	1.5	4.25	4.25
8	1:1	2:8	0.1	2	4	4
9	1:1	3:7	0.1	3	3.5	3.5
10	2:1	1.5:8.5	0.1	1.5	5.666	2.833
11	2:1	2:8	0.1	2	5.333	2.666
12	2:1	3:7	0.1	3	4.666	2.333
13	3:1	1.5:8.5	0.1	1.5	6.375	2.125
14	3:1	2:8	0.1	2	6	2

Droplet size distribution and emulsification time were studied as a function of oil concentration and S_{mix}. Oleic acid oil, cremophor, and Transcutol were pre-mixed and warmed for 3 minutes at 40 degrees Celsius in the water bath to ensure homogeneity during blending. The prepared SNE component blend and precisely weighed BLS were then combined. All prepared formulas contained the same amount of BLS (1% w/w). Formulas containing BLS were stirred at 500 rpm for 10 minutes and then at 1500 rpm for 20 minutes [15]. The prepared liquid was lastly sonicated by a probe Sonicator at 30% amplitude with a pulse duration of 2 seconds on and 2 seconds off for 5 minutes to equilibrate.

Characterization and assessment of SNEDDS loaded with bilastine

A tiny nanoemulsion was produced by diluting each formula 100 times with DW using a magnetic stirrer at 37 °C for mixing; the resulting nanoemulsions ' globular size was analyzed by the dynamic light scattering method using a globular size analyzer device (Malvern Zetasizer, USA), and the poly-dispersity index (PDI) was subsequently calculated [16]. The formulations' electrophoretic mobility was assessed and converted to zeta potential (ZP) by a Malvern Zetasizer with built-in software (Malvern Zetasizer, USA). The ZP value reveals the repellent forces between the droplets. The prepared SNE formulas' aqueous dispersion was made in the same way that globule size was measured [17,18].

Self-emulsification time and dispersibility

Liquid-SNE formulas (500 mg) were combined with 100 mL of 0.1N HCl while being stirred gently with a magnetic stirrer. The mixture was then visually observed. Based on how long it took the prepared formulations to fully disperse and produce nanoemulsions, an emulsification time was calculated [19]. The solutions that were produced were graded visually using the following system: Grade A has a clear appearance and forms quickly within one minute; Grade B forms in one minute and has a less clear appearance (i.e., a bluish translucent appearance); Grade C displays a white-bluish substance that forms in less than two minutes and looks somewhat like milk; Grade D reveals a grey, slightly oily emulsion that takes longer than two minutes to emulsify; and Grade E displays poor emulsification as evidenced by the presence of large oil globules on the surface [13].

Phase separation and dilution test robustness

To simulate in-vivo dilution behavior, the formulations were diluted 50, 100, and 1000 times with 0.1N HCl and deionized water in separate flasks. To ensure complete homogeneity, diluted systems were shaken using a magnetic stirrer at a temperature and speed that simulated body temperature, 37 ºC, and 100 rpm [20]. The nanoemulsions were then left alone for two hours. By measuring the percent transmittance at 650 nm, their optical clarity was calculated. To assess the physical

stability of the diluted SNE, it was also examined 24 hours later for any indication of drug precipitation or separation [21].

Thermodynamic stability

The prepared liquid-SNE formulations were subjected to stress conditions such as centrifugation and thermodynamic study to further assess physical stability and exclude unstable formulations. The SNE formulations were centrifuged at 3500 rpm for 30 minutes after being diluted 100 times with deionized water. The appearance and presence of any phase separation were then examined [22]. After that, the stabilized formulas underwent the heating/cooling cycles at two different temperatures (4 to 45 degrees Celsius), which were carried out for six cycles. At each temperature, formulas would stay at least 48 hr. Stable SNE formulas further underwent the freezing/thawing cycles at (-20 to 25 degrees Celsius). They were carried out for three cycles; each one was not less than 48 hr. Assessing stability would be based on BLS precipitation or phase separation and creaming [22]. The formulas that passed the physical stability study mentioned above were chosen for further analysis. Each formula that successfully passed the evaluation tests mentioned earlier was thoroughly mixed and dissolved in 100 ml of ethanol. A UV-visible spectrophotometer is utilized to detect drug content by measuring its absorbance following proper filtration and dilution to ensure product chemical stability [18].

In vitro drug release study

The in vitro drug release test was conducted using the USP dissolution apparatus, Type II, at 37 °C with a 100 rpm rotation speed and 300 mL of 0.1N HCl as the dissolving medium. The amount of truly free BLS was collected using the dialysis bag [23]. Dialysis membranes were soaked in freshly prepared 0.1N HCl for 24 hours at room temperature before the start of the in vitro release study [18]. The releasing medium was used to dilute the BLS powder (5.0 mg) and each liquid SNE formulation (0.505 g) ten times. Then, they were filled in a 10 x 3.5 cm dialysis membrane. The bag was fixed to the rotating paddle, both ends of which were tightly tied off to prevent leaks, and it was filled with the release medium [18]. A sample volume (5 mL) was taken at every predetermined time interval. To maintain a sink condition, an equivalent volume of 0.1N HCl was added to each collected sample. An UV spectrophotometer was set to its λ max used to analyze the collected samples for drug concentration.

Ex-vivo intestinal permeability study

The WMA Declaration of Helsinki - Ethical Principles for Medical Research, as well as the US National Academy of Science's guidelines for the use of laboratory animals, were both followed by the Search Ethics Committee, which approved these studies [24,25]. The animal house at the College of Pharmacy/University of Baghdad provided six male Wistar rats weighing between 200 and 250 g. The rats were not given any food or drink the day before the experiment; only water was allowed. Each rat was killed humanely by first receiving an anesthetic inhalation of diethyl ether. After the pain reflex disappeared, the cervical vertebrae were dislocated. The entire small intestine was removed after a 4-5 cm midline abdominal incision, leaving about 15 cm of the duodenum, and then an equal length of the jejunum segments was cut. Using a 10 ml syringe and cannula, the jejunum segments were cleaned with ice-cold normal saline solution. Silk thread was used to tie each segment's end. Then, different BLS-loaded formulas were injected into the intestinal sacs. Pure BLS powder was used as a control. Each of the formulas was mixed with 1 mL of phosphate buffer (pH 7.4). After that, the remaining ends of the segments were tied with silk thread and attached to the paddle of the dissolution device. The phosphate buffer saline (pH 7.4) dissolution medium had a 500 ml volume. The sink condition is considered in this test in which the concentration of BLS in the dissolution medium is less than 10% of its concentration in the intestinal sacs. The paddle's speed and temperature were set at 100 rpm and 37 °C, respectively. At predetermined time intervals, a 5 mL sample was taken, and it was immediately replaced with a fresh medium volume of the same size. After being gathered and filtered, the samples' BLS contents were estimated. Equation 1 was used to calculate the cross-sectional area of each intestinal sac (S), which came out to be 7.86 cm^2.

$$S = 2 \pi r h \text{ ----------Equation 1}$$

Assuming that the segments of the intestine were cylindrical. The sac had a 10 cm (h) length and a 0.125 cm (r) radius. Using equation 2, the apparent permeability coefficients (Papp) were determined.

$$Papp = (dQ/dt) / (S * C_0) \text{ ------ Equation 2}$$

Where the drug flux into the dissolution medium is (dQ/dt) / S. The steady-state rate (flux) can be determined by plotting the quantity accumulated as a result of drug permeation through the intestinal membrane vs. time. The flux is represented by the slope of the linear part of the graph. The initial drug concentration at the mucosal side is represented by C_0 [1]. Permeation enhancement by formulation was obtained through dividing the permeation rate at the steady state (flux) of the selected formulas by the flux of pure BLS powder. The cumulative BLS diffused into the acceptor jar was also calculated after 120 minutes. The extrapolation of the linear steady-state line to the time axis represented the lag time.

Statistical analysis

The experimental data were represented by using the standard deviation of triplicate samples. The data of the in-vitro dissolution study were evaluated using the similarity factor (f2). Scores on the f2 test ranged from 0 to 100. Two dissolution profiles are deemed similar when f2 is less than 50. This method is preferred for comparing the profile of dissolution when the points of dissolution time represent more than three or four [26].

RESULTS

The melting point of BLS was 203°C. Figure 1 shows the solubility profiles of BLS in different oils, stabilizers, and co-stabilizers.

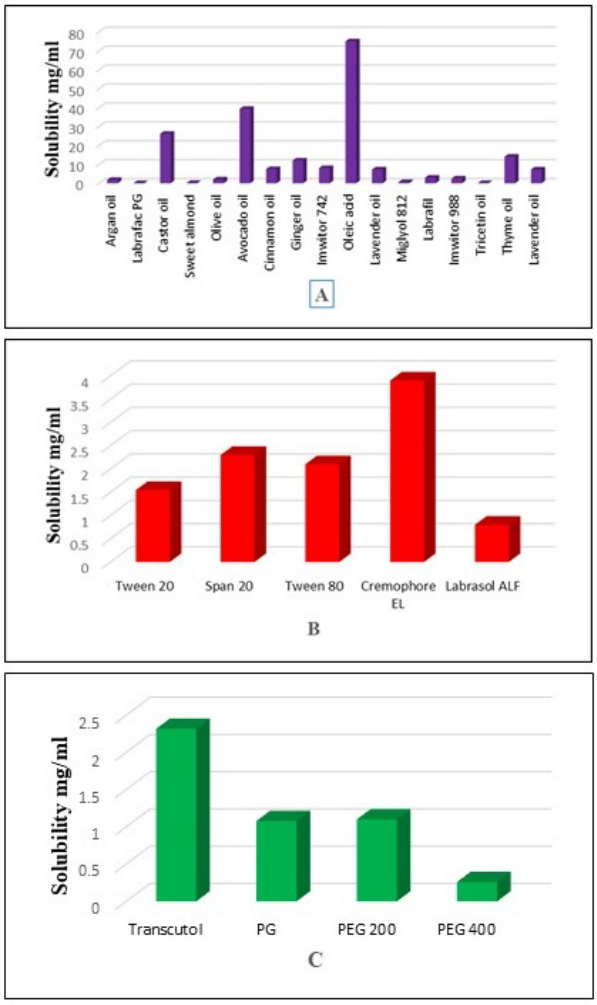

Figure 1: Histogram of bilastine solubility in various: **A**) oils, **B**) stabilizers, **C**) and co-stabilizers.

According to the screening study, the solubility of BLS was highest in oleic acid (75 mg/ml), Cremophor (3.9 mg/ml), and Transcutol (2.318 mg/ml). As a result, oleic acid, cremophor, and Transcutol were selected as the appropriate oils, stabilizers, and co-stabilizers. It was crucial for the oil, stabilizer, and co-stabilizer mixture used in the formulation of spontaneous nanoemulsion to disperse effectively in a matter of seconds while gently stirring. The number of inversions required for dispersion was measured to assess the capacity of different stabilizers and co-stabilizers to emulsify specific oils. Additionally, the percent transmittance of the dispersion after two hours indicates optical clarity, stability, and homogeneity. Oleic acid and stabilizer combination flask inversions and percent transmittance were shown in Table 2.

Table 2: Emulsification effectiveness of different stabilizers with oleic acid at a ratio of 1:1 w/w

Stabilizer	Stabilizer HLB value	Oleic acid	
		Flask inversions	T
Tween 20	16.7	9	33.8±0.25
Span 20	9	10	23.8±0.18
Tween 80	15	8	51.7±0.34
Cremophor EL	12-13	7	54.9±0.37
Labrasol ALF	14	10	21.2±0.45

Values were expressed as number and mean±SD.

Studies on emulsification with various co-stabilizers using Cremophor as the stabilizer and oleic acid as the oil in a ratio of 1:2:2 w/w display the effectiveness of different co-stabilizers in emulsifying Cremophor and oleic acid; it also shows that the percent transmittance increased when a co-stabilizer was added to the oil-stabilizer mixture compared to when the stabilizer was used alone, as seen in Table 2. The results of effective globule size, PDI and Zeta potential of Bilastine SNE dispersed in DW and 0.1N HCl are demonstrated in Table 3. According to these results, Transcutol® HP, propylene glycol, and PEG 200 all appeared to have a similar activity as co-stabilizers. The percent transmittance of Cremophor with Transcutol, propylene glycol, and PEG 200 was 97.1±2.8, 95.2±1.7 and 94.9±3.4, respectively. However, Transcutol was chosen due to its higher percentage of transmittance and higher BLS solubilizing potential, which improved drug loading capacity. The pseudoternary phase diagram was used to locate the nanoemulsion regions and the correct concentrations of three substances (oleic acid, cremophor, and Transcutol) that might form SNEDDS. To create pseudoternary phase diagrams, different ratios of Cremophor and Transcutol as S_{mix} were used, including 4:1, 3:1, 2:1, 1:1, 1:2, 2:1, and 3:1.

Table 3: The Effective globule size, PDI and Zeta Potential of Bilastine SNE Dispersed in DW and 0.1N HCl

Formula code	PS in DW (nm)	PDI in DW	PS in HCl (nm)	PD PDI in HCl	Zeta potential
F2	107.83±4.85	0.20±0.022	112.1±11.41	0.26±0.017	-30.96
F3	121±3.47	0.20±0.045	124.9±4.53	0.2±0.05	-42.69
F5	80.32±8.16	0.27±0.08	76.3±0.67	0.24±0.04	-33.74
F6	134.83±12.15	0.26±0.026	177.26±47.35	0.34±0.13	-20.93
F8	94.89±3.09	0.32±0.12	85.95±5.68	0.23±0.047	-24.85
F9	118.13± 6.07	0.23±0.02	102.79±7.35	0.26±0.0048	-30.46
F12	133.73±3.73	0.32±0.17	115.26±16.77	0.23±0.033	-26.85
F15	267.1±60.49	0.46±0.08	196.6±3.01	0.43±0.007	-17.56

Values were expressed as mean±SD. PS: median droplet size in nanometer. PDI: poly dispersity index. DW: distilled water.

The 4:1 phase diagram in Figure 2 had the narrowest shaded area, so this ratio was left out of the analysis. For each S_{mix} ratio, separate pseudoternary phase diagrams were made.

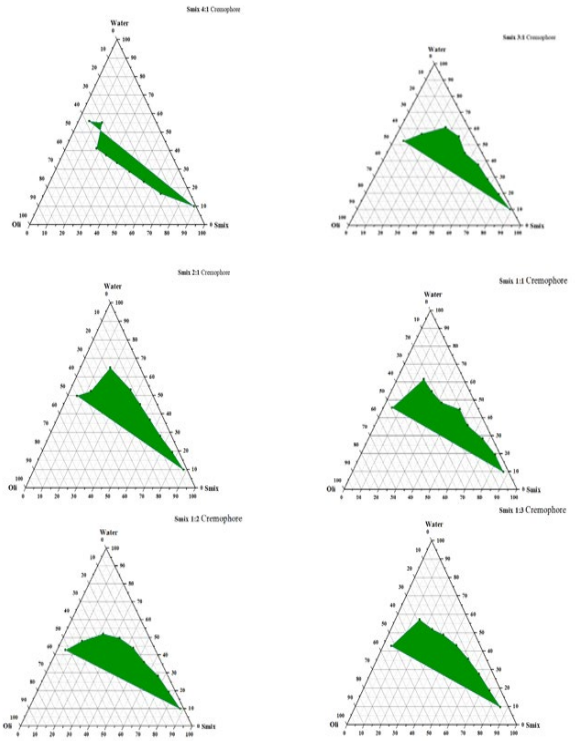

Figure 2: Nanoemulsion is depicted in color in pseudo-ternary phase diagrams of oleic acid, cremophor, and transcutol at various slurry ratios.

A nanoemulsion can be spontaneously formed upon gentle mixing during the aqueous titration and aided by the concentration and adsorption of stabilizers and/or co-stabilizers at the oil globule interface. In this study, fifteen formulas were developed by varying Smix and the percentage of oleic acid oil. The fifteen formulas that were prepared showed an initial uniform, clear, pale-yellow appearance. However, F1, F4, F7, F10, F11, F13, and F14 were not considered in the evaluation because they showed turbidity after 48 hours of storage, which may have resulted from higher drug loading than the drug's saturated solubility. The test results for measuring the average globule size and polydispersity index (PDI) for nanoemulsion in distilled water and 0.1N HCl are shown in Table 4. Table 4 shows that the mean globular size ranges from 80.32 to 267.1 nm and 76.3 to 196.6 nm in distilled water and 0.1 N HCl, respectively. All formulations that were evaluated had average globule sizes below 200 nm, which meet the requirements for nanoemulsion. Additionally, the PDI values for all formulations were below 0.5 and ranged from 0.20 to 0.46.

Table 4: Self-nano-emulsification and dispersibility grade time of liquid bilastine-loaded self- nanoemulsion

Formula code	T Emulsification (Sec)	Grade
F2	37 ± 1.5	A
F3	48 ± 3	B
F5	36 ± 1	A
F6	43 ± 1.5	A
F8	53 ± 0.5	A
F9	67 ± 1.2	B
F12	79 ± 2.1	B
F15	140 ± 3.7	C

The results confirmed the homogeneity of the dispersion and demonstrated its uniformity. The emulsification study is an essential indicator for describing how well the prepared systems produce homogenous nanoemulsions when diluted for oral ingestion. Therefore, the effectiveness could be assessed visually by measuring how long it took for the prepared formulations to completely transform into fine dispersion after mild agitation [19]. According to Table 4, the emulsification time ranged from 36 to 140 seconds. Following oral administration of the dosage form, GIT fluids would gradually dilute SNEDDS. The prepared formulas were subsequently tested for resistance to infinite dilution, which simulates in vivo conditions, and evaluated for resistance to various dilution folds. The reconstituted formulations demonstrated good nanoemulsion stability as exhibited 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, as shown in Table 5. The data from the study on thermodynamic stability is displayed in Table 6. F8, F9, F12, and F15 were the four formulas that displayed instability. As illustrated by Table 6, the drug content varied from 89% to 100%. The *in-vitro* release properties of the manufactured formulas and the pure BLS were evaluated in 0.1 N HCl.

Table 5: Data about dispersion stability and percent transmittance (%T) at various fold dilutions 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	24-hour stability in 0.1N HCl
F2	93.2	97.3	98.9	91.6	94.5	97.8	Stable
F3	83.4	89.3	96.7	85.6	90.4	95.7	Stable
F5	98.2	98.7	99.1	98.5	98.8	99.4	Stable
F6	75.3	86.5	90.7	81.3	87.6	96.2	Stable
F8	94.1	96.4	98.6	91.7	93.6	98.7	Stable
F9	79.6	88.9	97.3	82.4	91.5	97.8	Stable
F12	47.6	69.9	92.1	45.3	88.1	95.4	Unstable
F15	33.1	65.4	86.2	34.2	75.9	90.1	Unstable

Table 6: The results of a study on the thermodynamic stability of self-nanoemulsion loaded with bilastine

Formula Code	Centrifugation Test	Heating-cooling cycles test (45°C-4°C)	Freezing- thawing cycles (-20°C to 25°C)	Stability	Drug Content (%)
F2	√	√	√	Stable	95
F3	√	√	√	Stable	98
F5	√	√	√	Stable	99.8
F6	√	√	√	Stable	97
F8	√	√	X	Unstable	100
F9	√	X	-	Unstable	89
F12	√	√	X	Unstable	92
F15	√	X	-	Unstable	97

Figure 3A shows that all prepared formulations showed higher drug release than BLS pure powder, regardless of how the component ratios varied. All of the prepared liquid SNEDDS formulations displayed various release patterns in comparison to the pure drug (f = 50).

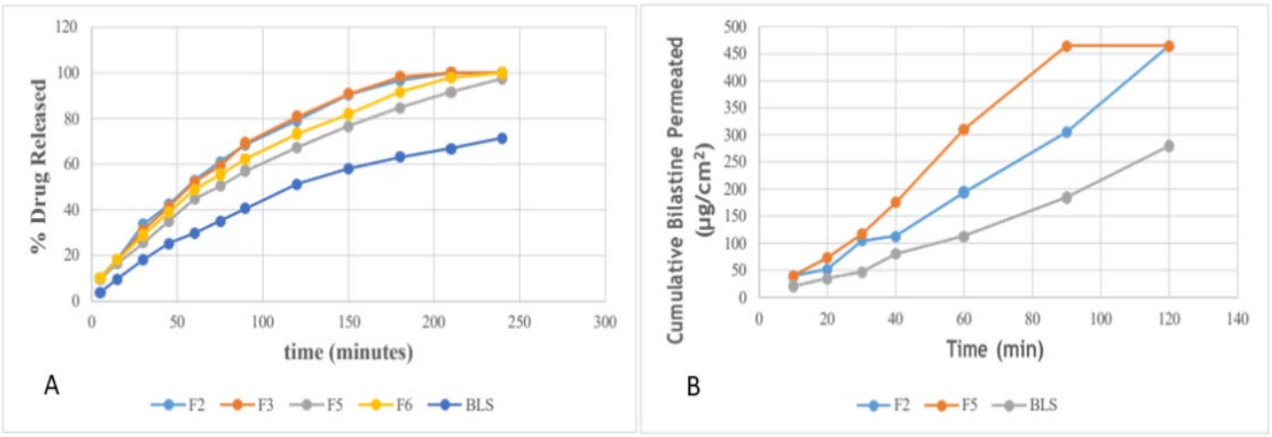

Figure 3: *In-vitro* and *Ex-vivo* analysis. **A**) Effect of stabilizer types on the release profile of loaded bilastine in 0.1N HCl maintained at 37 ºC temp; **B**) The cumulative bilastine drug release from F2, F5, and pure belastine powder at pH7 maintained in phosphate buffer pH 7.4 at 37 ºC temp.

Figure 3B shows the total amount of BLS that entered the Jejunum sac. The steady-state flux, permeability coefficient, and total amount that permeated from different BLS-loaded SNEs after 120 minutes were all found to be significantly higher than those from pure BLS suspension ($p< 0.05$). Table 7 provides a brief overview of the diffusion parameters. Formula F5 manifested the highest cumulative amount diffused at 120 min., flux, and permeability coefficient compared to F2 and pure BLS.

Table 7: The Diffusion parameters of bilastine permeability study from various bilastine–loaded SNEDDS and plane bilastine suspension

Formula code	Cumulative amount diffused at 120 min ($Q_{120\ min}$-$^{\mu g}$)	Flux (dQ/dt.S) (µg/min.cm^2)	Permeability coefficient $P_{app}*10^{-4}$ (cm/min)	Lag time (min)
F2	1273.69±3	3.69	7.38	13
F5	1646± 4.9	5.14	10.28	9
BLS	690.5±4.1	2.3	6.2	31

DISCUSSION

The capillary tube method was used for measuring the melting point to identify BLS and assess its purity. Pure BLS's powder melting point was at 203ºC, which is consistent with the melting point measurements given in the references, this indicating the purity of the drug [27]. To ensure the efficiency of SNEDDS in gastrointestinal fluid, an effective SNEDDS must spontaneously produce nanoemulsions. Therefore, choosing the right

ingredients to create SNEDDS is crucial. The study was carried out in a way that mimics the circumstances of the GIT. According to the literature [1], popular excipients were chosen for the formulation of SNEDDS because they were not affected by GIT changes in pH or ionic strength. Oleic acid showed a significantly better solubility profile compared to other oils, so it was selected for the formulation of BL as a SNEDDS. This can be related to the characteristic feature of the oleic acid structure that has a long-chain carboxylic acid with one double bond in the middle of the chain, between C9 and C10. Cremophor and Transcutol were also selected as stabilizers and co-stabilizers, respectively, for the preparation of BLS formulas according to the highest solubility of BLS in them. By calculating percent transmittance, which should not be less than 80%, the clarity of formed dispersion was standardized. The relationship between the amount of light intensity scattered and the optical clarity of fine dispersion is inverse. When particles in a dispersion are large or concentrated, they scatter more light, leading to a decrease in optical clarity. This results in a turbid or opaque appearance. Conversely, when particles are small or diluted, they scatter less light, leading to an increase in optical clarity. This results in a clear or transparent appearance. [28,29]. The safety of the stabilizer and the likelihood that some stabilizers will irritate GIT are two other crucial factors that must be considered when choosing a stabilizer [29]. Because Cremophor is non-toxic, non-irritating, and belongs to a class of non-ionic stabilizers that are generally regarded as safe and typically preferred over other counterparts, in addition to the higher solubility of the drug in it. The emulsification study for the selection of co-stabilizers and stabilizers also supports the use of Cremophor and Transcutol for the formulation of SNEDDS. The difference in HLB value and stabilizer structure may be responsible for the variation in the number of flask inversions and percent transmittance. In other words, the size and shape of the hydrophilic head, the number of tails, and the length of the tails all have an impact on the development of nanoemulsions [30]. Cremophor was chosen because of its higher percent transmittance and better solubility of BLS in it. Transcutol showed a higher percentage transmittance and higher BLS solubilizing potential, which may improve the drug loading capacity. These results may have been helped by co-stabilizers, especially those with short chains, which let oil move toward the hydrophobic tail of the stabilizer molecule, which made the interfacial tension much lower. The various curvatures (curvature refers to the shape or bending of the stabilizer film at the oil-water interface) required to produce nanoemulsions can be accomplished by increasing the fluidity of the hydrocarbon component of the surface film by intercalating themselves between stabilizer monomers. The curvature of the stabilizer film plays a critical role in determining the stability and structure of nanoemulsions. A specific curvature is required to form stable nanoemulsions [31]. Regarding the pseudo-ternary phase diagram, HLB values, which are a crucial component in the development of self-nanoemulsion, are taken into consideration when choosing components for an SNEDDS in addition to solubility. Even though stabilizers having 12-15 HLB values are thought to have spontaneous emulsification properties, the ideal HLB value for the O/W emulsion should be between 8 and 18 [32]. Therefore, when choosing the components for the ideal SNEDDS formulation, both solubility and HLB were taken into account. For the pseudo-ternary phase diagram, oleic acid was chosen as the oil, along with Cremophor EL (HLB = 12-13) and Transcutol as the stabilizer and co-stabilizer, respectively. The area of nanoemulsion formation and the most suitable ingredient percentage needed for a SNEDDS formulation are both determined by the pseudo-ternary phase. Transparent isotropic regions are those that self-nanoemulsifying. According to water titration, the shaded region in the diagram represents the area where a system without any active ingredients forms a self-nanoemulsion [33]. Because it controls the rate and extent of drug release as well as absorption, the droplet size of the emulsion is an essential factor of self-emulsification performance [7]. Additionally, PDI, which stands for the size distribution width, typically describes the quality of homogenous dispersion. A small range of droplet sizes, homogeneity, and mono-dispersion of the self-nanoemulsion, as well as long-term stability, are described by a low PDI value [34]. High zeta potential between adjacent droplets indicates high electrostatic repulsion of similarly charged particles. So, ZP may be related to the stability of colloidal dispersion by stopping particles from sticking together and coalescing in the GIT or while they are being stored [35]. According to Hernández and Goymann, nanoparticle stability necessitates ZP values above 8–9 mVolt [36]. Formulations with a ZP value of more than +30 mVolt or less than -30 mVolt are typically considered electrically stable [34]. The measured zeta potential and the concentration of oil or S_{mix} were not correlated. This may be because the fact that the zeta value was not a straightforward function of a single factor but rather may have involved several complex interactive factors. The prepared formulas have been exposed to stress conditions to further ensure their stability against creaming, coalescence, flocculation, sedimentation, and phase separation, as nanoemulsions differ from conventional emulsions in terms of thermodynamics and kinetics. Therefore, after centrifugation and abrupt temperature changes, such as heating-cooling cycles and freezing-thawing cycles, the prepared formulas were visually evaluated [37]. While the temperature changes caused instability in some formulations because the nanoemulsion was occasionally thermodynamically unstable, all formulas passed the centrifugation test because it was kinetically

stable [38]. Table 6 shows that a certain amount of oleic acid, Cremophor, and Transcutol was needed to make thermodynamically stable SNE. The drug content results are within the acceptable range as stated by the United States Pharmacopoeia requirement of drug content [39]. Traditional dissolution tests help depict how SNEDDS disperse in the media of the dissolution. However, these traditional tests are insufficient for the simulation of the *in vivo* dissolution and deciding the precise profile of drug release. This may be attributed to the inability to differentiate between the quantities of the drug that are dissolved and those that are entrapped in O/W nanoemulsion droplets that formed upon SNE dispersion in an aqueous medium [13]. By allowing only the dissolved drug to permeate, the dialysis bag approach allowed for a more precise assessment of the release of the drug from the SNEDDS platforms. A dialysis membrane with a molecular weight cutoff of 8000 to 14000 Da was used in this study to make sure that the particles that were exposed to the dissolution medium had a lot of surface area [40]. All tested formulations were diluted with 0.1N HCl to reduce their adhesion to the membrane [41]. The rapid self-emulsification capabilities of SNEDDS, as seen in Figure 3, and their ability to produce nanoemulsions with small droplet sizes upon dilution may be the trigger of the apparent improvement in the in vitro drug release profiles. [41].

Conclusion

The study showed that bilastine-loaded SNEDDS with 20% oleic acid, 27% cremophor, and 53% Transcutol had good globule sizes and thermodynamic stability in the nanometric system range. When compared to pure bilastine powder, all formulations demonstrated increased *in vitro* drug dissolution profiles, and ex vivo studies show the SNEDDS components' enhancing properties and may offer the possibility of greater absorption and bioavailability.

Conflict of interests

The authors declared no conflict of interest.

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Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

REFERENCES

1. Nair AB, Singh B, Shah J, Jacob S, Aldhubiab B, Sreeharsha N, et al. Formulation and evaluation of self-nanoemulsifying drug delivery system derived tablet containing sertraline. *Pharmaceutics*. 2022;14(2):336. doi: 10.3390/pharmaceutics14020336.
2. Dolly SA, Talele AN, Prajapati AP, Narkhede SB. Formulation development and evaluation of sublingual drug delivery system of bilastine for allergic rhinoconjunctivitis. *IAJPS*. 2021;8(4):166-181. doi: 10.5281/zenodo.4710585.
3. Falcon RM, Caoili SE. Immunologic, genetic, and ecological interplay of factors involved in allergic diseases. *Front Allergy*. 2023;4:1215616. doi: 10.3389/falgy.2023.1215616.
4. Leceta A, Sologuren A, Valiente R, Campo C, Labeaga L. Bilastine in allergic rhinoconjunctivitis and urticaria: a practical approach to treatment decisions based on queries received by the medical information department. *Drugs Context*. 2017;6:212500. doi: 10.7573/dic.212500.
5. Zhang K, Wang Q, Yang Q, Wei Q, Man N, Adu-Frimpong M, et al. Enhancement of oral bioavailability and anti-hyperuricemic activity of isoliquiritigenin via self-microemulsifying drug delivery system. *AAPS Pharm SciTech*. 2019;20:1. doi: 10.1208/s12249-019-1421-0.
6. Tong Y, Zhang Q, Shi W, Wang J. Mechanisms of oral absorption improvement for insoluble drugs by the combination of phospholipid complex and SNEDDS. *Drug Deliv*. 2019;26(1):1155-1166. doi: 10.1080/10717544.2019.1686086.
7. Rathore C, Hemrajani C, Sharma AK, Gupta PK, Jha NK, Aljabali AA, et al. Self-nanoemulsifying drug delivery system (SNEDDS) mediated improved oral bioavailability of thymoquinone: Optimization, characterization, pharmacokinetic, and hepatotoxicity studies. *Drug Deliv Transl Res*. 13(1), 292-307. doi: 10.1007/s13346-022-01193-8.
8. Hazzaa SA, Abd-Alhameed SN. Formulation and evaluation of optimized Zaltoprofen lyophilized tablets by Zydis technique. *Iraqi J Pharm Sci*. 2017;26(1):40-49. doi: 10.31351/vol26iss1pp40-49.
9. Ghareeb MM, Neamah AJ. Formulation and characterization of nimodipine nanoemulsion as ampoule for oral route. *Int J Pharm Sci Res*. 2017;8(2):591. doi: 10.13040/IJPSR.0975-8232.8(2).591-02.
10. Nasr AM, Gardouh AR, Ghonaim HM, Ghorab MM. Design, formulation and in-vitro characterization of Irbesartan solid self-nanoemulsifying drug delivery system (S-SNEDDS) prepared using spray drying technique. *J Chem Pharm Res*. 2016;8(2):159-183.
11. Abushal AS, Aleanizy FS, Alqahtani FY, Shakeel F, Iqbal M, Haq N, et al. Self-nanoemulsifying drug delivery system (SNEDDS) of apremilast: In vitro evaluation and pharmacokinetics studies. *Molecules*. 2022;27(10):3085. doi: 10.3390/molecules27103085.
12. Mahmoud H, Al-Suwayeh S, Elkadi S. Design and optimization of self-nanoemulsifying drug delivery systems of simvastatin aiming dissolution enhancement. *Afr J Pharm Pharmacol*. 2013;7(22):1482-1500. doi: 10.5897/AJPP2013.2987.
13. Al-Tamimi DJ, Hussein AA. Formulation and characterization of self-microemulsifying drug delivery system of tacrolimus. *Iraqi J Pharm Sci*. 2021;30(1):91-100. doi: 10.31351/vol30iss1pp91-100.
14. Alothaid H, Aldughaim MS, Yusuf AO, Yezdani U, Alhazmi A, Habibullah MM, et al. A comprehensive study of the basic formulation of supersaturated self-nanoemulsifying drug delivery systems (SNEDDS) of albendazolum. *Drug Deliv*. 2021;28(1):2119-2126. doi: 10.1080/10717544.2021.1986601.
15. Chandan C, Maheshwari RK. Mixed solvency concept in reducing stabilizer concentration of self-emulsifying drug delivery systems of candesartan cilexetil using D-optimal mixture design. *AJP*. 2013;7(2). doi: 10.4103/0973-8398.115960.
16. Sabri LA, Hussein AA. Comparison between conventional and supersaturable self-nanoemulsion loaded with nebivolol: Preparation and in-vitro/ex-vivo evaluation. *Iraqi J Pharm Sci*. 2020;29(1):216-225. doi: 10.31351/vol29iss1pp216-225.
17. Ali HH, Hussein AA. Oral nanoemulsions of candesartan cilexetil: Formulation, characterization and in vitro drug release studies. *AAPS Open*. 2017;3(1):4. doi: 10.1186/s41120-017-0016-7.
18. Bhagwat DA, Swami PA, Nadaf SJ, Choudhari PB, Kumbar VM, More HN, et al. Capsaicin loaded solid SNEDDS for enhanced bioavailability and anticancer activity: In-vitro, in-silico, and in-

vivo characterization. *J Pharm Sci*, 2021;110(1):280-291. doi: 10.1016/j.xphs.2020.09.066.
19. Ochoa D, Román M, Belmonte C, Martín-Vilchez S, Mejía-Abril G, Abad-Santos F, et al. Pharmacokinetics and safety of a bilastine once-daily, preservative-free, ophthalmic formulation. *Adv Ther*. 2021;38(7):4070-408. doi: 10.1007/s12325-021-01801-y.
20. Salim FF, Rajab NA. Formulation and characterization of piroxicam as self-nano emulsifying drug delivery system. *Iraqi J Pharm Sci*. 2020;29(1):174-183. doi: 10.31351/vol29iss1pp174-183.
21. Teaima M, Hababeh S, Khanfar M, Alanazi F, Alshora D, El-Nabarawi M. (2022). Design and optimization of pioglitazone hydrochloride self-nanoemulsifying drug delivery system (SNEDDS) incorporated into an orally disintegrating tablet. *Pharmaceutics*. 2022;14(2):425. doi: 10.3390/pharmaceutics14020425.
22. Verma R, Kaushik A, Almeer R, Rahman MH, Abdel-Daim MM, Kaushik D. (2021). Improved pharmacodynamic potential of rosuvastatin by self-nanoemulsifying drug delivery system: An in vitro and in vivo evaluation. *Int J Nanomedicine*, 2021;16:905-922. doi: 10.2147/IJN.S311078.
23. Shrestha B, Dunn L. The declaration of Helsinki on medical research involving human subjects: A review of seventh revision. *J Nepal Health Res Counc*. 2020;17(4):548-552. doi: 10.33314/jnhrc.v17i4.1042.
24. Garber JC, Barbee RW, Bielitzki JT, Clayton LA, Donovan JC, Hendriksen CFM, et al. Guide for the care and use of laboratory animals. Washington, DC: National Academy Press; 2011.
25. Huang YB, Tsai YH, Yang WC, Chang JS, Wu PC. Guidance for industry, dissolution testing of immediate release solid oral dosage forms. *Biol Pharm Bull*. 2004;27(10):1626-1629. doi: 10.1155/2013/197398.
26. Čebzan D, Pavlović A, Kokanović J, Trijić S, Jovanović V, Mladenović A, et al. Dissolution profile comparison using similarity factor F2 in sertralin tablets. *Dissolution Technol*. 2006;56(5):694-695. doi: 10.14227/DT060399P15.
27. Rathi SG, Chaudhari DB, Vaghela SS, Kamani KR. Physicochemical characterization and dissolution enhancement of bilastine by solid dispersion. *Int J Pharm Sci Rev Res*. 2021;69(1):194-200. doi: 10.47583/ijpsrr.2021.v69i01.028.
28. Khatri P, Shao J. Mechanism and structural factors of lipid and stabilizer in the formation of self-emulsified nanoemulsion. *J Pharm Sci*. 2018;107(8):2198-2207. doi: 10.1016/j.xphs.2018.03.017.
29. Jain K, Kumar RS, Sood S, Gowthamarajan K. Enhanced oral bioavailability of atorvastatin via oil-in-water nanoemulsion using aqueous titration method. *J Pharm Sci Res*. 2013;5(1):18.
30. Amin MM, El Gazayerly ON, Abd El-Gawad NA, Abd El-Halim SM, El-Awdan SA. Effect of formulation variables on design, in vitro evaluation of valsartan SNEDDS and estimation of its antioxidant effect in adrenaline-induced acute myocardial infarction in rats. *Pharm Dev Technol*. 2016;21(8):909-920. doi: 10.3109/10837450.2016.1162630.
31. Usta DY, Timur B, Teksin ZS. Formulation development, optimization by Box-Behnken design, characterization, in vitro, ex-vivo, and in vivo evaluation of bosentan-loaded self-nanoemulsifying drug delivery system: A novel alternative dosage form for pulmonary arterial hypertension treatment. *Eur J Pharm Sci*. 2022;174:106159. doi: 10.1016/j.ejps.2022.106159.
32. Inugala S, Eedara BB, Sunkavalli S, Dhurke R, Kandadi P, Jukanti R, et al. Solid self-nanoemulsifying drug delivery system (S-SNEDDS) of darunavir for improved dissolution and oral bioavailability: In vitro and in vivo evaluation. *Eur J Pharm Sci*. 2015;74:1-10. doi: 10.1016/j.ejps.2015.05.001.
33. Saifullah S, Kanwal T, Ullah S, Kawish M, Habib SM, Ali I, et al. Design and development of lipid modified chitosan containing muco-adhesive self-emulsifying drug delivery systems for cefixime oral delivery. *Chem Phys Lipids*. 2021;235:105052. doi: 10.1016/j.chemphyslip.2021.105052.
34. Mekhilef SF, Hussein AA. (2018). Novel combination for self-nanoemulsifying drug delivery system of candesartan cilexetil. *Iraqi J Pharm Sci*. 2018;27(2):123-134. doi: 10.31351/vol27iss2pp123-134.
35. Fitria A, Hanifah S, Chabib L, Uno AM, Munawwarah H, Atsil N, et al. (2021). Design and characterization of propolis extract loaded self-nano emulsifying drug delivery system as immunostimulant. *Saudi Pharm J*. 2021;29(6):625-634. doi: 10.1016/j.jsps.2021.04.004.
36. Villalobos-Hernández JR, Müller-Goymann CC. Novel nanoparticulate carrier system based on carnauba wax and decyl oleate for the dispersion of inorganic sunscreens in aqueous media. *Eur J Pharm Biopharm*. 2005;60(1):113-122. doi: 10.1016/j.ejpb.2004.11.002.
37. Reddy MR, Gubbiyappa KS. Formulation development, optimization and characterization of Pemigatinib-loaded supersaturable self-nanoemulsifying drug delivery systems. *Future J Pharm Sci*. 2022;8(1):45. doi: 10.1186/s40502-022-00367-7.
38. The United States Pharmacopoeia (USP) 30 N. NF 25. (2006). The United States Pharmacopeial Convention Inc. USA: Rockville, 1191-1193, 2110.
39. Wu W, Wang Y, Que L. Enhanced bioavailability of silymarin by self-microemulsifying drug delivery system. *Eur J Pharm Biopharm*. 2006;63(3):288-294. doi: 10.1016/j.ejpb.2006.02.003.
40. Bakhle SS, Avari JG. Development and characterization of solid self-emulsifying drug delivery system of cilnidipine. *Chem Pharm Bull*. 2015;63(6):408-417. doi: 10.1248/cpb.c14-01047.
41. Park EJ, Choi SA, Min KA, Jee JP, Jin SG, Cho KH. Development of alectinib-suspended SNEDDS for enhanced solubility and dissolution. *Pharmaceutics*, 2022;14(8):1694. doi: 10.3390/pharmaceutics14081694.