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Development and characterization of bilastine nanosuspension for enhanced dissolution in orodispersible films

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ABSTRACT

Bilastine, a second-generation antihistamine, is commonly prescribed for managing allergic rhinoconjunctivitis and urticaria due to its prolonged action. However, its therapeutic potential is constrained by poor water solubility and low oral bioavailability. This study aimed to enhance bilastine dissolution and patient compliance by formulating a nanosuspension-based orodispersible film (ODF). An anti-solvent precipitation method was employed to produce nanosuspension using different hydrophilic stabilizers (Soluplus®, Poloxamer 188, and PEG 6000). The influence of formulation parameters, such as the stabilizer ratio, the anti-solvent ratio, stirring speed, and the stabilizer type, on particle size and polydispersity index (PDI) was optimized using an experimental design approach. The optimal formulation, with a 1:1 stabilizer-to-drug ratio using Soluplus®, a 6:1 anti-solvent to solvent ratio, and a stirring rate of 820 rpm, yielded nanoparticles with a mean particle size of 83.8 nm and a narrow PDI of 0.019. This formulation also significantly enhanced the drug's dissolution rate in phosphate buffer pH 6.8, releasing 92.02% of bilastine within 90 minutes. Further characterization of the lyophilized nanoparticles using FESEM, FTIR, and XRD, confirmed their amorphous nature and drug compatibility. The optimized nanosuspension was subsequently incorporated into ODFs via the solvent-casting technique, with the optimal film formulated with a 1:1 ratio of PVA and HPMC E5 as the film-forming polymers, demonstrating a rapid disintegration time of 18 seconds and releasing 93.16% of bilastine within 6 minutes. These results confirm the successful formulation of bilastine into ODFs, significantly improving its dissolution compared to the pure drug.

1. Introduction

Oral delivery is the most convenient and widely preferred route in drug therapy, owing to its safety, high patient compliance, ease of administration, cost-effectiveness, and scalability. However, its primary limitation lies in the challenges associated with drug bioavailability [1,2]. The release of drugs is a critical factor that determines their bioavailability when taken orally, especially for drugs categorized under Biopharmaceutical Classification System (BCS) class II, which have low solubility but high permeability. Enhancing the drug release characteristics of these medications can potentially increase their bioavailability and minimize adverse effects [3]. The conventional strategy for improving the dissolution rate of poorly soluble drugs typically involves particle size reduction through micronization. However, achieving further enhancement in dissolution rate and bioavailability necessitates shifting

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from micronization to nanonization. This transition requires the development of innovative technological approaches and solutions to overcome the physicochemical and stability challenges inherent to nanostructures [1].

Nanosuspension is a biphasic colloidal dispersion of extremely fine solid particles in an aqueous medium stabilized with surfactant and/or polymer. It has been proven to enhance the effectiveness of medications with low water solubility by enabling faster dissolution of smaller particles. This improvement boosts both the rate and magnitude of absorption [3,4]. The main advantages of nanosuspension that make it an integral part of nano-carriers are improved bioavailability, reduced toxicity, and ease of administration through different routes. Many techniques may be used to produce nano-sized particles, including top-down techniques that involve turning big particles into smaller ones, and bottom-up techniques where the drug in its solution precipitates by introduction into an anti-solvent [5]. In the process of designing, developing, and optimizing nanoparticle production at an industrial scale, it is imperative to tackle various challenges, especially those associated with instability [1].

Orodispersible films (ODFs) appear to be an excellent alternative for solidifying drug nanosuspensions without causing agglomeration, effectively transforming the suspended drug into a solid dosage form suitable for patients of all ages [6]. ODFs are thin polymeric strips characterized by their ability to rapidly disintegrate in the tongue without requiring chewing or swallowing facilitating immediate drug release [2].They offer several advantages compared to traditional dosage forms, including ease of self-administration and the elimination of the need for water during intake, which can enhance patient adherence. They are also stable, effective, and provide improved bioavailability by reducing the impact of first-pass metabolism. Additionally, the development process for ODFs is relatively straightforward [7]. Film manufacturing techniques commonly include solvent casting, hot-melt extrusion, and rolling. Of these methods, solvent casting is the most prevalently employed by researchers for film production [2,7].

Bilastine is a specific, prolonged-action second-generation H1-receptor antagonist. Initially authorized in the European Union in 2010, it is used to manage symptoms of allergic rhinoconjunctivitis and urticaria [8]. It is a BCS class II drug with an absolute oral bioavailability of 60.67%, which decreases by approximately 30% due to food-drug interactions in the presence of food [9,10]. Abbas & Abd Al Hammid reported that the marketed tablets released only 62.27% of the drug [11]. Therefore, efforts were undertaken to improve its dissolution rate and oral bioavailability by different methods, including formulating it as solid dispersion using polyvinyl pyrrolidone (PVP K30) and poloxamer188 (PLX188) and as a self-nano emulsifying drug delivery system [12,13]. This study aimed to develop a bilastine nanosuspension (BLA-NS) utilizing the anti-solvent precipitation method with Soluplus®, PLX188, and PEG6000 as stabilizers, and to incorporate the optimized nanosuspension directly into orodispersible films via solvent casting to enhance dissolution and enable rapid drug action regardless of feeding state.

2. Materials and methods

2.1. Materials

Bilastine (Wuhan HSN Pharmaresearch CO. Ltd., China), Soluplus® (BASF, Germany), poloxamer188 (PLX 188) (Eastman Chemical Company, USA), Polyethylene glycol 6000 and 400 (PEG6000 and PEG400), Polyvinyl alcohol (PVA), KH_2PO_4, and Na_2HPO_4 (HiMedia, India), Hydroxypropyl methylcellulose (HPMC E5), Pullulan (purchased from China), glycerin and Mannitol (Hopkin & Willims, UK), methanol (chem-lab, Belgium), and Dialysis membrane 8000-14000 Da (MYM company, USA).

2.2. Methods

2.2.1. Saturation solubility determination

The solubility of the pure drug was assessed using the shake-flask method. Excess bilastine was dissolved in phosphate buffer (pH 6.8) and distilled water, then shaken for 72 hours at 25°C (water) and 37°C (phosphate buffer). The samples were filtered with a 0.45 μm syringe filter, diluted, and analyzed via UV-visible spectrophotometry at 274 nm [12].

2.2.2. Preparation of bilastine nanosuspension

Bilastine nanosuspension (BLA-NS) was prepared by an anti-solvent precipitation technique. Firstly, dissolve 10 mg of bilastine in 3 ml methanol (solvent). The resulting drug solution was dropped using a syringe with a surgical needle (gauge 25) at a steady slow rate of 0.5 ml/min (over 6 minutes) into deionized water (anti-solvent) in the presence of a stabilizer (Soluplus®, poloxamer188, or PEG6000). The volumes of deionized water were adjusted to get ratios of 3:1, 5:1, and 7:1 anti-solvent to solvent. Dropping was performed with continuous stirring at different rates (500, 1000, and 1500 rpm) on a magnetic stirrer at room temperature, followed by an additional hour of stirring to promote organic solvent evaporation [14].

Various BLA-NS formulations were developed depending on the design of experiment approach. This approach sought to reduce the total number of experiments while clarifying the impact of different factors to achieve the optimum nanoformulation according to statistical calculations [15].

2.2.3. Optimization of bilastine nanosuspension

The optimization of BLA-NS was carried out using four factors at three different levels within the D-optimal design framework, using Design-Expert software (version 13, Stat-Ease Inc., Minneapolis, MN, USA), resulting in 28 experimental trials. The independent variables included the stabilizer-to-drug ratio (referred to as stabilizer ratio, A), the anti-solvent-to-solvent ratio (anti-solvent ratio, B), stirring rate (C), and stabilizer type (D) as outlined in Table 1. Their effects on particle size (PS) and polydispersity index (PDI) were assessed.

The optimal BLA-NS formulation was selected based on the desirability function to achieve the smallest PS and PDI. The highest-scoring solution was lyophilized using a vacuum freeze dryer without adding a cryoprotectant to ensure analytical clarity. The resulting nanoparticles were then used for subsequent analytical studies [16].

2.2.4. Characterization of bilastine nanosuspension

2.2.4.1. Particle size and polydispersity index. The PS and PDI of each formulation were measured using dynamic light scattering (DLS) with a Zetasizer (Malvern Instruments, UK). Nanosuspension liquid formula samples were measured in triplicate at room temperature, with a detection angle of 90° [17,18].

2.2.4.2. Drug content. The bilastine content in each formula was measured to validate the method's effectiveness and ensure formulation quality. A specified volume of each formula was diluted with methanol and measured spectrophotometrically at its λmax (275nm) in triplicate. Eq. (1) was utilized to determine the % drug content [12,19].

$$Drug\ content\ \% = \frac{Calculated\ drug\ content}{Theoretical\ drug\ content} \times 100 \quad (1)$$

2.2.5. Characterization of the optimal formula

2.2.5.1. In vitro drug release. The optimized formula of BLA-NS was evaluated for the in vitro dissolution using a dialysis membrane (MWCO 8000-14000 Da) and set to paddle of USP dissolution apparatus-Type II applying a rotation rate of 50 rpm. Furthermore, the release of pure drug and physical mixture at same conditions of the nanosuspension formula was also tested. Phosphate buffer (pH 6.8) was utilized as a dissolution environment in a volume of 900 mL at 37 ± 0.5°C. 5 mL volume was withdrawn at specified intervals, and substituted with fresh buffer, then measured spectrophotometrically at λmax of 274 nm [12,17].

A comparison was conducted between the release patterns of BLA-NS formula versus bilastine pure powder and physical mixture, by using similarity factor $f2$. DDSolver add-ins program was used for dissolution profile comparison.

2.2.5.2. Determination of entrapment efficiency. The entrapment efficiency (EE%) of the selected BLA-NS formulation was evaluated by the ultrafiltration method using an Amicon® Ultra centrifugal filter with a 10 kDa molecular weight cut-off [20]. A 4 mL sample of BLA-NS was placed in the Amicon tube and centrifuged at 5000 rpm for 30 minutes. Using the direct method, the resulting bilastine nanoparticles were diluted with methanol and analyzed by UV spectrophotometry at 275 nm. EE% was calculated using Eq. (2) [21].

$$EE\% = \frac{Obtained\ bilastine\ amount}{The\ amount\ of\ bilastine\ present\ in\ the\ formulation} * 100 \quad (2)$$

2.2.5.3. Zeta potential evaluation of nanosuspension. The surface charge properties were analyzed to evaluate the stability of the prepared nanosuspension. The zeta potential of the optimized BLA-NS formulation was measured using a Zetasizer Nano ZS (Malvern Instruments, Worcestershire, UK) [14].

2.2.5.4. Field emission scanning electron microscopy (FESEM). The surface morphology of the optimized BLA-NS formula and pure bilastine were evaluated by using field emission scanning electron microscope (FESEM). The sample was placed onto double-sided carbon tapes that were affixed to the specimen mount of the FESEM (INSPECT F50, FEI Company, The Netherlands.) The examination was conducted by employing various levels of magnification [22].

2.2.5.5. Fourier transform-infrared spectroscopy (FT-IR). Fourier transform infrared (FTIR) spectra of pure bilastine, the selected stabilizer, the lyophilized optimum formula, and its physical mixture were acquired with the use of the FTIR spectrometer (7600) (lambda scientific systems, Inc, Australia). Potassium bromide was used to compress samples. The resulting spectrum range was between 4000-400 cm-1 wavenumbers [17].

2.2.5.6. Powder X-ray diffraction (PXRD). Powder X-ray Diffraction (PXRD) analyses were performed on pure bilastine, stabilizer, optimum formula, and its physical mixture to determine their crystalline nature. The test was conducted at a speed rate of 5°/min to scan samples over a 2θ range of 3–50 [22].

Table 1
Experimental design for optimization of BLA-NS.

Independent variables	Level 1	Level 2	Level 3
(A) stabilizer ratio	1	2	3
(B) anti-solvent ratio	3	5	7
(C) stirring rate	500	1000	1500
(D) stabilizer type	Soluplus®	PLX 188	PEG6000

2.2.5.7. Preliminary stability assessment of BLA-NS. A one-month period stability study can detect potential issues, such as particle aggregation, sedimentation, or changes in drug content, which could impact drug performance [4]. The stabilities were investigated by storing the samples at 4 ∘C and room temperature. After storage for 7, 15, and 30 days, the stability was assessed by measuring particle size, PDI, and drug content of BLA-NS as a quality assurance measure [18,23].

2.2.6. Preparation of ODF

The solvent casting method was used to prepare orodispersible films of the optimized BLA-NS formula. Polyvinyl alcohol (PVA), hydroxypropyl methylcellulose (HPMC E5), and Pullulan were selected as the film-forming polymers. The specified amount of polymer was dissolved in hot deionized water (60°C) with continuous stirring at 300 rpm. After cooling, a plasticizer (either glycerin or PEG 400, the most commonly used plasticizer to enhance film flexibility) was added at 20% w/w of the polymer amount [24] and stirred for one hour. Mannitol and citric acid were incorporated as sweetener and saliva stimulant, respectively. The freshly prepared BLA-NS, ensuring a 10 mg dose per film, was then incorporated into the polymer solution with continuous stirring.

The homogeneous mixture was poured into a 7 cm Petri dish and dried at room temperature. Once dried, the film was peeled, cut into 2×3 cm pieces, and stored for evaluation. Six BLA-NS-loaded ODF formulations were prepared, as shown in Table 2. The amounts in Table 2 represent the total quantities used in the film preparation. A plain ODF (p7) containing the pure drug was also prepared to compare the release profile with the nanosuspension-loaded films [25].

2.2.7. Evaluation of ODFs

2.2.7.1. Physical appearance and surface texture. The films were visually examined to evaluate their color, clarity, surface smoothness, and the presence of any air bubbles [21,26].

2.2.7.2. Weight variation test. To assess weight variation, three evenly cut film pieces (2 × 3 cm) from each successfully produced batch were individually weighed using a digital balance, followed by calculating the average weight [21].

2.2.7.3. Uniformity of film thickness measurement. The thickness of the films was measured using a digital Vernier caliper, with dimensions recorded at both the central region and the corners of each film. The mean thickness values were then calculated [26].

2.2.7.4. Folding endurance test. The number of folds serves as an indicator of the brittleness of ODFs and is crucial for assessing their suitability for storage and handling. This measurement is conducted by repeatedly folding the film at the same point at a 180° angle until it breaks. A film that withstands 300 folds is considered to have excellent flexibility [7].

2.2.7.5. Surface pH determination. The pH of the ODFs must fall within the oral cavity pH range of 5.5 to 7.4 to prevent mucosal irritation. The pH of the films was measured by placing the film in a petri dish, moistening it with distilled water at room temperature, and then using a digital pH meter to measure the pH by contacting the surface of the film [7,27].

2.2.7.6. Drug content of ODF. A film piece was placed in 15 mL of methanol and stirred for 30 minutes. Afterward, samples were withdrawn and filtered using a 0.45 µm syringe filter. The absorbance of the filtrate was then measured at 275 nm [12,21].

2.2.7.7. In vitro disintegration time. To measure disintegration time, a film piece was placed in a glass Petri dish containing 10 mL of phosphate buffer (pH 6.8) and subjected to gentle shaking. The time at which the film began to disintegrate or break was recorded as the disintegration time [7,28].

2.2.7.8. In vitro dissolution test. An in vitro dissolution study was conducted using a USP type II apparatus. A single film piece was placed in 900 mL of phosphate buffer (pH 6.8) as the dissolution medium, with the system set to 50 rpm and maintained at 37°C for 15 minutes. At predetermined intervals, 5 mL samples were withdrawn and replaced with an equal volume of fresh dissolution medium to maintain sink conditions. The samples were then filtered through a 0.45 µm syringe filter and analyzed spectrophotometrically at 274 nm [26].

Table 2
Composition of BLA-NS orodispersible films.

Film code	BLA-NS (mg)	BLA (mg)	PVA (mg)	HPMC E5 (mg)	Pullulan (mg)	Glycerin (mg)	PEG 400 (mg)	Mannitol (mg)	Citric acid (mg)
p1	128		320			64		36	25
p2	128		320				64	36	25
p3	128			320			64	36	25
p4	128				320	64		36	25
p5	128		213.4	106.6			64	36	25
p6	128		160	160			64	36	25
p7		64	160	160			64	36	25

2.2.8. Statistical analysis

The results from the experimental work are presented as the mean ± standard deviation (SD) based on three measurements. A one-way analysis of variance (ANOVA) was used to assess the significance between the various formulations, with statistical significance set at a level of $p < 0.05$.

3. Results and discussion

3.1. Saturation solubility

The saturation solubility of bilastine was found to be 0.3429 ± 0.0017 mg/ml in distilled water, and 0.51825 ± 0.0807 mg/mL in phosphate buffer pH 6.8. These solubility values demonstrate that bilastine is only very slightly soluble in both water and phosphate buffer under the specified conditions [29]. Therefore, the study was directed to enhance the solubility in these media as a preliminary study to be prepared as an orodispersible film.

3.2. Particle size and polydispersity index

The average PS and PDI of various NS batches prepared are provided in Table 3.

As illustrated in the Fig. 1, the particle size varied from 90.45 nm (F20) to 131.56 nm (F1). Additionally, the polydispersity index (PDI) ranged from 0.03554 (F16) to 0.4149 (F6). The lowest values were obtained in formulations containing Soluplus®.

3.3. Drug content

As shown in Fig. 1, the total drug content of all BLA-NS formulas ranged from 93.5% to 102.7% demonstrating the effectiveness of this method.

3.4. Optimization of BLA-NS using D-optimal design

Response surface methodology originated in experimental design, aimed at identifying the optimal variables to achieve a specific target response while minimizing the number of required experiments [30].

3.4.1. Effect of formulation variables on particle size

The quadratic model provided the best fit for the data ($p < 0.0001$) with an insignificant lack-of-fit ($p = 0.4237$), showing reliable predictive power with a predicted R^2 of 0.8518 and an adjusted R^2 of 0.9169.

The primary formulation variables—A (stabilizer ratio), B (anti-solvent ratio), C (stirring rate), and D (stabilizer type)—all were

Table 3
Experimental design, along with responses, for BLA-NSs.

F code	Factor 1 A: stabilizer ratio	Factor 2 B: anti-solvent ratio	Factor 3 C: stirring rate	Factor 4 D: stabilizer	Response 1 p. size	Response 2 PDI
F1	2	7	1500	PLX188	131.56±5.577	0.2612±0.057
F2	2	3	500	Soluplus®	124.43±8.224	0.056±0.0009
F3	2	5	1000	Soluplus®	96.54±3.36	0.039±0.034
F4	2	5	1500	PEG6000	108.61±14.094	0.2612±0.0663
F5	2	3	1000	PLX188	101.06±12.913	0.3074±0.0415
F6	3	3	1500	PLX188	124.6±9.676	0.4194±0.0142
F7	3	7	1500	Soluplus®	96.26±2.295	0.1084±0.0035
F8	2	5	1500	PEG6000	111.1±16.63	0.272±0.086
F9	3	3	500	PLX188	114.76±14.528	0.3459±0.1351
F10	1	5	1500	PLX188	117.1±2.695	0.2183±0.0160
F11	1	3	1500	PEG6000	99.78±5.861	0.311±0.0304
F12	3	7	500	PEG6000	113.1±7.543	0.287±0.0903
F13	2	5	500	PEG6000	104.93±4.4049	0.2616±0.0256
F14	1	7	500	PLX188	114.86±19.523	0.2942±0.0243
F15	3	5	1000	PLX188	107.65±9.334	0.328±0.134
F16	1	5	1500	Soluplus®	97.34±4.936	0.0355±0.01
F17	1	7	1000	PEG6000	110.7±10.454	0.3205±0.047
F18	2	3	1000	PLX188	104.96±4.53	0.293±0.118
F19	3	3	1000	Soluplus®	114.53±3.035	0.045±0.0107
F20	2	7	500	Soluplus®	90.45±4.796	0.0356±0.0023
F21	1	3	500	Soluplus®	111.3±1.0408	0.0935±0.0190
F22	2	3	1500	Soluplus®	116.6±0.5	0.1444±0.0195
F23	2	5	1000	Soluplus®	97.6±4.81	0.0371±0.011
F24	2	5	500	PLX188	112.9±3.207	0.307±0.0249
F25	2	5	500	PEG6000	107.66±7.791	0.291±0.0327
F26	3	5	1000	PLX188	113.5±15.483	0.314±0.0514
F27	3	3	1000	PEG6000	106±5.188	0.197±0.0187
F28	3	5	500	Soluplus®	104.6±2.128	0.081±0.0082

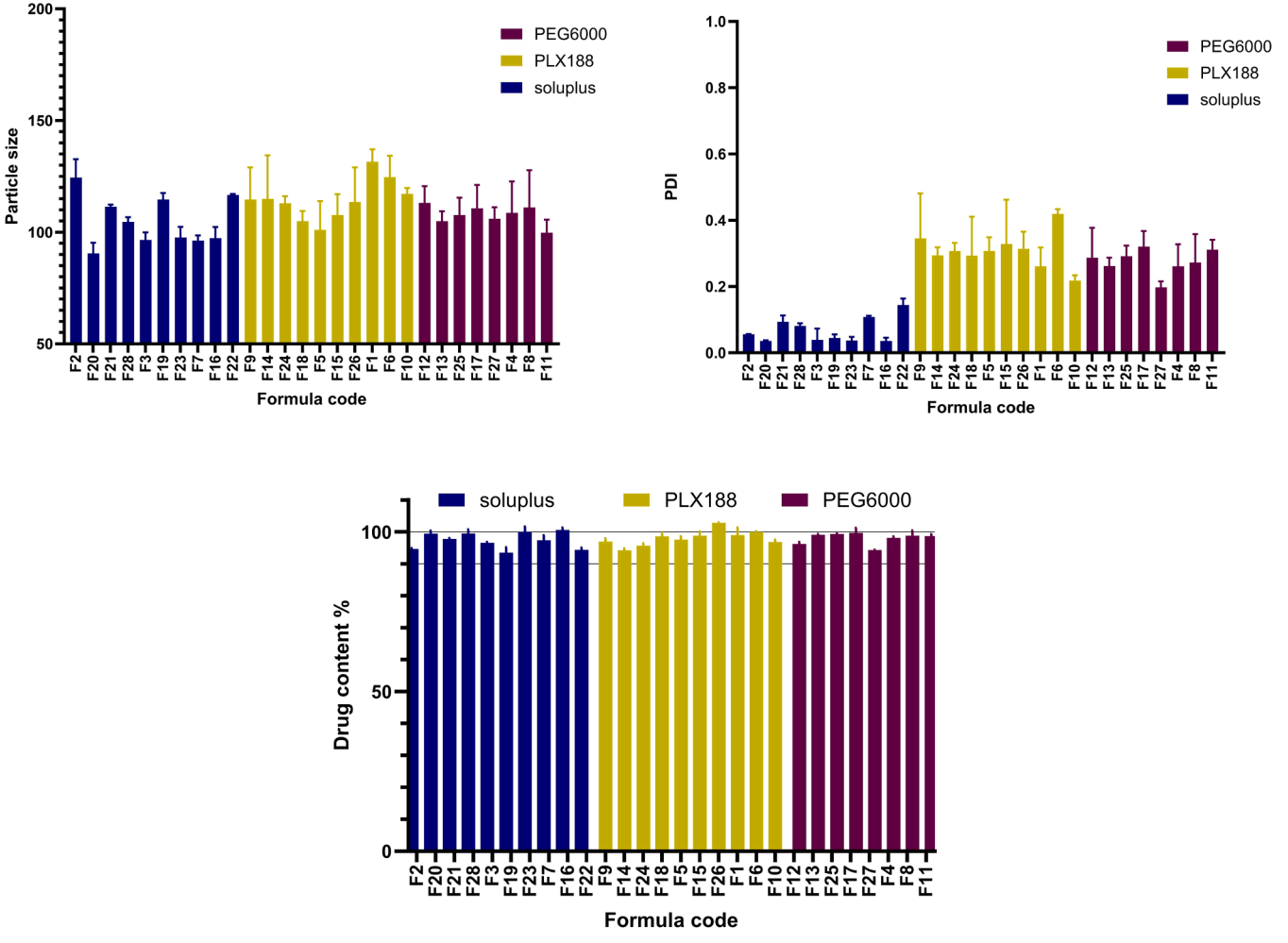

Fig. 1. PS, PDI and drug content of BLA-NS formulas.

significant model terms, along with their interactions (AB, BC, BD, and CD) and squared terms B^2 and C^2. The stabilizer ratio ($p = 0.0002$) and type ($p < 0.0001$) had the greatest impact on nanoparticle size, with a notable interaction between anti-solvent ratio and stabilizer type (BD) ($p < 0.0001$), indicating a combined influence beyond their individual effects. Additionally, the stirring rate had a nonlinear effect, and its impact becomes more apparent when considering its squared term C^2 ($p < 0.0001$) compared to its linear term ($p = 0.0072$)

3.4.2. Effect of formulation variables on PDI

The quadratic model provided the best fit for the data ($p < 0.0001$), with an insignificant lack-of-fit ($p = 0.0657$), indicating a reliable model. The predicted R^2 value (0.8849) closely matched the adjusted R^2 (0.9726).

Significant model terms included A (stabilizer ratio), D (stabilizer type), interactions (AC, AD, BC, BD), and the squared term C^2. The stabilizer type had the greatest impact on nanoparticle polydispersity ($p < 0.0001$). The stirring rate exhibited a significant nonlinear effect through its squared term ($p = 0.0024$), while its linear effect was not significant.

Based on the aforementioned results, it is evident that the effect of independent variables on PDI follows a similar pattern to their effect on the PS. The lowest PS and PDI were achieved when the stabilizer ratio was reduced towards the lower limit of the studied range (Fig. 2). This finding can be explained by the fact that a smaller amount of the stabilizer was sufficient to surround the formed nanoparticles, maintaining their stability at small sizes and preventing aggregation [19]. Conversely, increasing the stabilizer concentration led to a rise in the viscosity of the anti-solvent solution, which may hinder particle movement and result in more coating of drug particles, thereby promoting their growth [31].

Additionally, increasing the anti-solvent ratio resulted in a further reduction of PS and PDI (Fig. 2), which is consistent with previously documented results. When maintaining the same solute amount in the system, increasing the anti-solvent ratio raises the degree of supersaturation. This enhanced supersaturation accelerates the nucleation process, leading to a reduction in particle size [32].

An intermediate stirring rate was found to be optimal for minimizing PS and PDI (Fig. 2), as excessively high speeds can lead to particle collisions and aggregation, while low speeds result in insufficient dispersion and larger particle sizes [33]. This result was in agreement with a previous study by Khafeef HK, and Rajab NA who found that moderate stirring speed is recommended to maintain the desired particle size while minimizing the formation of aggregates [17].

Soluplus® proved to be the most effective stabilizer. It is a graft copolymer exhibiting amphiphilic properties, making it a highly efficient surfactant and wetting agent. It reduces the interfacial tension between the hydrophobic surface of drug particles and the aqueous anti-solvent, thereby helping to maintain the small particle size of the nanosuspension [34].

3.4.3. Determination of selected BLA-NS

BLA-NS were optimized using the optimal values for the variables obtained through numerical optimization based on the desirability function [23,30]. The suggested optimal formula was a 1:1 stabilizer ratio with Soluplus®, a 6 anti-solvent ratio, and a stirring rate of 820 rpm. The predicted outcomes were a PS of 87.895 nm and a PDI of 0.014, while the obtained actual values from the preparation of the optimal formulation process were a PS of 83.816 ± 0.903 nm and a PDI of 0.019 ± 0.00916. The close match between the observed and predicted responses for BLA-NS suggests that the design effectively anticipated the results.

3.5. Characterization of the optimized BLA-NS formula

3.5.1. In vitro release

As illustrated in Fig. 3, over 90 minutes, the release profile of the BLA-NS formulation showed significant improvement compared to the pure drug and physical mixture (*f2* = 27.00 and 19.04 respectively) in a phosphate buffer medium (pH 6.8), indicating distinct differences in dissolution behavior.

The optimized formulation achieved a release percentage of 92.02 ± 1.35%, whereas the pure drug and physical mixture had release percentages of 51.503 ± 8.853% and 34.36 ± 7.01%, respectively. This suggests that the NS formulation effectively enhances drug release, which can be attributed to the unique features of nanoparticles (augmented surface area and expected amorphous nature) [17,35].

3.5.2. The entrapment efficiency

The percentage of entrapment efficiency indicates how effectively the stabilizer surrounds the active ingredient. For the selected formula, the EE% was 89.94 ± 3.61%. The high entrapment efficiency is attributed to the appropriate selection and concentration of stabilizer [36]

3.5.3. Zeta potential evaluation

The zeta potential of the optimum formula was (-4.59 mV) owing to the use of a non-ionic stabilizer in a formulation that frequently yields lower zeta potential values. Steric stabilization relies on the presence of polymer or surfactant layers that create a physical barrier around nanoparticles, preventing them from aggregating due to steric hindrance. Therefore, even with a low zeta potential, the formulation can still be stable if the steric stabilizer is effective [16,37].

3.5.4. Field emission scanning electron microscopy (FESEM)

The optimized BLA-NS formulation exhibited uniform, spherical particles with nano-scale diameters (Fig. 4A). This morphology

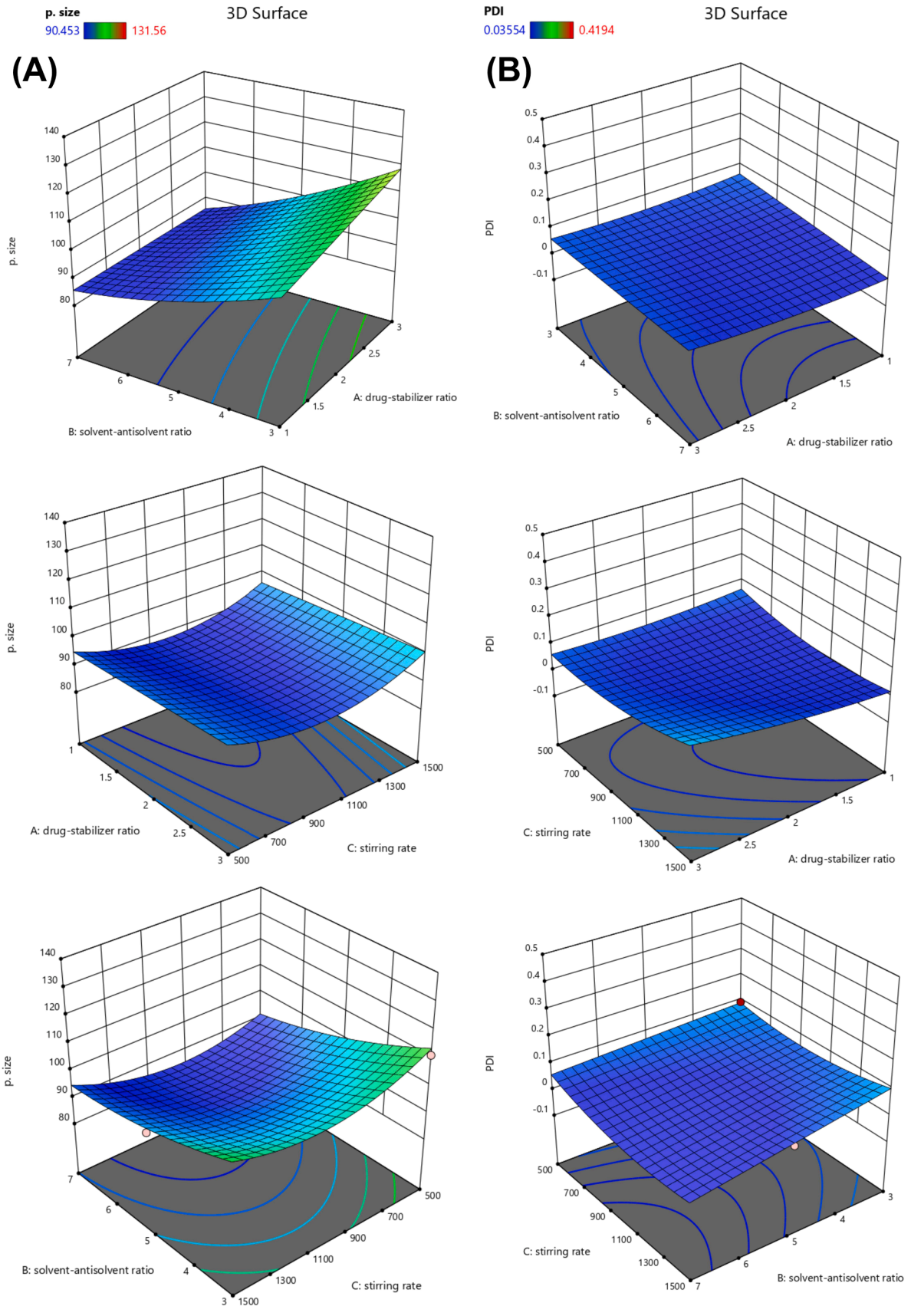

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Fig. 2. Three-dimensional graphs for the effect of independent variables on PS (A) and PDI (B).

contrasts with the defined crystalline structure of raw bilastine (Fig. 4B), indicating a transformation in particle structure and size that supports the formulation's nanosuspension characteristics [11,16].

3.5.5. Fourier transform-infrared spectroscopy (FT-IR)

As shown in Fig. 5, bilastine displayed characteristic peaks (in cm^{-1}): O-H stretching at 3404, C-H stretching at 2968, 2926, and 2855, C=O stretching at 1665, and C-N stretching for aromatic amines around 1121, consistent with previous studies [29].

The FTIR spectrum of Soluplus® shows characteristic peaks for hydroxyl groups at 3449, C-H stretching at 2924 and 2858, intense ester carbonyl C=O stretching at 1734 and 1642, and C-O-C stretching at 1456, which agreed with previous studies [38].

The FTIR spectrum of the physical mixture shows the absence of the drug's O-H stretching, likely masked by Soluplus®. The C=O and C-N stretching peaks shift slightly to 1661 and 1119 cm^{-1}, with reduced intensity in some bilastine peaks, likely due to dilution from the mixing process.

The FTIR spectrum of the lyophilized nanosuspension shows preserved functional groups with reduced intensity due to dilution with the stabilizer. The broad peak at 3435 cm^{-1} suggests hydrogen bond formation between Soluplus® and bilastine, enhancing solubility and mutual affinity, indicating no significant interaction between the drug and polymer [39].

3.5.6. Powder X-ray diffraction (PXRD)

Fig. 5 presents PXRD diffractograms. Pure bilastine showed sharp peaks at 2θ values of 11.35°, 17.25°, 19°, and 19.85°, confirming its crystalline structure, consistent with previous studies [11]. The physical mixture still displayed bilastine's characteristic peaks but with reduced intensity. In contrast, the lyophilized optimized formula showed the disappearance of these peaks, indicating the conversion of bilastine into an amorphous form [22].

3.5.7. Preliminary stability of BLA-NS

Fig. 6 shows the particle size and PDI of BLA-NS after one month of storage at 4°C and room temperature. At room temperature, particle size increased from 86.57 nm to 91.61 nm, with a 2.3-fold rise in PDI. At 4°C, changes were minimal, with particle size increasing to 89.28 nm and PDI rising slightly from 0.036 to 0.058. These results suggest Soluplus effectively stabilizes bilastine nanoparticles [31], with refrigeration being the optimal storage condition. Drug content remained stable at 4°C, with a slight increase at room temperature likely due to solvent evaporation, which poses no significant stability concern.

3.6. Evaluation of ODFs

3.6.1. Physical appearance and surface texture

All prepared films were transparent, homogenous, with smooth surfaces. Films prepared with PVA (p1, p2) were flexible and easily handled. The film prepared with HPMC alone (p3) was brittle and cracked when attempting to peel it from the petri dish. However, when combining HPMC with PVA (in p5 and p6), the resulting film was flexible and readily handled. Pullulan film (p4) was delicate and easily prone to tearing. P3 and p4 were excluded from further evaluation tests.

*3.6.2. Weight **Variation***

The average weights of ODFs as shown in Table 4 were uniform and passed the weight variation test with small ±SD.

3.6.3. Uniformity of film thickness

The thickness of films of all formulas was found to vary from 0.11 to 0.16 mm which lay within the accepted values (50–1000 μm) [21], as shown in Table 4.

3.6.4. Folding endurance

Folding endurance was observed to exceed the recommended threshold of 300 for all tested films.

3.6.5. Surface pH

All the tested films demonstrated an acceptable surface pH range of 6.1 to 6.3, as shown in Table 4, suggesting they are unlikely to irritate the oral mucosa [27].

3.6.6. Drug content

All of the formulas were found to contain an almost uniform quantity of the drug, The acceptable range for content uniformity is between 85% and 115% [26].

3.6.7. In-vitro disintegration time

The disintegration times of the tested ODFs ranged from 17 to 20 seconds, falling within the typical range of 5 to 30 seconds [7], as presented in Table 4. A comparison of plasticizer types in formulations P1 and P2 revealed that PEG 400 facilitated faster disintegration, making it the preferred choice for subsequent formulations.

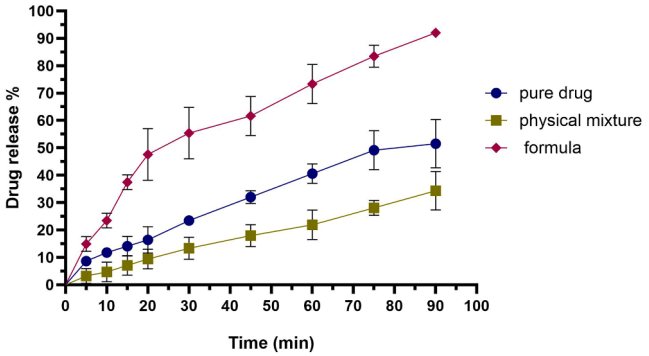

Fig. 3. Release profile of the BLA-NS, bilastine, and physical mixture.

3.6.8. *In-vitro dissolution test*

Fig. 7 depicts the dissolution profiles of p1, p2, p5, p6, and p7 ODFs in phosphate buffer (pH 6.8). The similarity factors comparing the release profile of the pure bilastine film (p7) with those containing BLA-NS (p1, p2, p5, and p6) were 27.20, 21.79, 23.57, and 15.27, respectively. These results indicate that incorporating bilastine as a nanosuspension significantly improved its release compared to the pure drug.

Furthermore, the dissolution study demonstrated that PEG 400 not only improved disintegration times but also accelerated dissolution, establishing it as the preferred plasticizer for the final ODF formulations [26].

The p6 formulation exhibited the fastest drug release, with 93.16 ± 1.79% released within 6 minutes. Its similarity factors were distinct from p1, p2, and p5, at 33.69, 43.72, and 37.09, respectively. Consequently, p6, formulated with a 1:1 ratio of PVA and HPMC E5, was identified as the optimal choice (Fig. 8). These polymers, known for their high water absorption capacity, enhance film swelling, disintegration, and drug release, consistent with the findings of Kadhum RW and Abd-Alhameed SN [25,40].

3.7. *Conclusion*

The solvent anti-solvent precipitation method effectively produced BLA-NS with enhanced dissolution rates. This approach proved to be effective, cost-efficient, and easy to implement. The formulation was optimized using Design-Expert® software with a D-optimal design. It revealed that the best results can be achieved using Soluplus® as a stabilizer, with a decreased stabilizer ratio, an increased anti-solvent ratio, and a moderate stirring rate. The close alignment between the observed and predicted responses suggests that the design accurately anticipated the outcomes. The optimized formula was monodispersed, nanosized particles of amorphous nature with narrow PDI, with improved dissolution rates.

BLA-NS was successfully loaded into ODFs using the solvent-casting method with various polymers to enhance dissolution and improve patient compliance. The PVA and HPMC E5-based film (p6) was identified as the best formulation, showing quick disintegration and fast dissolution.

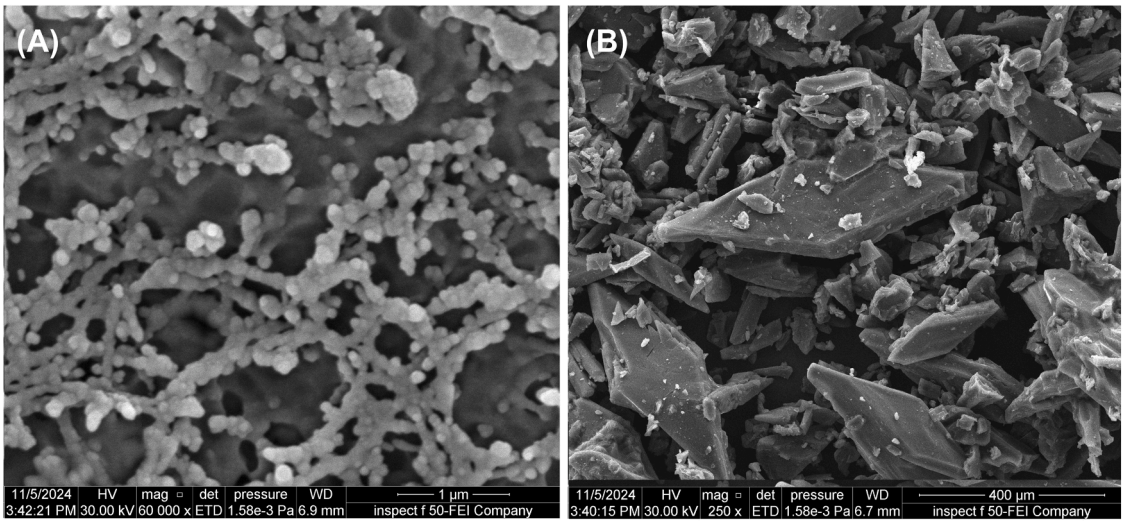

Fig. 4. FESEM of lyophilized optimum formula (A) at 1 µm scale, and pure drug (B) at 400 µm scale.

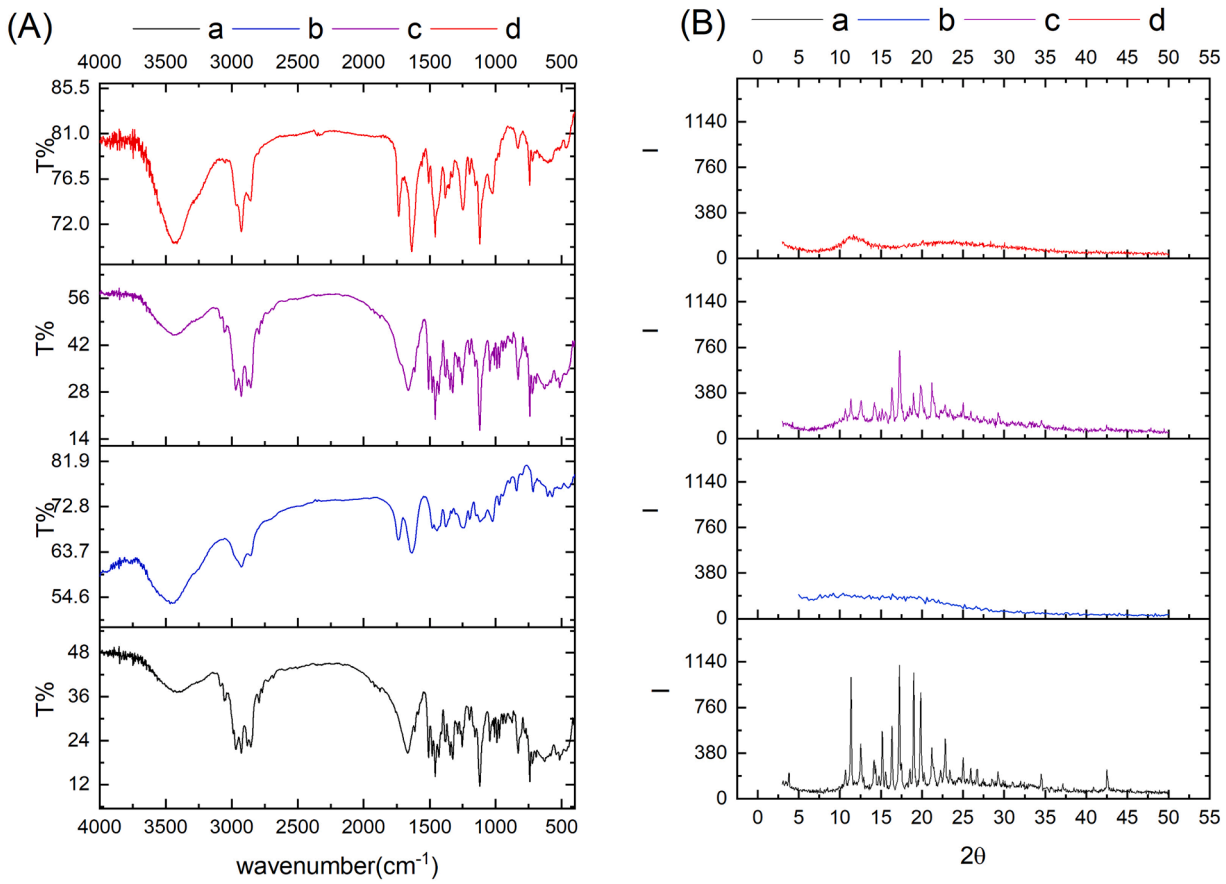

Fig. 5. FTIR spectrum (A), and PXRD diffractogram (B) of bilastine (a), Soluplus® (b), physical mixture (c), and lyophilized formula (d).

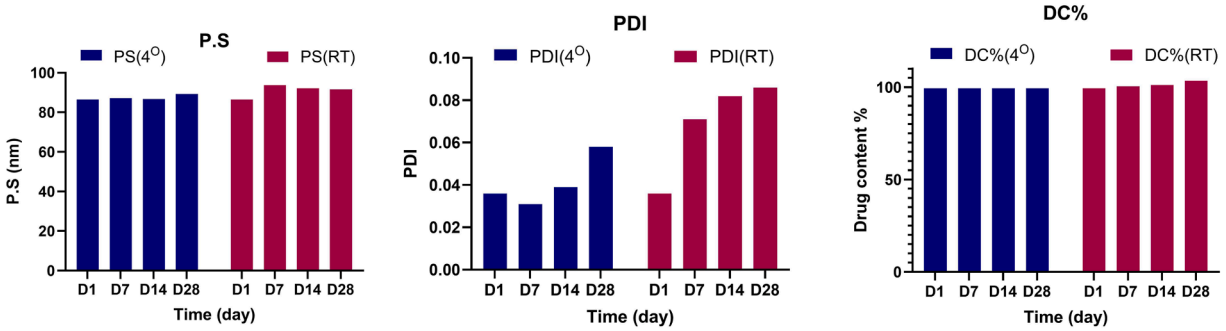

Fig. 6. Changes in the PS, PDI, and drug content (DC) of BLA-NS stored at 4 ∘C and room temperature for a one-month duration.

Table 4
Evaluation of bilastine orodispersible films.

Film code	Film thickness (mm)	Wt variation (mean ± SD)	Folding endurance	Disintegration time (sec)	Surface pH	Drug content (%)
p1	0.11 ± 0.005	92.6 ± 0.953	> 300	20	6.2	100.71 ± 8.11
p2	0.13 ± 0.011	92.93 ± 1.721	> 300	17	6.1	94.33 ± 3.44
p5	0.14 ± 0.005	94.76 ± 1.305	> 300	19	6.2	95.01 ± 3.27
p6	0.15 ± 0.005	93.6 ± 1.345	> 300	18	6.3	96.76 ± 1.58

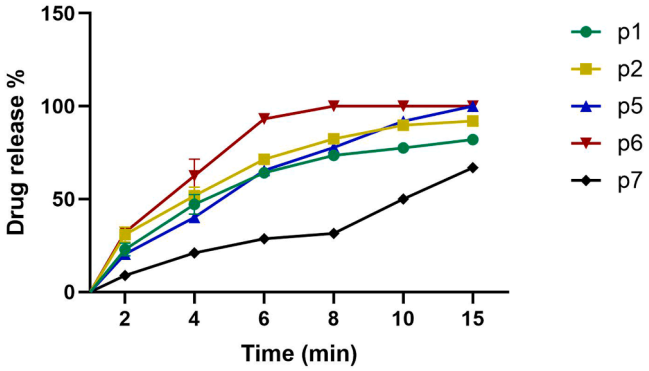

Fig. 7. Release profile of ODF formulations.

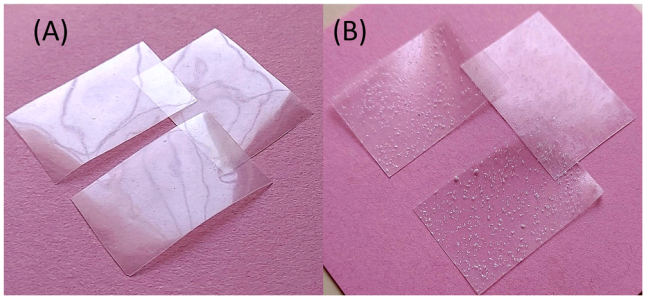

Fig. 8. P6 ODF (A) containing BLA-NS and P7 ODF (B) prepared with pure bilastine.

CRediT authorship contribution statement

Sarah Adnan Oudah: Writing – original draft, Resources, Methodology, Formal analysis. **Eman B.H. Al-Khedairy:** Writing – review & editing, Validation, Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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