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RESEARCH ARTICLE

Competency of Lyophilization and Spray Drying Techniques to Improve the Solubility of Bosentan Monohydrate: A Comparative Study

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SUMMARY

The present study focused on comparing the efficacy of two novel techniques, lyophilization and spray drying, which were proposed to overcome the solubility drawbacks of the highly effective antihypertensive drug, bosentan monohydrate. Solid dispersion approach is the most globally acknowledged and successful method for improving solubility. Poloxamer 188 was used as the carrier to prepare the solid dispersions. The results indicated that the particle size, solubility, and dissolution profiles of formulated amorphous systems varied significantly. Lyophilized solid dispersions demonstrated the highest level of solubility in the prepared solid dispersions. The solid dispersion formulations FL10 and FS10 prepared using lyophilization and spray drying techniques were optimized using a 32 full factorial design approach. The resulting amorphous solid dispersions were characterized using Fourier-transform infrared spectroscopy (FTIR), particle size analysis, differential scanning calorimetry (DSC), X-ray diffraction (XRD), scanning electron microscopy (SEM), and transmission electron microscopy (TEM). The optimized solid dispersion (FL10) prepared via lyophilization had an average particle size of 450.9 nm in particle size analysis. X-ray diffraction analyses of both FL10 and FS10 revealed a decrease in peak intensity compared to the drug and polymer, indicating the transformation of the crystalline form to amorphous. The outcomes of this study allow us to conclude that even though lyophilization and spray drying can be used to enhance solubility, lyophilization showed superior results.

Key Words: *Solid dispersion, lyophilization, spray drying, solubility enhancement, hypertension*

Bosentan Monohidratın Çözünürlüğünü Artırmak için Liyofilizasyon ve Püskürtmeli Kurutma Tekniklerinin Yeterliliği: Karşılaştırmalı Bir Çalışma

ÖZ

Bu çalışma, yüksek etkili antihipertansif bir ilaç olan bosentan monohidratın çözünürlük sorunlarının üstesinden gelebilmek için 2 yeni teknik olan liyofilizasyon ve püskürterek kurutma tekniklerinin etkileri üzerine odaklanmıştır. Katı dispersiyon yaklaşımı, çözünürlük arttırmak için dünya çapında en çok kabul gören başarılı bir yöntemdir. Poloxamer 188, katı dispersiyonları hazırlamak için taşıyıcı olarak kullanılmıştır. Sonuçlar göstermektedir ki formüle edilmiş amorf sistemlerin partikül boyutu, çözünürlüğü ve çözünme profilleri önemli ölçüde değişmiştir. Liyofilize katı dispersiyonlar, hazırlanan katı dispersiyonlarda en yüksek çözünürlük seviyesini göstermiştir. Liyofilizasyon ve püskürterek kurutma teknikleri kullanılarak hazırlanan katı dispersiyon formülasyonları FL10 ve FS10, 32 tam faktöriyel tasarım yaklaşımı kullanılarak optimize edilmiştir. Hazırlanan amorf katı dispersiyonlar Fourier-transform kızılötesi spektroskopisi (FTIR), partikül büyüklüğü analizi, diferansiyel taramalı kalorimetri (DSC), X-ışını difraksiyonu (XRD), taramalı elektron mikroskobu (SEM) ve transmisyon elektron mikroskobu (TEM) kullanılarak karakterize edilmiştir. Liyofilize edilmiş optimum katı dispersiyonların (FL10) ortalama partikül büyüklüğü 450.9 nm'dir. Hem FL10 hem de FS10'un X-ışını kırınım analizleri, ilaca ve polimere kıyasla tepe yoğunluğunda bir azalma ortaya çıkarmıştır ve bu, kristalli formun amorfa dönüşümünü göstermetedir. Bu çalışmanın sonuçları, çözünürlüğü arttırmak için liyofilizasyon ve püskürterek kurutma kullanılabilse de liyofilizasyonun daha iyi sonuçlar gösterdiği sonucuna varmamızı sağlar.

Anahtar Kelimeler: *Katı dispersiyon, liyofilizasyon, püskürterek kurutma, çözünürlüğü artırma, hipertansiyon*

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INTRODUCTION

Hypertension refers to persistently increased blood pressure (BP) in the systemic arteries. According to the Global Burden of Disease study, non-optimal blood pressure continues to be the single most significant risk factor contributing to the global burden of disease and all-cause mortality, accounting for 9.4 million deaths and 212 million lost healthy life years (8.5% of the global total) each year (Forouzanfar, 2015). Worldwide, hypertension is the most prevalent preventable risk factor for cardiovascular disease (CVD), chronic kidney disease, and cognitive impairment, and is the single leading cause of death and disability of all reasons (Forouzanfar, 2015).

One type of hypertension that affects the lungs and the heart is pulmonary arterial hypertension (PAH), which falls under the pulmonary hypertension classification. PAH is defined as a persistent elevation of pulmonary arterial pressure to greater than 25 mm Hg at rest or greater than 30 mm Hg during exercise, with a mean pulmonary capillary wedge pressure and a left ventricular end-diastolic pressure less than 15 mm Hg (Gaine, 1998). This differ from having normal blood pressure. When the small arteries in the lungs become narrowed or obstructed in a patient with PAH, blood has a more challenging time flowing through them, increasing the blood pressure in the lungs (Farber, 2004). PAH is a well-established multifactorial clinical condition that is severe and occasionally fatal. Because endothelial dysfunction, vasoconstriction, inflammatory responses, and platelet aggregation are the primary pathophysiological arms of PAH, specific therapeutic techniques have been developed to suppress these disorders. These therapies are highly effective at treating the disease.

Bosentan Monohydrate (BM) is a dual endothelin receptor antagonist that can be effectively used to treat PAH by inhibiting the action of endothelin molecules that cause blood vessel narrowing and hypertension in the absence of endothelin molecules. It improves patients' exercise capacity and slows the rate of clinical deterioration. Patients with PAH have elevated endothelin levels in their plasma and lung tissue, which is a potent vasoconstrictor (McLaughlin, 2006). BM prevents endothelin from binding to its receptors, neutralizing its detrimental effects. It is a water-insoluble compound having 50% absolute bioavailability (Dingemanse, 2004). It possess several limitations like insufficient absorption, fluctuating bioavailability, and gastrointestinal toxicity due to its water insolubility. As a result, increasing drug solubility is vital for achieving the desired drug concentration in the systemic circulation.

Solid dispersion is an effective technique to enhance solubility and thus increases the bioavailability of drugs. Reduced particle size, improved wettability, and increased porosity are just a few of the advantages of solid dispersion (Singh, 2011). Solid dispersions can easily be formulated into tablets or capsules for oral administration and ensure the stability of the drugs even in amorphous form (Chiou, 1971). Solid dispersions can be prepared by numerous techniques like solvent evaporation method, fusion method, melting method, kneading method, co-grinding method, melt agglomeration and hot-melt extrusion, etc. All the methods have been reported to enhance the solubility of poorly water-soluble drugs. Still, an attempt has been made in the present study to compare the efficacy of lyophilization and spray drying techniques.

MATERIALS AND METHODS

Bosentan monohydrate was purchased from Pure Chem Pvt. Ltd., Gujarat. Poloxamer 188 was purchased from Alfa Aesar, Massachusetts, United States. Methanol was obtained from Qualikems Fine Chem. Pvt. Ltd., Gujarat. Chloroform was purchased from Merck Specialities Pvt. Ltd., New Delhi. All other chemicals used were of analytical grade.

Solubility study

The solubility of BM in various solvents was evaluated using the shake flask method. An excess of the drug was added to screw-capped vials containing 10 ml of each solvent and then kept in a water bath shaker at 37±1°C for 48 h. Then the saturated solutions

were filtered through 0.45 µm sized membrane filters and analyzed using a UV spectrophotometer (UV-1800, Shimadzu Corporation, Kyoto, Japan) at 268 nm and resolution 0.2 nm (Patel, 2008). The method was validated for linearity, accuracy, and precision. The linearity range was found to be 5-40 µg/ml, with a % RSD(Relative standard deviation) value less than two, which indicates the method is precise. The method's sensitivity was found by determining LOD (lower limit of detection) and LOQ (lowest limit of quantification). The LOD and LOQ values were 0.95 and 2.88 µg/ml, respectively.

Drug-excipient compatibility studies

While designing the solid dispersions, it is imperative to consider the compatibility of drugs and polymers used within the systems. It is therefore, necessary to confirm that the drug does not show any incompatibility with the polymer under experimental conditions (40±5°C and 75±5% RH) for at least three weeks. The desired quantity of drug with specified excipient poloxamer 188 (P188) was taken in the ratio of 1:5 and mixed thoroughly, sieved, and filled in dried vials. The vials were examined daily at regular intervals for discoloration, clump formation, and liquefaction. Also, the FTIR spectra of pure drug, polymer, and solid dispersion were obtained to determine the compatibility. For FTIR, a sample of approximately 4 mg was kept in an FTIR spectrometer, and the spectra were recorded (Maximiano, 2011).

Preparation of solid dispersion

Lyophilization (freeze-drying method)

Solid dispersions were prepared by the freeze-drying method. BM was dissolved in a sufficient quantity of methanol to form phase I. Similarly, (P188) were dissolved separately in water to form phase II, and both phases were mixed. Methanol was evaporated and the resulted solution was frozen in a quick/deep freezer at −20°C and was then lyophilized in a freeze dryer (LyoQuest-55 Azbil Telstar Technologies, Terrassa, Spain) at temperatures of −30°C to −40°C and a vacuum of 0.200 mbar. The freeze-dried mass was then sieved through sieve no. 86 and stored in a desiccator (Betageri, 1995; Abdul-Fattah, 2002).

Spray drying method

Solid dispersions were also prepared by the spray drying method. BM was dissolved in a sufficient quantity of methanol to form phase I. Similarly, P188 was dissolved separately in water to form phase II, and both phases were mixed and sonicated for 2 minutes. The resultant solutions are then spray-dried using a spray dryer (Spray Mate Lab Spray Dryer, JISL, Mumbai, India) at inlet temperatures of 90°C to 110°C with an outlet temperature of 80°C at a feed rate of 10 ml/min and aspiration speed of 35 mbar. The spray-dried mass was then sieved through sieve no. 86 and stored in a desiccator (Paradkar, 2004; Ha, 2014).

Characterization of solid dispersion formulation

The drug content of all the prepared solid dispersions was determined by dissolving solid dispersions equivalent to 10 mg of BM according to their ratio prepared in methanol. It was then diluted to obtain a theoretical concentration of 10 µg/ml. The solution was then filtered through membrane filters and analyzed with a UV spectrophotometer. Then the percentage yield of each formulation was determined according to the final weight of solid dispersions.

$$Percentage\ yield = \frac{Practical\ weight\ of\ solid\ dispersion}{Theoretical\ weight\ of\ solid\ dispersion} \times 100$$

Solubility of prepared solid dispersion formulations

The solubility of solid dispersion in distilled water was evaluated. Excess of the solid dispersions was added to screw-capped vials containing 10 ml of distilled water and then kept on a water bath shaker at 37±1°C for 48 h. Then the saturated solutions were filtered through 0.45 µm sized membrane filters and analyzed using a UV spectrophotometer.

***In vitro* drug release study**

In vitro drug release of prepared solid dispersions and the pure drug was performed in triplicate using a dissolution apparatus (DS 8000, Labindia Analytical

Instruments Pvt. Ltd., Navi Mumbai, India) in PBS pH 6.8 at 37±0.5°C using USP type II apparatus at 100 rpm. Powdered solid dispersions equivalent to 62.5 mg of BM were added to the dissolution medium. At appropriate time intervals, 5 ml of the sample was withdrawn and replaced with a fresh dissolution medium to maintain the sink conditions. The withdrawn samples were filtered using a membrane filter and analyzed for drug content using a UV spectrophotometer. The dissolution efficiency (DE%) after 120 min was determined via the trapezoidal method and was calculated as the percentage area of a rectangle divided by the area of 100% dissolution at a particular time (Potluri, 2011; Krupa, 2017).

Experimental design of solid dispersions

Optimization of the lyophilization process and spray drying process

A 3^2 full factorial design (Design-Expert version 11; State Ease Inc., USA) was used to determine the optimized formulation to target percentage yield and highest percentage dissolution efficiency in the case of lyophilized and spray-dried solid dispersions. In this design, two factors were evaluated, each at three levels and experimental trials were performed at all nine possible combinations. For the lyophilization process, the drug to polymer ratio (X_1) and temperature (X_2) was considered as independent variables, whereas, DE_{120}% and Yield% were taken as dependent variables. In the case of the spray drying process, the drug to polymer ratio (X_1) and inlet temperature (X_2) were considered as independent variables, whereas, the DE_{120}% and Yield% were taken as dependent variables. In both cases, a checkpoint batch was prepared to prove the validity of the evolved mathematical model. In addition, contour plots were used to graphically represent the effect of independent variables (Singh, 2017).

The desirability of all solid dispersions using optimization software

A numerical optimization technique utilizing the desirability functions approach was used to generate the optimum settings for the process conditions of both preparation methods of solid dispersions. All response variables were optimized using the desirability functions approach with the Design-expert software version 11. The solid dispersion having the maximum desirability value was considered as the optimal formulation.

Characterization of optimized formulations using different techniques

IR spectral analysis

The chemical interactions and compatibility of BM, P188, and optimized formulations were determined using FTIR analysis. The samples were mixed with 80 mg of dry potassium bromide (KBr), and the mixture was compressed into discs. Later, the discs were scanned in the wavelength of 4000-500 cm^{-1}.

Particle size and size distribution analysis

The particle size of the optimized solid dispersions was determined using a particle size analyzer (Malvern Zetasizer Nano ZS90, United Kindom). The samples were suspended in triple distilled water and subjected to particle size analysis. This method also depicted the polydispersity index, which is a measure of uniformity in size distribution.

Differential scanning calorimetry (DSC)

DSC analysis was performed using an automatic differential scanning calorimeter (DSC822e, Mettler Toledo, Ohio, United States). Each sample of 3 mg was weighed and analyzed in pierced aluminum pans at a heating rate of 10°C/min and temperature range of 10 to 300°C.

X-ray diffraction (XRD)

The crystalline nature of the drug can be confirmed using an X-ray diffractometer (D/max r-B, Rigaku, Japan). XRD analysis of drug, polymer, and solid dispersions was performed using Cu-Kα radiation at an of angle 2θ range from 5° to 80° with a step size of 0.02°, a step time of 18.7 min, and a scanning speed of 5°/min.

Scanning electron microscopy (SEM)

A scanning electron microscope (SUPRA-55; Zeiss, Germany) was used to examine the morphology of pure drug, polymer, and prepared solid dispersions at an accelerating voltage of 10 kV and an aperture of 20 μm. The sample powder was mounted on a brass stub with graphite glue and then slathered with gold under vacuum before being viewed under SEM.

Transmission electron microscopy (TEM)

The morphology (particle shape and size) of the optimized solid dispersion was determined using TEM. The solid dispersion was dispersed in triple distilled water. A drop was placed on a carbon-coated copper grid and dried before being examined under a transmission electron microscope (HRTEM, JEM 2100, JEOL Ltd., Tokyo, Japan), which was operated at a 200 kV accelerating voltage and a beam current of 100 μa (Ricarte, 2015).

Stability study of optimized solid dispersion

During the preparation of solid dispersions, the drug undergoes a transition from crystalline form to amorphous form. But during storage, they are widely reported to recrystallize. So, accelerated stability studies were conducted. The optimized solid dispersions were stored at room temperature for 3 months. The dispersions were analyzed for changes in physical appearance and drug content after a period of 0, 30, 45, 60, and 90 days. After a storage period of 3 months, X-ray diffraction and particle size studies were conducted to determine any changes in particle size and crystallinity.

Statistical analysis

The data is provided as the mean and standard deviation of three sets of results. Analysis of variance (ANOVA) was used to examine the statistical difference between solubility and dissolution efficiency, followed by Tukey's test (Sigma stat 3.5; STATCON). At the 0.05 level of probability, significance was determined. A 3^2 full-factorial design (Design-Expert version 11; State Ease Inc., USA) was used to investigate the influence of formulation variables on the optimization process to obtain the desired formulation.

RESULTS AND DISCUSSION

Solubility profiles

The solubility of the BM in a variety of solvents was determined, including distilled water, methanol, phosphate buffer 6.8, and phosphate buffer 1.2. The lowest solubility of the BM was found to be 10.19±0.3 μg/ml in distilled water, while the highest solubility was found to be 3090.7±15.6 μg/ml in methanol. The solubility of the BM in phosphate buffer 6.8 and phosphate buffer 1.2 was found to be 48.1±2.1 and 10.54±0.5, respectively. The results show that the drug is completely soluble in methanol, insoluble in water, and shows pH-dependent solubility as reported (Krupa, 2017).

Drug-excipient compatibility studies

In the drug-excipient compatibility study, the desired quantity of drug with excipients was kept under observation for three weeks for any physical changes. There were no physical changes for three weeks. As shown in Figure 1, FTIR confirmed that characteristic peaks of BM and P188 seem to be preserved in prepared solid dispersion, which proves that there was no chemical interaction between the drug and the excipient.

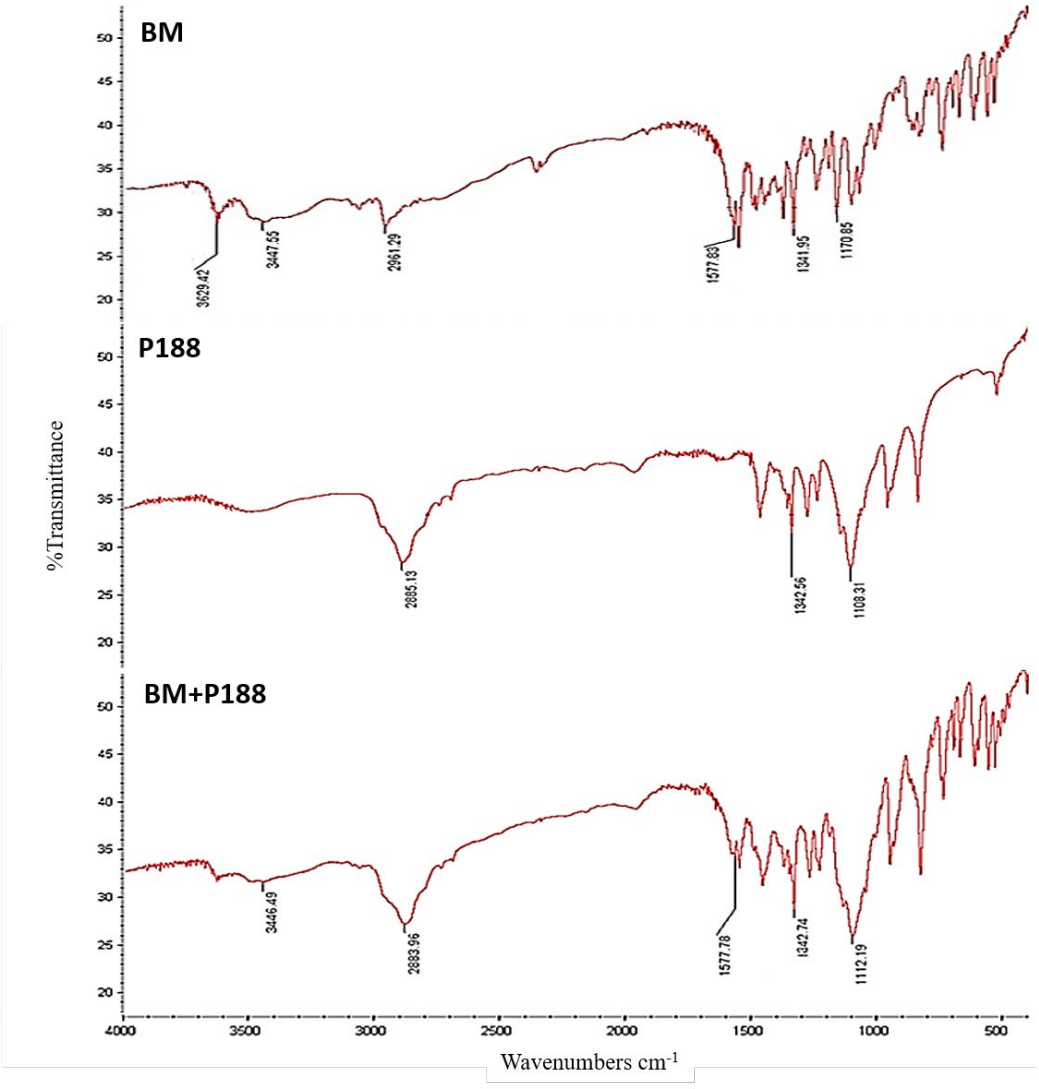


Figure 1. FTIR of bosentan monohydrate, poloxamer 188, and bosentan monohydrate-poloxamer 188

BM: Bosentan monohydrate, P188: Poloxamer 188

Preparation of solid dispersions

The solid dispersions were prepared by using lyophilization and spray drying methods. Lyophilized solid dispersions were prepared at the drug to polymer ratios of 1:1, 1:2, and 1:3 and at temperatures of -30°C, -35°C, and -40°C. Whereas, spray-dried solid dispersions were prepared with the drug to polymer ratios of 1:1, 1:2, and 1:3 at 90°C, 100°C, and 110°C inlet temperatures. Nine formulations using each technique were prepared where lyophilized solid dispersions were coded as FL1, FL2,...FL9 and similarly spray-dried solid dispersions were coded as FS1, FS2,...FS9. Compositions of all solid dispersions are shown in Table 3.

Then the percentage yield (Yield %) and percentage drug content (DC %) of each formulation were determined. As shown in Table 3, it was found that as the amount of polymer increased, the Yield % decreased. It might be due to the sticky nature of P188. Solid dispersion formulations prepared by

using lyophilization have shown a higher Yield % than the solid dispersion formulation prepared by the spray drying method. All formulations have shown an average DC % of 95% regardless of their Yield %.

The solubility of all solid dispersions prepared using lyophilization and spray drying was evaluated in distilled water. As depicted in Table 1, both methods demonstrated a significant increase in solubility as the amount of polymer increased. However, there was no significant increase in solubility after the drug to polymer ratio 1:2 in either method. When the preparation methods were compared, lyophilization outperformed spray drying at the same drug to polymer ratios. This may be because the lyophilization process produces a porous and fluffy product, increasing the surface area and thus the surface free energy, resulting in increased solubility (Betageri, 1995).

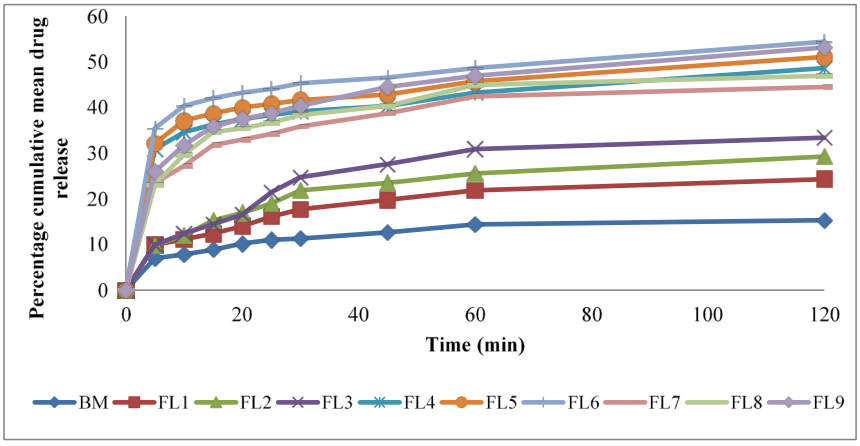
Table 1. Composition and characterization of prepared solid dispersions of bosentan monohydrate

Composition			Characterization			
Formulation code	**Drug: Polymer ratio**	**Temperature/ inlet temperature (℃)**	**%Yield**	**%DC**	**Saturation solubility (µg/ml)**	**%DE_{120}**
Bosentan monohydrate (Pure Drug)						
BM	-	-	-	-	10.19±0.3	
Lyophilization						
FL1	1:1	-30	93.7±1.8	98.1±1.9	213±1.9	19.5±1.03
FL2	1:1	-35	94.6±1.3	97.1±1.0	219±2.35	23.9±1.07
FL3	1:1	-40	93.1±0.8	99.0±1.5	222±1.26	26.62±0.80
FL3	1:2	-30	88.3±0.4	96.8±1.4	308±2.73	41.36±1.99
FL4	1:2	-35	87.1±1.1	99.0±1.4	310±4.28	43.72±1.55
FL5	1:2	-40	86.1±0.6	99.4±1.9	313±2.82	46.9±0.55
FL6	1:3	-30	79.2±1.1	97.3±1.6	326±4.16	38.43±0.46
FL8	1:3	-35	78.1±1.6	98.5±0.9	327±3.47	40.64±1.48
FL9	1:3	-40	77.6±0.9	97.9±1.1	329±2.62	43.94±1.97
Spray drying method						
FS1	1:1	90	74.2±0.8	97.2±1.3	195±2.76	21.85±0.39
FS2	1:1	100	73.3±1.1	94.9±1.8	201±1.22	23.8±0.64
FS3	1:1	110	74.9±0.6	95.7±1.9	205±1.76	25.86±1.68
FS3	1:2	90	61.3±1.5	98.5±1.1	296±4.16	38.63±1.46
FS4	1:2	100	63.4±0.9	98.2±1.4	302±2.89	40.61±1.18
FS5	1:2	110	62.2±1.0	97.1±1.5	308±3.01	41.75±0.94
FS6	1:3	90	60.5±0.2	96.3±1.2	322±3.98	38.2±1.12
FS8	1:3	100	59.3±1.8	96.1±1.1	326±3.56	38.71±1.58
FS9	1:3	110	58.4±0.9	95.4±1.1	328±3.15	39.53±0.89

Data are expressed as mean ±SD (n=3); Yield %: Percentage yield, DC %: percentage drug content, DE_{120}%: Dissolution efficiency after 120 min

***In vitro* dissolution studies**

In the case of BM, it showed a percentage cumulative drug release of 15.30%, whereas all solid dispersions showed an extended drug release rate over the period of 120 min. In the case of lyophilized solid dispersions, it showed percentage cumulative drug release ranging from 24.31% to 54.34%, whereas, in the case of spray-dried solid dispersions, they have shown the percentage cumulative drug release ranging from 25.13% to 44.88%, as shown in Figure 2. Over a period of 120 min, neither pure drug nor solid dispersions have shown a 100% cumulative drug release. In all solid dispersions, as the amount of polymer increased, the DE_{120}% also increased as shown in Table 3. When we compared the solid dispersions, the lyophilized solid dispersions have shown an increased DE_{120}% compared to the spray-dried solid dispersions at the same drug to polymer ratios. This increase in the dissolution rate can be attributed to an increase in solubility, which was found to be better in the case of spray-dried solid dispersions. To deep root these findings, the formulations were further optimized and tested for other parameters like particle size analysis, differential scanning calorimetry (DSC), X-ray diffraction (XRD), scanning electron microscopy (SEM), and transmission electron microscopy (TEM) (Dangre, 2017).



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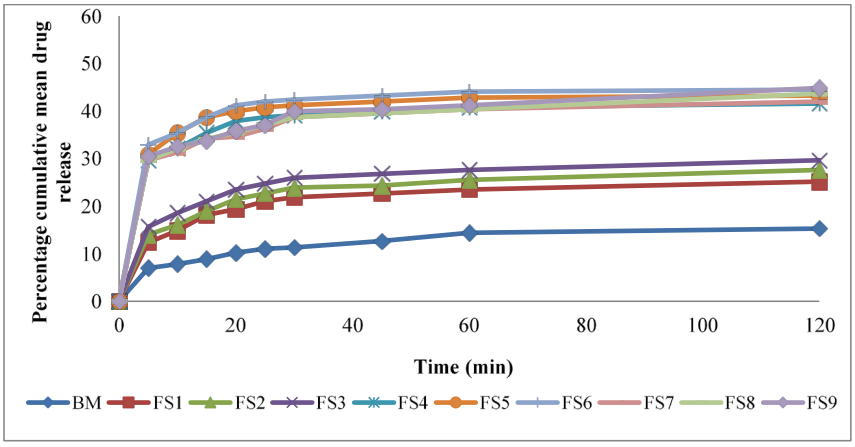

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Figure 2. Graphical representation of dissolution release profile of pure bosentan monohydrate and solid dispersions prepared by (A) lyophilization and (B) spray drying

Experimental design of solid dispersions

Optimization of the lyophilization method

A 3^2 full factorial design approach was used to determine the optimized formulation having maximum Yield % and maximum DE_{120}% by using design expert software (Design-Expert 11). Drug to polymer ratio (X_1) and temperature (X_2) were taken as two independent variables. A statistical model incorporating both interactive and polynomial terms was used to estimate the response by using the equation.

$$Y= b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_1^2X_1^2 + b_2^2X_2^2$$

Y is the dependent variable (Y_1 = Yield % and Y_2 = DE_{120}%), b_0 is the arithmetic mean response of all 9 runs, b_1 and b_2 are estimated coefficients for X_1 and X_2, respectively. Here X_1 and X_2 provide the average result on varying a single factor at one time, whereas X_1X_2 is the interaction term that illustrates how the response changes when 2 factors are changed simultaneously. Both polynomial terms i.e., $(X_1)^2$ and $(X_2)^2$ are included in determining nonlinearity.

The blueprint and results of lyophilized solid dispersions are shown in Table 2. There was a significant difference in Yield% (94.6±1.3% to 77.6±0.9%) and DE_{120}% (19.5±1.03% and 46.9±0.55%) in all the prepared formulations.

Table 2. Blueprint of 3^2 full factorial design (lyophilized solid dispersions)

Formulation code	Variable levels in coded form		Yield%	DE_{120}%
	X_1	X_2		
FL1	-1	-1	93.7±1.8	19.5±1.03
FL2	-1	0	94.6±1.3	23.9±1.07
FL3	-1	1	93.1±0.8	26.62±0.80
FL4	0	-1	88.3±0.4	41.36±1.99
FL5	0	0	87.1±1.1	43.72±1.55
FL6	0	1	86.1±0.6	46.9±0.55
FL7	1	-1	79.2±1.1	38.43±0.46
FL8	1	0	78.1±1.6	40.64±1.48
FL9	1	1	77.6±0.9	43.94±1.97
TCP (check point)	**0.19**	**1.0**	**90.8±0.9**	**43.2±0.61**
Coded values	**Actual values**			
	X_1 (Ratio)	**X_2 (Temp.)**		
-1	1:1	-30°C		
0	1:2	-35°C		
1	1:3	-40°C		

Data are expressed as mean ±SD (n=3); Yield%: Percentage yield, DE_{120}%: Dissolution efficiency after 120 min

Table 3. Regression analysis data of lyophilized solid dispersions

Response	Yield%		DE_{120}%	
	FM	RM	FM	RM
b_0	92.40	91.97	43.97	36.11
b_1	-4.25	-4.25	8.83	8.83
b_2	-0.23	-0.23	3.03	3.03
b_{11}	-0.25		-11.82	
b_{22}	-0.40		0.03	
b_{12}	0.25		-0.40	

Yield%: Percentage yield, DE_{120}%: Dissolution efficiency after 120 min, FM: Full model, RM: Reduced model

It is depicted from Table 2 that both the chosen independent variable have a significant effect on Yield% and DE_{120}%. The fitted equation (full and reduced model) relating different responses, Yield%, and DE_{120}% to the transforming factor is revealed in Table 3.

The polynomial equations can be utilized to draw conclusions from the magnitude of coefficient and positive or negative sign. The results of the ANOVA as depicted in Table 4 were executed to identify the insignificant factors. The value of correlation was near 1 for both Yield% and DE_{120}%, thereby indicating a

good fit for all the dependent variables. Among both dependent variables, regression analysis suggests that coefficients b_{11}, b_{22}, and b_{12} ($P \geq 0.05$) were insignificant in predicting Yield% and DE_{120}%. Hence these terms were omitted from the full model to generate the reduced model.

Both coefficients b_1 and b_2 bear a negative sign as shown in multiple linear regression analysis (reduced model), which indicates that upon increasing the drug to polymer ratio or temperature, Yield% decreases. On the contrary, the increase in drug to polymer ratios and the temperature increased the DE_{120}% as the coefficients b_1 and b_2 bear positive signs.

Table 4. Analysis of variance of the full model and the reduced model for the dependent variables in the case of lyophilized solid dispersions of bosentan monohydrate

Full model	For Yield%				
	df	**SS**	**MS**	***f***	**R^2**
Regression	5	109.40	21.88	33.43	0.9824
Residual	3	1.96	0.65		
Reduce model					
Regression	2	108.70	54.35	122.67	0.9761
Residual	6	2.66	0.44		
Full model	**For DE_{120}%**				
	df	**SS**	**MS**	***f***	**R^2**
Regression	5	803.17	160.63	492.87	0.9988
Residual	3	0.977	0.032		
Reduce model					
Regression	2	523.01	261.51	5.58	0.9504
Residual	6	281.13	46.86		

df: Degree of freedom, SS: Sum of squares, MS: Mean of squares, *f:* Fischer's ratio, R: Regression coefficient

Optimization of formulation variables of lyophilization method

The optimization of lyophilized solid dispersions' components (Drug to polymer ratio and temperature) was done to target the Yield% and DE_{120}% of 93% and 45%, respectively. The optimized amount determined with the help of software is depicted in surface response curves as shown in Figure 3. A checkpoint batch (TCP) was prepared at $X_1 = 0.19$ level and $X_2 = 1.0$ level at which Yield% and DE_{120}% were 90.8±0.9 and 43.2±0.61, respectively. The optimized batch (TCP) depicted the expected results. The desirability of the optimized batch was 0.914727.

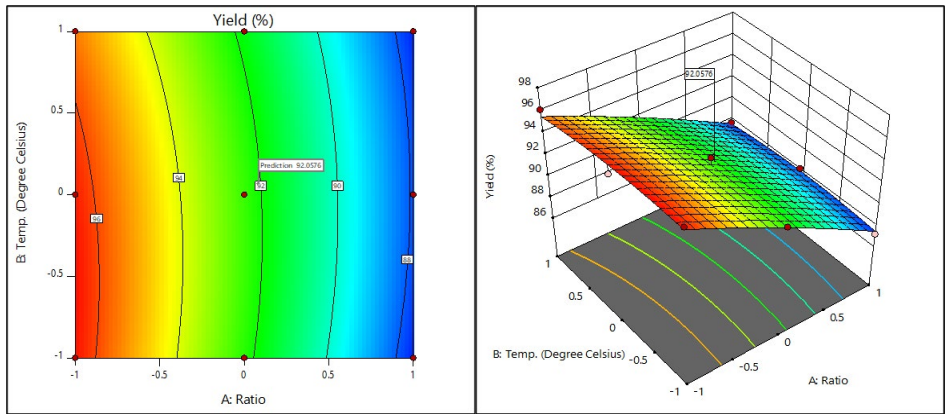
From the optimization conducted, it has displayed the optimized data in a coding form. The coded responses to X_1 and X_2 from the input data in the 3^2 full factorial design were 0.19 and 1.0, respectively. The responses were decoded and were found to be 1:2.2 and -40, i.e., for maximum %Yield and $\%DE_{120}$, the drug: polymer ratio and the temperature of the lyophilization method should be 1:2.2 and -40°C, respectively. To evaluate the prediction capability of the models and to verify the optimization process, lyophilized solid dispersions were prepared based on optimal process variable settings.

Optimization of the spray drying process

Using design expert software (Design Expert 11), a 3^2 full factorial design approach was used to find the optimal formulation with maximum Yield% and DE_{120}%. Two independent variables were used and they are drug: polymer ratio and inlet temperature. A statistical model incorporating both interactive and polynomial terms was used to estimate the response by using the equation.

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_1^2X_1^2 + b_2^2X_2^2$$

Y is the dependent variable (Y_1 = Yield% and Y_2 = DE_{120}%), b_0 is the arithmetic mean response of all 9 runs, b_1 and b_2 are estimated coefficients for X_1 and X_2, respectively. Here X_1 and X_2 provide the average result on varying a single factor at one time, whereas X_1X_2 is the interaction term that illustrates how the response changes when 2 factors are changed simultaneously. To determine nonlinearity, both polynomial terms $(X_1)^2$ and $(X_2)^2$ are used.



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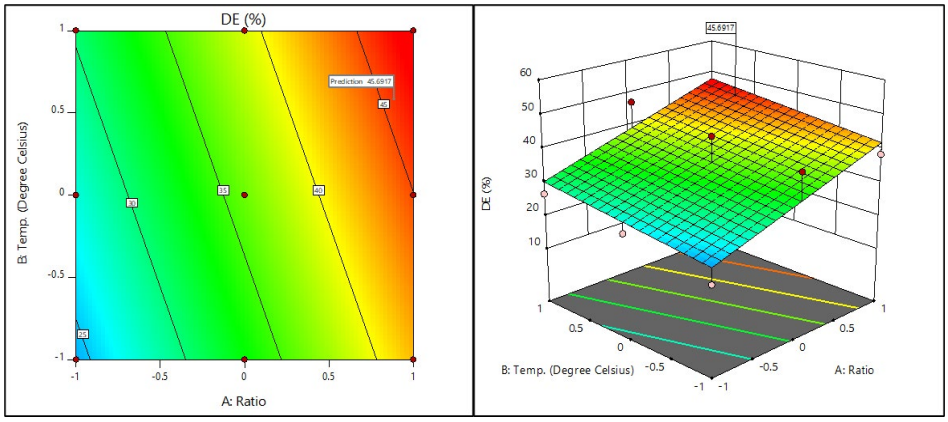

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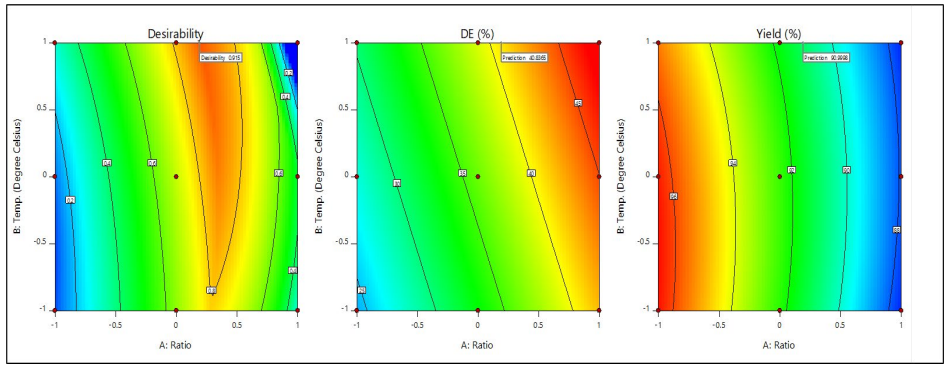

C

Figure 3. Response surface plots of (A) percentage yield, (B) percentage dissolution efficiency, and all response surface plots of (C) lyophilized solid dispersions using optimization software

The blueprint and results of spray-dried solid dispersions are shown in Table 5. There was a significant difference in Yield% (74.2±0.8% to 58.4±0.9%) and DE_{120}% (21.85±0.39% and 39.53±0.80%) in all the prepared formulations.

Table 5. Blueprint of 3^2 full factorial design (spray-dried solid dispersions)

Formulation code	Variable levels in coded form		Yield%	DE_{120}%
	X_1	X_2		
FS1	-1	-1	74.2±0.8	21.85±0.39
FS2	-1	0	73.3±1.1	23.8±0.64
FS3	-1	1	74.9±0.6	25.86±1.68
FS4	0	-1	61.3±1.5	38.63±1.46
FS5	0	0	63.4±0.9	40.61±1.18
FS6	0	1	62.2±1.0	41.75±0.94
FS7	1	-1	60.5±0.2	38.2±1.12
FS8	1	0	59.3±1.8	38.71±1.58
FS9	1	1	58.4±0.9	39.53±0.80
TCP (check point)	**0.29**	**0.99**	**68.4±0.7**	**40.2±0.5**
Coded values	**Actual values**			
	X_1 (Ratio)	**X_2 (Inlet Temp.)**		
-1	1:1	90°C		
0	1:2	100°C		
1	1:3	110°C		

Data are expressed as mean ±SD (n=3); Yield%: Percentage yield, DE_{120}%: Dissolution efficiency after 120 min.

It is portrayed clearly from Table 5 that both the chosen independent variables have a significant effect on Yield% and DE_{120}%. Table 6 shows the fitted equation (full and reduced model) relating various responses, Yield %, and DE_{120}% to the transforming factor.

Table 6. Regression analysis data of spray-dried solid dispersions

Response	Yield%		DE_{120}%	
	FM	**RM**	**FM**	**RM**
b_o	68.51	69.69	40.38	34.33
b_1	-5.70	-5.70	7.49	7.49
b_2	-0.46	-0.46	1.41	1.41
b_{11}	0.23		-9.00	
b_{22}	1.53		-0.07	
b_{12}	0.30		-0.67	

Yield%: Percentage yield, DE_{120}%: Dissolution efficiency after 120 min, FM: Full model, RM: Reduced model

The polynomial equations can derive conclusions based on the coefficient magnitude and positive or negative sign. ANOVA results, as shown in Table 7, were used to identify insignificant factors. The correlation value was close to one for both Yield% and DE_{120}%, indicating a good fit for all dependent variables. Regression analysis showed that coefficients b_{11}, b_{22}, and b_{12} ($P \geq 0.05$) were insignificant in predicting Yield% and DE_{120}% among both dependent variables. As a result, these terms were omitted from the full model to generate the reduced model.

Both coefficients b_1 and b_2 bear a negative sign as shown in multiple linear regression analysis (reduced model), which indicates that upon increasing the drug: polymer ratio or inlet temperature, Yield% decreases. On the contrary, the increase in drug to polymer ratios and inlet temperature increased the DE_{120}% as the coefficients b_1 and b_2 bear positive signs.

Table 7. ANOVA results of the full model and the reduced model for the dependent variables in the case of spray-dried solid dispersion of bosentan monohydrate

Full model	For Yield%				
	df	**SS**	**MS**	***f***	**R^2**
Regression	5	201.42	40.28	18.28	0.9882
Residual	3	6.61	2.20		
Reduce model					
Regression	2	196.25	98.12	49.97	0.9434
Residual	6	11.78	1.96		
Full model	For DE_{120}%				
	df	**SS**	**MS**	***f***	**R^2**
Regression	5	512.36	102.47	1590.10	0.9996
Residual	3	0.19	0.06		
Reduce model					
Regression	2	348.38	174.19	6.37	0.9797
Residual	6	164.18	27.36		

df: Degree of freedom, SS: Sum of squares, MS: Mean of squares, *f*: Fischer's ratio, R: Regression coefficient

Optimization of formulation variables of spray drying method

The optimization of spray-dried solid dispersions components (drug to polymer ratio and inlet temperature) was done to target the Yield% and DE_{120}% of 70% and 41%, respectively. The optimized amount determined with the help of software is depicted in surface response curves as shown in Figure 4. A checkpoint batch (TCP) was prepared at X_1 = 0.29 level and X_2 = 0.99 level at which Yield% and DE_{120}% was 68.4±0.7 and 40.2±0.5, respectively. The optimized batch (TCP) depicted the expected results. The desirability of the optimized batch was 0.917003.

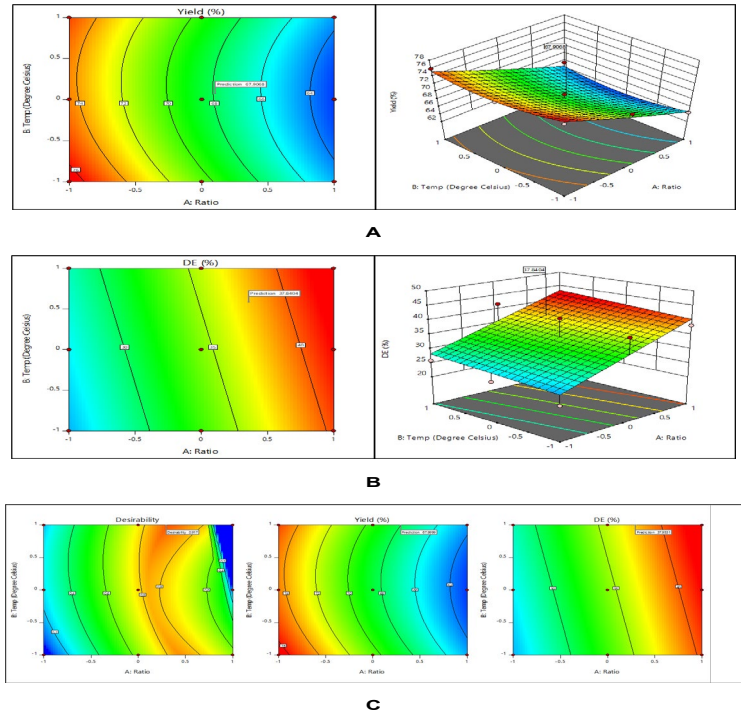

Figure 4. Response surface plots of (A) percentage yield, (B) percentage dissolution efficiency, and all response surface plots of (C) spray-dried solid dispersions using optimization software

From the optimization conducted, it has shown optimized data in a coding form. Based on the input data, it showed a 0.29 and 0.99 coded response to X_1 and X_2 in the 3^2 full factorial design. The responses were decoded and found to be 1:2.3 and 109.9, respectively. In other words, for maximum %Yield and $\%DE_{120}$, the drug: polymer ratio and spray drier inlet temperature should be 1:2.3 and 109.9°C, respectively. To evaluate the prediction capability of the models and to verify the optimization process, spray-dried solid dispersions were prepared based on optimal process variable settings.

Characterization of optimized formulations

IR spectral analysis

Initially, the FTIR spectrum of a drug can be used to determine the functional groups in that compound. As shown in Figure 5, FTIR spectra of pure bosentan showed characteristic peaks at 3629.42 cm^{-1} for O-H stretch, 3447.55 cm^{-1} for N-H stretch, 2961.29 cm^{-1} for C-H stretch aliphatic, 1577.83 cm^{-1} for N-H bend, 1341.95 cm^{-1} for S=O, and 1170.85 cm^{-1} for sulfonamide. The FTIR spectra of formulations showed a slight shift in the peaks 2883 cm^{-1} and 2880 cm^{-1} (C-H stretch aliphatic) for FL10 and FS10, respectively, without any other significant changes. This could be due to possible intermolecular hydrogen bonding in the formulations (Dangre, 2017). The IR spectrum of poloxamer 188 is characterized by principal absorption peaks at 2885.13 cm^{-1} (C-H stretch aliphatic), 1342.56 cm^{-1} (in-plane O-H bend), and 1108.31 cm^{-1} (C-O stretch). Characteristic peaks of bosentan monohydrate and poloxamer 188 seemed to be preserved in the prepared solid dispersion, which proved that there was no chemical interaction between the drug and the excipient.

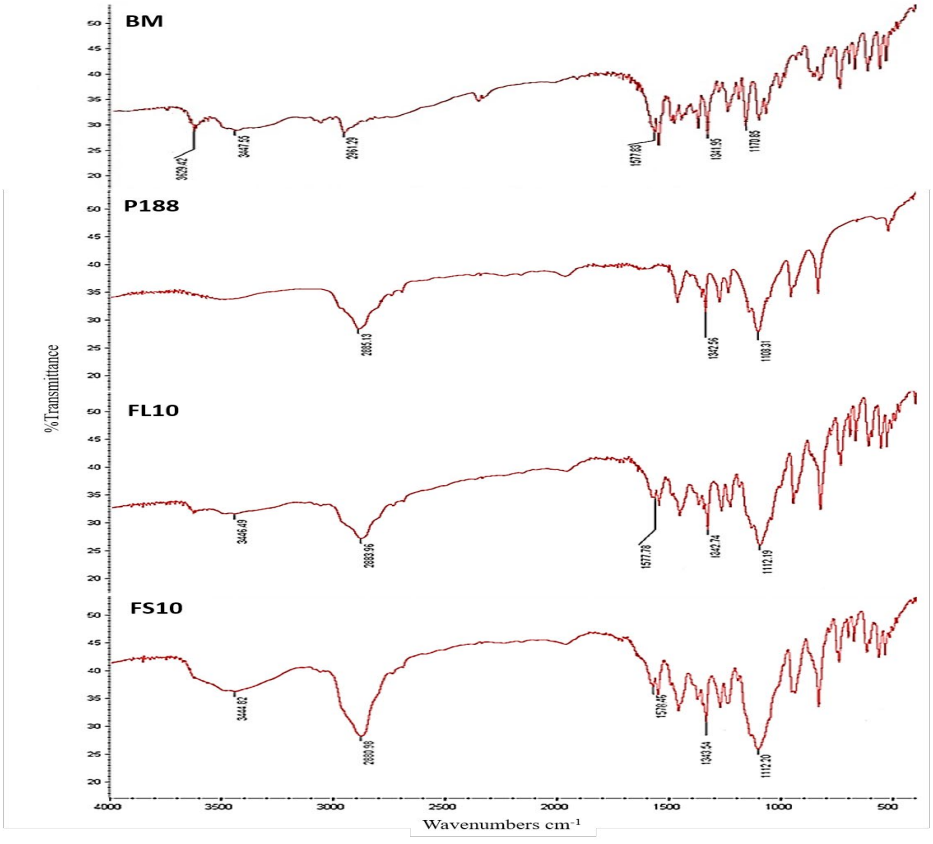

Figure 5. FTIR spectral analysis of bosentan monohydrate (BM), poloxamer 188 (P188), and optimized formulations FL10 and FS10

Particle size and size distribution analysis

The particle size of the optimized solid dispersions was determined using a particle size analyzer. The samples were dissolved in triple distilled water and subjected to particle size analysis. This method also depicts the polydispersity index (PDI), which is a measure of uniformity in size distribution. As shown in Figure 6, the average particle size and the PDI of FL10 were 450.9 nm and 0.401, respectively. And in the case of the FS10, the average particle size and the PDI were found to be 550.8 nm and 0.590, respectively.

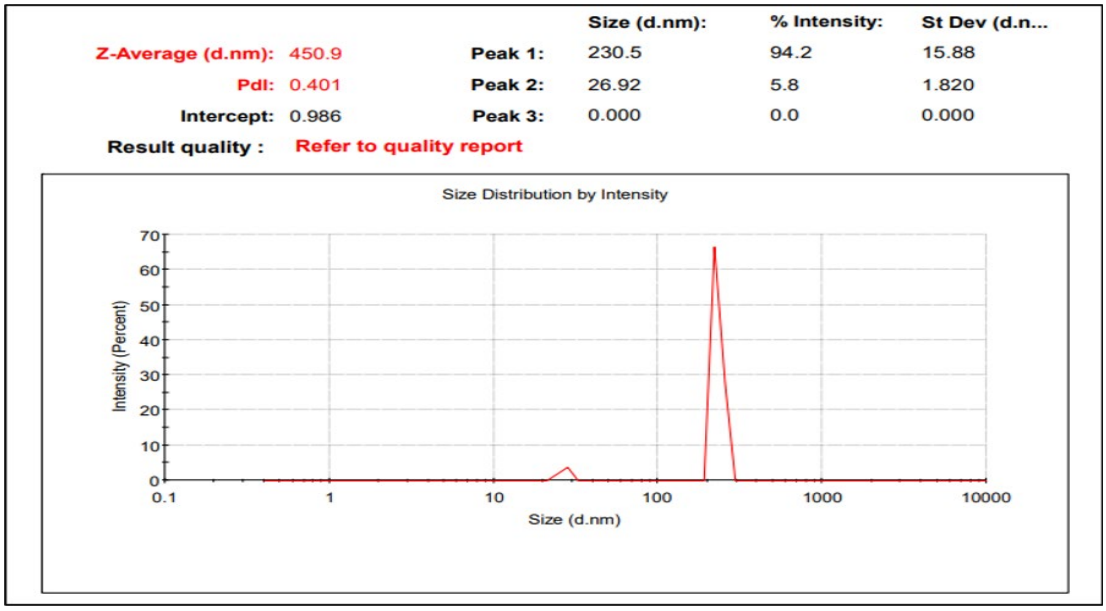

Figure 6. Particle size and size distribution of solid dispersions (A) FL10 and (B) FS10

Differential scanning calorimetry (DSC)

DSC curves of pure drug, P188, and prepared solid dispersions (FL10 and FS10) with P188 are shown in Figure 7. For pure BM, a sharp endothermic peak is observed at 128.64°C, characterizing the melting point of BM, which indicates that the pure drug was in crystalline form. P188 showed a melting endothermic peak at 58.07°C. Upon the formation of solid dispersions of drug with P188, there was a disappearance of the drug melting endotherm in the solid dispersions, which could be due to the amorphous form of BM in the solid dispersions. But in both solid dispersions, the sharp peak corresponding to polymer remained and was at a slightly lower temperature than that of pure P188 (58.07°C). It might be due to the reason that drug molecules get dispersed in the P188 matrix of the solid dispersions and the thermal property was changed, or it might be due to the formation of eutectic mixtures in solid dispersions leading to the depression of melting point (Zhai, 2017). Further, to deep root these findings, XRD of pure drug and its solid dispersions was carried out.

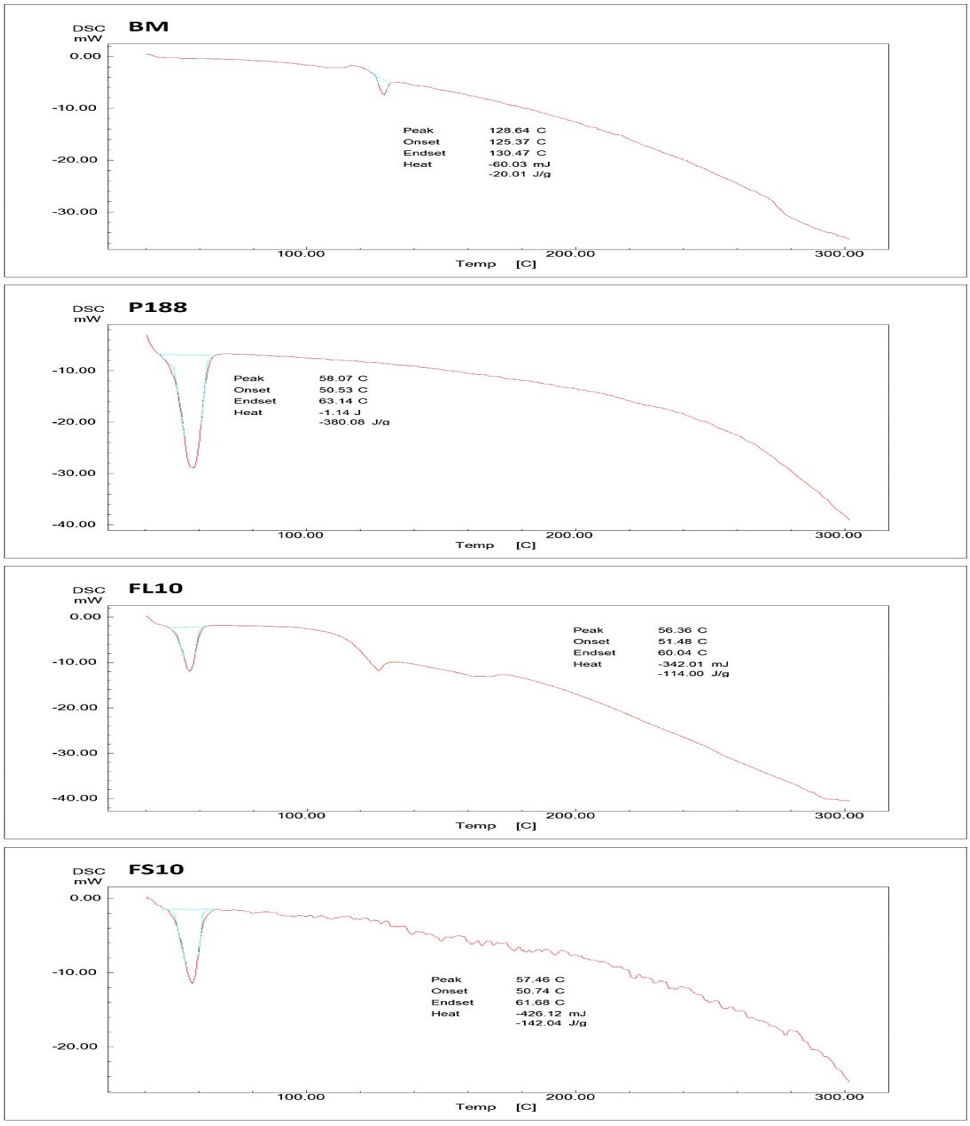

Figure 7. DSC of bosentan monohydrate (BM), poloxamer 188 (P188), and optimized solid dispersions prepared with poloxamer 188 by lyophilization and spray drying methods (FL10 and FS10)

X-ray diffraction (XRD)

X-ray diffraction patterns were used to confirm the crystalline nature of the drug. As shown in Figure 8, pure BM exhibited distinct sharp peaks at 2θ diffraction angles of 18.42°, 9.1°, 22.5°, and 16.48°, which were intense and displayed sharp intensities of 7535, 4153, 4090, and 3495, respectively, indicating its crystalline nature. In comparison, P188 showed sharp crystalline peaks at 23.18° and 19°. Both solid dispersions prepared with P188 exhibited the disappearance of some high-intensity drug peaks and a reduction in the intensity of polymer peaks. Compared to FS10, the lyophilized FL10 solid dispersion showed low-intensity peaks of corresponding drug and polymer. Hence, there was a reduction in crystallinity in both solid dispersions prepared using both methods. The disappearance or decrease in intensity of the peaks at the same diffraction angles in solid dispersions prepared with P188 indicates that BM may have undergone a transition from crystalline to amorphous form or crystallinity was reduced. Moreover, the high-intensity peaks of P188 indicating its crystalline nature also got diminished.

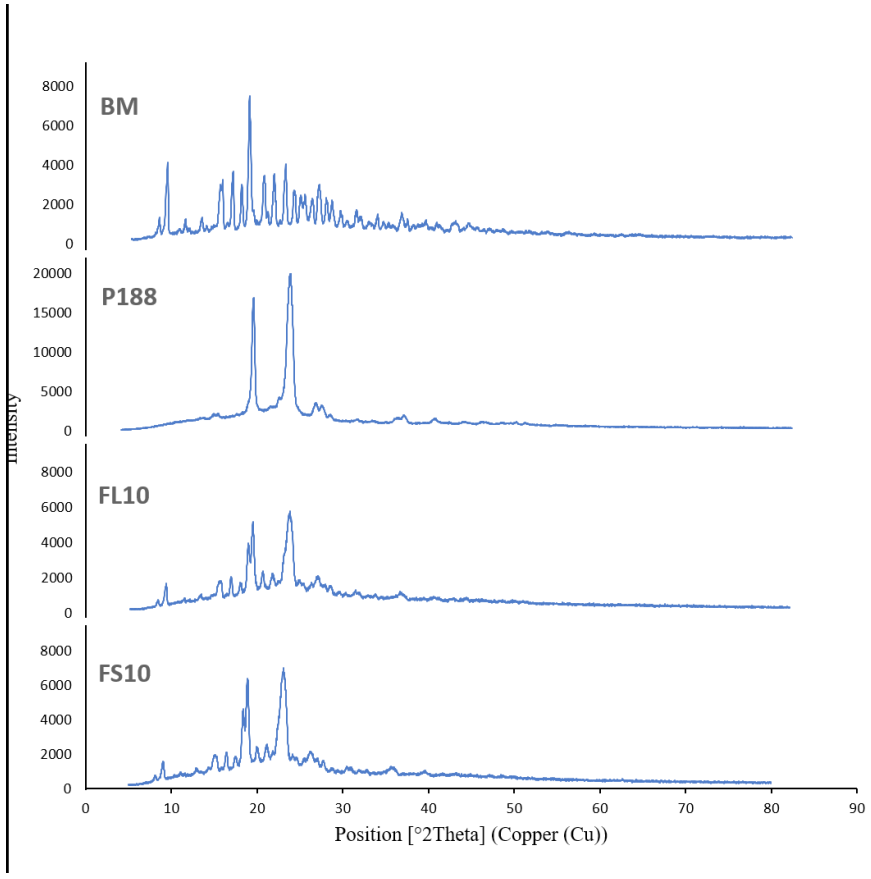

Figure 8. XRD of bosentan monohydrate (BM), poloxamer 188 (P188), and optimized solid dispersions prepared with poloxamer 188 by lyophilization and spray drying methods (FL10 and FS10)

Scanning electron microscopy (SEM)

The SEM photomicrographs of pure BM, P188, and optimized solid dispersions prepared using lyophilization and spray drying methods (FL10 and FS10) are shown in Figure 9. The pure drug appeared as crystals, whereas P188 and solid dispersions revealed amorphous particles. Solid dispersion prepared with P188 using lyophilization technique (FL10) showed the formation of a porous and fluffy product that increases the surface area and in turn, the surface free energy, resulting in higher solubility and dissolution. For further analysis of FL10 and to confirm its amorphous state, it was subjected to SEM analysis at higher magnification as shown in Figure 10.

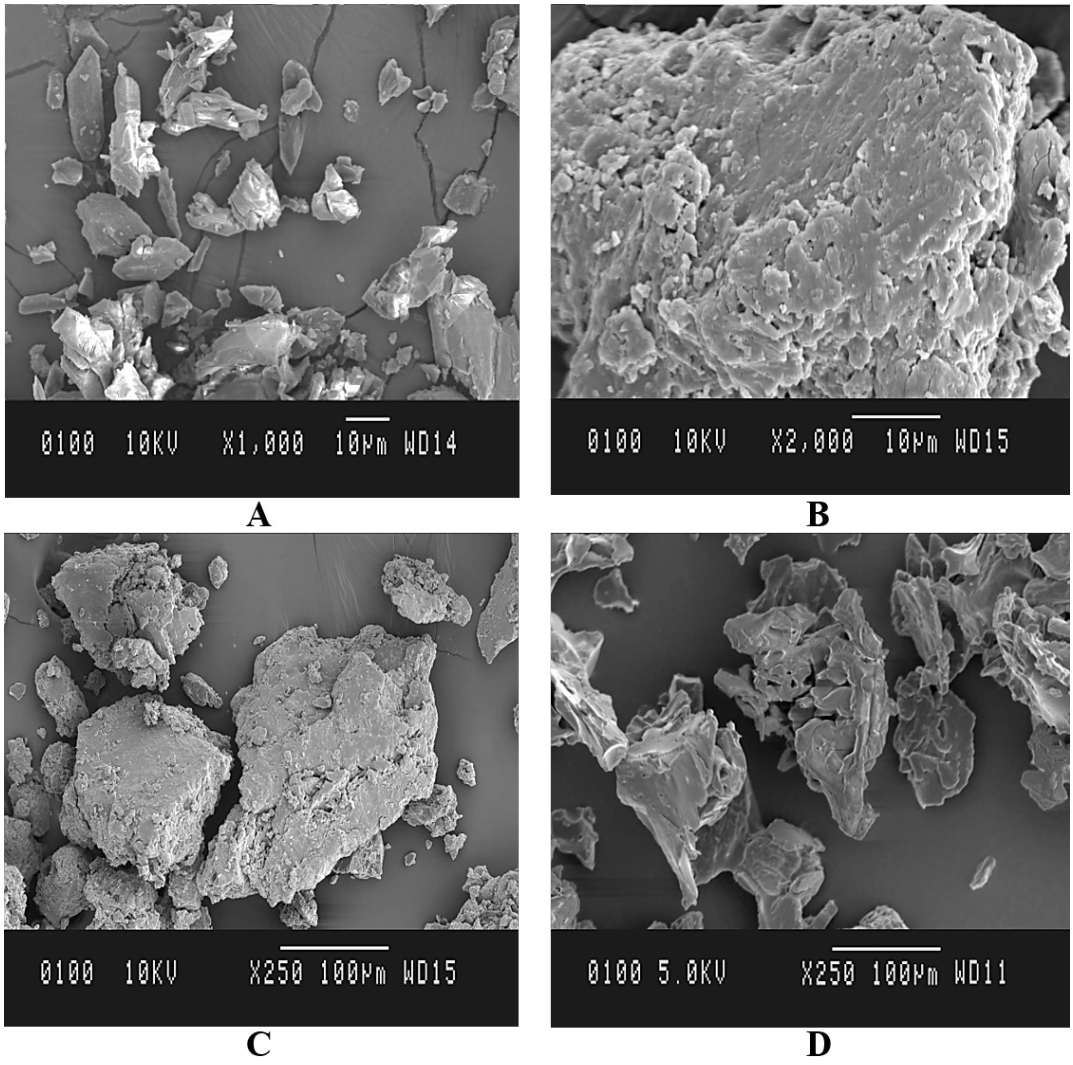

Figure 9. SEM of (A) pure bosentan monohydrate, (B) poloxamer 188, (C) solid dispersion FL10, and (D) solid dispersion FS10

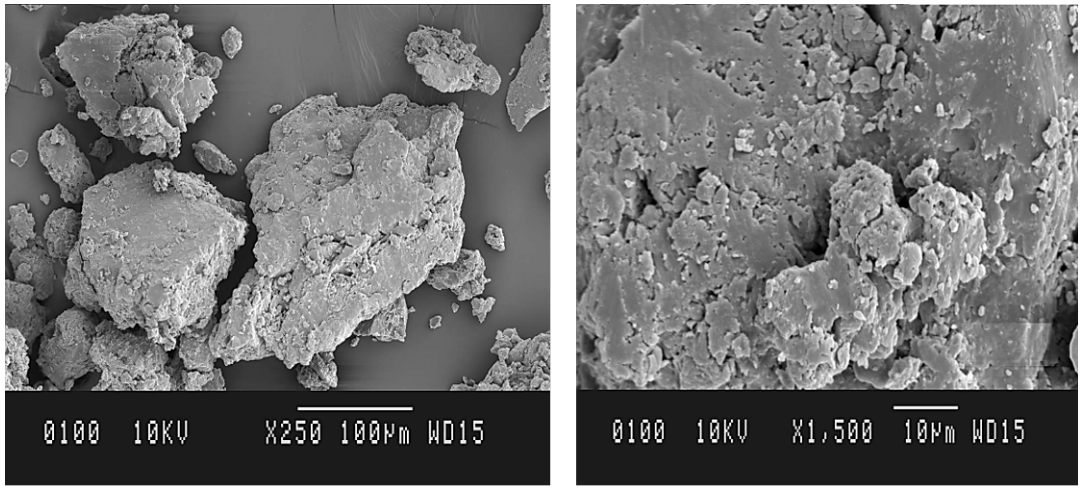

Figure 10. SEM of optimized solid dispersion formulation FL10 in magnifications of (A) 250x and (B) 1500x

Transmission electron microscopy (TEM)

As FL10 showed improved properties in previous tests, A TEM of FL10 was carried out to further determine its particle shape and particle size. As shown in Figure 11, the particle size of optimized solid dispersion FL10 prepared with P188 was found to be 505.68 nm. The reduced particle size of solid dispersion FL10 confirms why lyophilized solid dispersions were showed improved solubility and dissolution rate as compared to the solid dispersion prepared using the spray drying method. Additionally, FL10 demonstrated an acceptable shape, indicating that it may exhibit good flow properties.

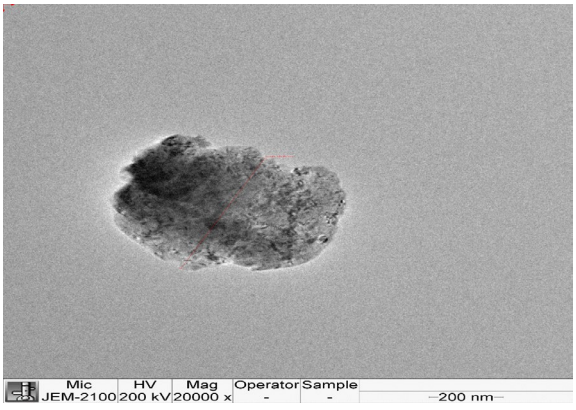

Figure 11. TEM of solid dispersion formulation FL10 prepared with poloxamer 188

Stability study of optimized solid dispersion

An accelerated stability study of optimized solid dispersion FL10 at room temperature was conducted for 3 months and any physical changes were analyzed. The results of stability studies of optimized solid dispersions of BM are shown in Table 8. There were no changes observed in the physical appearance of the solid dispersions during the storage period of 3 months. The drug content also showed no significant difference. Two characterization studies, i.e., XRD and particle size analysis were conducted to determine any change in the amorphous nature of prepared dispersions during storage. In the case of XRD studies of optimized dispersion, no sharp endothermic peaks were observed and the high-intensity peaks of the corresponding drug were absent. No significant changes were observed in both analyses up to 3 months of storage.

Table 8. Stability study of optimized solid dispersion FL10

Formulation code	Characterization	Days				
		0	30	45	60	90
FL10	Physical appearance	No change	No change	No change	No change	No change
	%DC	98.9 ±1.1	97.8 ±1.3	97.08 ±2.4	96.89 ±1.3	96.51 ±1.6
	XRD	-	-	-	-	No significant changes in the intensity of peaks
	Particle size analysis	-	-	-	-	No significant change in particle size

Data are expressed as mean ±SD (n=3); %DC: Percentage drug content, XRD: X-ray diffraction.

CONCLUSION

Solid dispersions of bosentan monohydrate were prepared to enhance its aqueous solubility and determine the effect of using lyophilization or spray drying method on solubility enhancement. Solid dispersions were prepared by using poloxamer 188 as the carrier. It was found that the particle size and solubility of the dispersions were significantly affected by the type of method used. According to the analytical study results, the lyophilization technique was more effective at preparing solid dispersions. The optimized solid dispersion prepared with poloxamer 188 by the lyophilization technique (FL10) showed a smaller particle size and was subjected to different characterization studies. The findings of this study substantiate the notion that solid dispersion reduces the particle size and crystallinity of a drug while increasing its aqueous solubility. The study also demonstrates that the lyophilization technique is better as compared to spray drying due to improved solubility, dissolution, reduced particle size, a significant reduction in crystallinity as indicated by XRD and formation of more amorphous product as indicated by SEM. Hence, it can be concluded that lyophilization technique shows superior results as compared to spray drying in the preparation of solid dispersions.

CONFLICTS OF INTEREST

No conflict of interest is declared by the authors. The authors alone are responsible for the content and writing of the paper.

AUTHOR CONTRIBUTION STATEMENT

Research hypothesis (Kaur L., Singh G., Chemban S.A.). Experimentation and data collection (Chemban S.A., Kaur A., Singh L). Draft of the text (Chemban S.A., Dhawan R.K., Kaur L.) Interpretation of the data and use of software (Kaur L., Singh G., Dhawan R.K., Chemban S.A., Singh L.). Text reviewed (Kaur L., Singh G., Dhawan R.K.). Statistical analysis (Kaur L., Singh G., Chemban S.A.). Literature research (Chemban S.A., Singh L., Singh G.). Writing, review & editing (Kaur L., Singh G., Dhawan R.K., Singh L.)

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