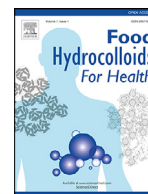




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# Food Hydrocolloids for Health

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## Introducing novel hybridization technique for solubility enhancement of Bosentan formulation

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### ABSTRACT

Bosentan is choice of drug for pulmonary arterial hypertension. It belongs to BCS class-II category. Due to poor solubility in aqueous media, treatment leads to frequent dosing & increasing cost of therapy subsequently. One cannot unsee the shortcomings of commonly existing solubility enhancement techniques such as physical and chemical modifications of the drug, salt formation, solid dispersion, complexation because of the need for sophisticated equipment, decreased yield, incomplete removal of organic solvents, etc. The novel hybridization technique is a fusion of complexation and liquisolid technique. In this research work, Bosentan was complexed with captisol in the first step. In the second step, the complex was dispersed in non-volatile solvent & mixed with the carrier using mortar-pestle followed by the addition of coating material to form a free-flowing powder. It was then compressed into tablets by mixing with other excipients. The formulation was characterized by phase solubility study, DSC, FTIR, and XRD to confirm drug-excipients compatibility, crystallinity etc. The phase solubility study showed ~28.85 fold increase in solubility. From screening, Avicel PH101 & Parteck SLC 500 as carrier and coating material respectively & PEG-600 as non-volatile solvent were selected. Explotab as super-disintegrant and Pearlitol 100SD as direct compressible excipient were used. Optimization by 3<sup>2</sup> factorial design gave Bosentan tablet with disintegrate in time of 7–8 min and showed 87.264±0.823% drug release in 30 min higher than marketed tablet i.e. 61.750±1.226%. From the stability study, the Novel hybridization technique was proved as a choice for solubility enhancement.

### Introduction

Cardiovascular disease (CVD) causes almost 18 million deaths per annum. Along with strong evidence, hypertension associated with high blood pressure have a high-risk factor for CVD. Among the types of hypertension, pulmonary arterial hypertension (PAH) is perilous with 20% mortality. It results in right coronary failure and death (Chopra & Ram, 2019; Fuchs & Whelton, 2020; Tsai, Sung & de Jesus Perez, 2016; Vadlamudi et al., 2017). For the effective management of pulmonary arterial hypertension, the newest endothelin receptor antagonist (Bosentan) is more preferred due to higher tissue penetration and greater affinity and longer duration (Hoepfer et al., 2017). Various clinical data have suggested that bosentan is a well-tolerated and effective molecule (Lan et al., 2018).

Bosentan is marketed under the trade name Tracleer. In the current market, 62.5 mg and 125 mg bosentan tablets and film-coated tablets respectively are available (Krupa et al., 2017). But certain limitations like the requirement of frequent dosing, gastric stability, poor water solubil-

ity (BCS class II drug), half-life of about 5 h, and only 50% bioavailability are resulting in its low therapeutics outcomes. Focusing on all these issues, physicochemical properties of bosentan become an interest of research to develop effective oral formulation (Azim et al., 2012).

Numerous methods are available for dissolution and solubility enhancement of BCS class-II drugs (Bremmell & Prestidge, 2019; Chaudhary & Patel, 2012; Sravya et al., 2013). The complexation method provides a complete coating of a drug molecule with a complexing agent and liquisolid system renders formulation as dry free-flowing, non-adherent and compressible powder by the use of appropriate carriers and coating materials. The combination of these two methods is called the novel hybridization technique, which imparts advantages of both the methods and becomes advantageous to improve dissolution efficiency of bosentan (Wang et al., 2017; Zhang et al., 2018).

Design of experiments (DoE) and multivariate statistical data analysis are crucial fundamentals of QbD, established by the recent International Conference of Harmonization Q8 guideline (Saydam & Takka, 2018).

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In the present research work, systematic optimization of formulation and evaluation of bosentan formulations were conducted using basic QbD tool i.e. full factorial design. The issue of poor solubility and dissolution were addressed by a complexation, liquisolid, and novel hybridization technique (combination of complexation & liquisolid technique). Use of novel components such as Captisol (Sulfobutylether  $\beta$ -cyclodextrin) for solubility enhancement of drug by encapsulation, Parateck SLC 500 (Mesoporous silica) as particle structure modifier to convert drug in amorphous form, Kolliphor EL (version of polyethoxylated castor oil) as emulsifier and non-ionic solubilizer, Pearlitol SD 100 (Mannitol) as direct compression excipient and Explotab (Sodium Starch Glycollate) as a super-disintegrant were screened and evaluated for betterment of dissolution efficacy of bosentan. Interaction between drug and excipients were studied using DSC & FTIR study. Screening of drug:  $\beta$ -CD ratios, carrier and coating material were investigated. The dissolution properties of bosentan formulation by all 3 methods were also investigated and compared with marketed formulation and crystallinity of formulation was investigated by XRD study.

## Materials and methods

### 2.1. Materials

Bosentan was received as a gift sample from Alembic Pharmaceuticals Ltd., Vadodara, Gujarat, India. Sulfobutylether  $\beta$ -cyclodextrin (Captisol) from Cydex, Gangwal, Mumbai, Maharashtra, India, Kolliphor EL (version of polyethoxylated castor oil) from BASF Pvt. Ltd., Mumbai, Maharashtra, India, Parateck SLC 500 (Mesoporous silica) from Merck Life Science Pvt. Ltd., Mumbai, Maharashtra, India, Pearlitol SD 100 (Mannitol) from Signet Chemical Corporation Pvt. Ltd., Mumbai, Maharashtra, India and Explotab from Rettenmaier India Pvt. Ltd., Mumbai, Maharashtra, India were obtained as a gift sample. Double distilled water was prepared in the laboratory for study. All materials used for the study conformed to USP 24 standards and were purchased from Sigma-Aldrich, Mumbai (India).

### 2.2. Methods

#### 2.2.1. Phase solubility study

According to Spireas and Sadu (1998), the phase solubility studies of bosentan were determined in the Bosentan-Captisol inclusion complex

in different ratios (1:1, 1:2 & 1:3) (Spireas & Sadu, 1998). Saturated solutions in 6.8 pH phosphate buffer were prepared by adding an excess amount of bosentan containing complex and rotated for 48 h at 100 RPM to reach equilibrium at 25 °C using an orbital shaker. The filtered supernatants were further diluted if necessary with 6.8 pH phosphate buffer and analyzed spectrophotometrically using a UV/visible spectrophotometer (Shimadzu UV-1650 PC (E) 230 V, Tokyo, Japan) at 272 nm (Maulvi et al., 2011). The solubility of bosentan in the respective Bosentan-captisol inclusion complex was calculated using the bosentan calibration curve (Dangre, Sormare & Godbole, 2017) (Fig. 1). Each result were in triplicate for calibration. Gibbs free energy ( $\Delta G^\circ$ ), the thermodynamic parameter was determined by the Eq. (1).

$$\Delta G^\circ = -2.303RT \log(S_c/S_o) \quad (1)$$

Where,  $\Delta G^\circ$  = Gibbs free energy of transfer,  $R$  = Gas constant (8.314 J/K.mole),  $T$  = Temperature in kelvins &  $S_c/S_o$  = Ratio of molar solubility of Bosentan with Captisol.

#### 2.2.2. Complexation method

Bosentan & captisol (Drug:  $\beta$ CD) inclusion complex was prepared in 1:1 molar ratio by using the co-solvents evaporation method. Bosentan was dissolved into ethanol (80%), while Captisol was dissolved into water (20%). The resulting solution was evaporated at 45 °C and the dried complex was pass through sieve no.44 (Chaudhary & Patel, 2012; Sravya et al., 2013). Powdered Complex was compressed into a tablet by direct compression method by using Avicel pH 101 as a diluent, pregelatinized starch as a disintegrating agent and magnesium stearate and talc as a lubricant. Batch L1 was prepared by the complexation method.

#### 2.2.3. Liquisolid technique

The liquisolid formulation was obtained by mixing the drug with non-volatile solvent and then the drug solution was mixed with carrier excipients where coating of the drug molecule with thin film was formed. The resulting liquid medication-carrier system was adsorbed on a coating agent to get a dry, free-flowing and nonadherent powder that can be easily compacted into tablets. So screening of non-volatile solvents, carrier and coating material was essential (Elkordy et al., 2012).

2.2.3.1. Screening of non-volatile solvents. For screening, various non-volatile solvents such as Tween 20, Tween 40, Tween 60, Tween 80,

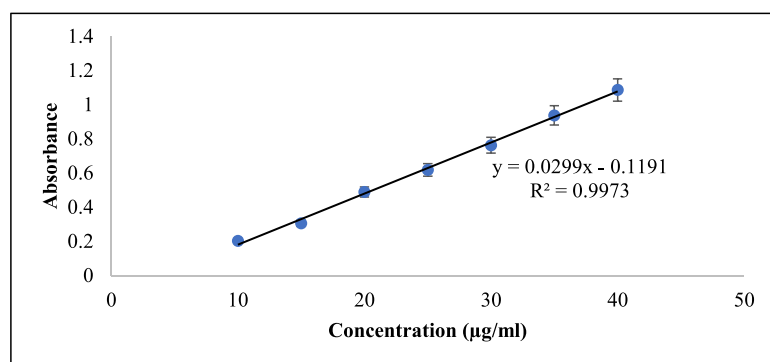


Fig. 1. Bosentan Calibration Curve (Each result is in triplicate).

Table 1

Variables (CQAs and CMAs) of full factorial design with coded and actual values of CMAs.

CMAs	Coded and Actual Values		
	Low (-1)	Medium (0)	High (+1)
Amount of Explotab (X1)	26.5 mg	39.75 mg	53 mg
Amount of Pearlitol SD 100 (X2)	26.5 mg	53 mg	79.5 mg
CQAs	Target		
The disintegration time (in a minute) ( $Y_1$ )	Below 30 min		
Cumulative drug released after 30 min (%) ( $Y_2$ )	Maximum		

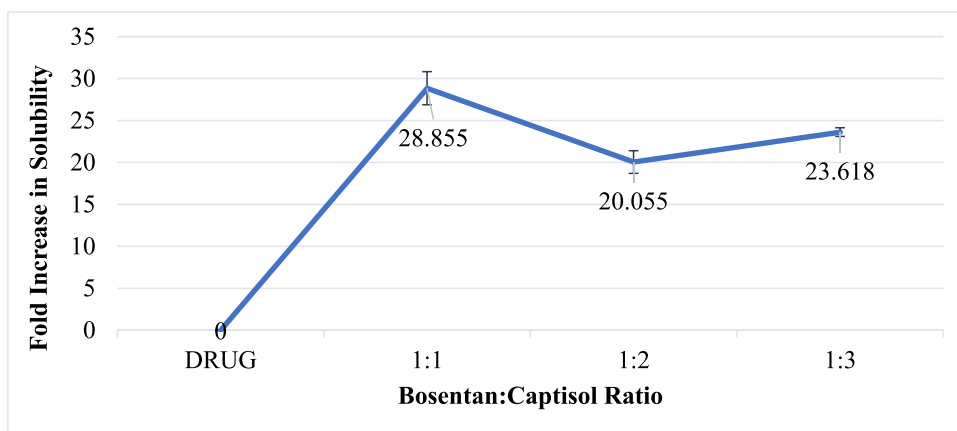


Fig. 2. Phase Solubility curve.

Table 2  
Gibbs free energy.

Bosentan: Captisol molar ratio	Solubility* (mg/ml)	Gibbs free energy* ( $\Delta G^\circ$ )(J/K.mol)	Fold increase in solubility*
Bosentan	0.055±0.001	-	-
1:1	0.469±0.002	-5531.884±42.061	28.855±1.976
1:2	0.338±0.002	-4689.037±39.669	20.055±1.348
1:3	0.353±0.023	-4877.283±234.283	23.618±0.521

\* All values are mean ± SD; n = 3.

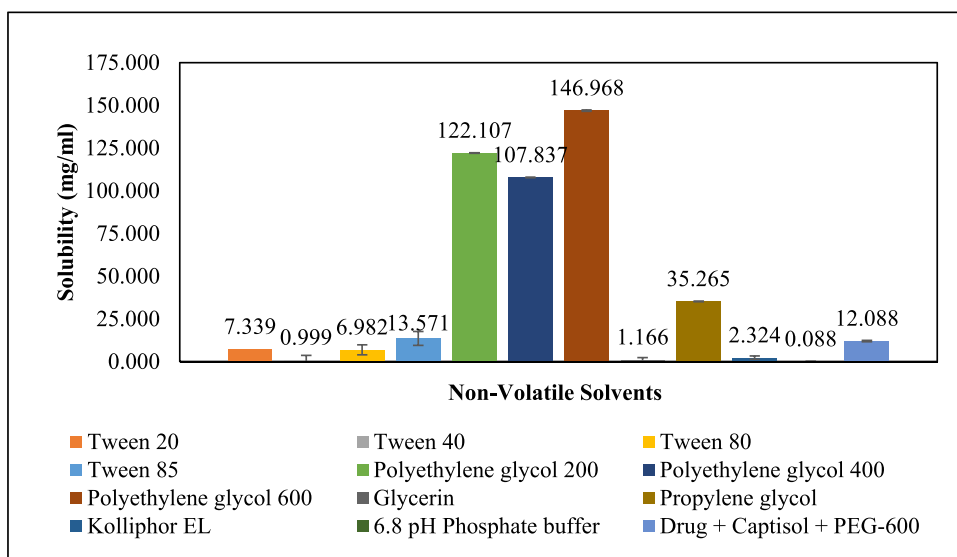


Fig. 3. Screening of Non-Volatile solvents.

Table 3  
Liquid-retention potentials ( $\Phi$ -Value) with the corresponding liquid load factor ( $L_f$ ).

Coating Materials	Syloid 244 FP	Sylsilia 350 F CP	Syloid XDP 3150	Pardeck SLC 500					
$\Phi$ -Value	0.865±0.011	0.945±0.018	1.017±0.023	1.557±0.024					
Carrier	Avicel pH 101	Avicel pH 101	Avicel pH 101	Avicel pH 101					
Carrier:Coating Materials	1:1	2:1	1:1	2:1	1:1	2:1	1:1	2:1	2:1
$L_f$	0.899 ± 0.014	0.471 ± 0.008	0.979 ± 0.020	0.511 ± 0.011	1.050 ± 0.026	0.547 ± 0.014	1.591 ± 0.026	0.817 ± 0.014	

Tween 85, Polyethylene glycol 200, Polyethylene glycol 400, Polyethylene glycol 600 and Kolliphor EL were selected and excess quantity of drug and inclusion complex were added into it. These suspensions were kept for 48 hrs on an orbital shaker at room temperature and then filtered & analysed for best non-volatile solvent [16].

2.2.3.2. Screening of carrier and coating materials. Carrier & coating material selection were based on liquid load factor and flowable liquid re-

tention potential. The flowable liquid retention potential ( $\Phi$ -value) of a powder means maximum amount of a non-volatile liquid retained inside powder bulk to maintain adequate flowability. The liquid load factor ( $L_f$ ) is the mass ratio of the liquid medication to the carrier powder in the liquid-solid formulation (W/W). To calculate  $L_f$ , non-volatile solvent was dropwise added to 10 g carrier powder followed by blending for 1 min and carrier powder was evaluated for flowability.  $L_f$  and  $\Phi$ -value were determined by using Eq. (2) and (3) respectively to find out the



**Fig. 4.** Bosentan formulations by solubility enhancement techniques.

**Table 4**  
Response values of full factorial design.

Batch No.	Variable levels in coded form		Response Values	
	X1	X2	Disintegration time (minute) (Y <sub>1</sub> )	Cumulative drug released at 30 min (%) (Y <sub>2</sub> )
L1	NA	NA	6.600±0.408	77.461±1.065
L2	NA	NA	8.760±0.033	71.808±0.936
F1	-1	-1	9.600±0.216	82.300±1.466
F2	0	-1	9.900±0.294	71.685±0.827
F3	+1	-1	8.480±0.054	47.315±0.740
F4	-1	0	10.100±0.163	72.810±0.805
F5	0	0	7.500±0.016	87.264±0.823
F6	+1	0	9.200±0.216	84.543±1.031
F7	-1	+1	8.500±0.082	84.369±1.287
F8	0	+1	8.000±0.163	66.743±1.018
F9	+1	+1	8.600±0.163	68.870±0.648

**Table 5**  
Predicted and Experimental values of optimized and checkpoint batches.

Batch code	X <sub>1</sub>	X <sub>2</sub>	Predicted value		Experimental value	
			Y <sub>1</sub>	Y <sub>2</sub>	Y <sub>1</sub>	Y <sub>2</sub>
Optimized batch	40.657	56.350	7.63	87.14	7.500±0.163	86.690±0.901
Check point batch (I)	35.628	69.623	8.30	84.68	8.343±0.065	83.948±0.871
Check point batch (II)	46.458	39.348	8.20	82.65	8.183±0.029	80.974±0.946

X<sub>1</sub> = Amount of Explotab (in mg), X<sub>2</sub> = Amount of Pearlitol SD 100 (in mg), Y<sub>1</sub> = Disintegration time (in min), Y<sub>2</sub> = % Cumulative drug released at 30 min.

**Table 6**  
Stability Study Results.

Formulation	Testing interval	Content uniformity (%)	% Cumulative drug released
Optimized formulation (F5)	Initial	95.226±2.818	87.264±0.823
	10 days	95.085±0.147	86.564±0.623
	20 days	94.917±0.139	86.364±0.923
	30 days	94.854±0.080	86.164±1.122

best suitable carrier and coating material ratios (Bonthagarala et al., 2019; Kurek et al., 2016).

$$Lf = \Phi_{carrier} + \Phi_{coating} \cdot (1/R) \quad (2)$$

$$\Phi \text{ value} = \text{Weight of liquid medication} / \text{Weight of powder} \quad (3)$$

Where, Lf= Liquid load factor, R=Ratio between weights of carrier and coating materials

**2.2.3.3. Liquisolid method.** Bosentan was dispersed into a suitable non-volatile solvent. Then carrier was added into a mortar and mixed with the above solution for 10 to 20 min with the help of a pestle. Then coating material was added and mixed with the above mixture to form a free-flowing powder. This mixture was mixed with other excipients & compressed into tablets using a tablet compression machine using the same components as discussed in Section 2.2.3. Batch L2 was prepared by the liquisolid method (Anzilago et al., 2019; Singh et al., 2012).

#### 2.2.4. Novel hybridization technique

Novel hybridization technique was implemented by the combination approach of complexation with liquisolid technique. Here, a complex of Bosentan-captisol (1:1 molar ratio) was dispersed in the non-volatile solvent & then the carrier was added into mortar and pestle and mixed with it for 10 to 20 min. Then coating materials were completely mixed with the above mixture to form a free-flowing powder. This free-flowing powder was mixed with other excipients & compressed into tablets by using a tablet compression machine using the same components discussed in Section 2.2.3 (Pile & Chemate, 2019). Formulation of bosentan by novel hybridization technique was optimized using 3<sup>2</sup> full factorial design discussed in Section 2.2.6.

#### 2.2.5. Optimization of formulation by experimental design

A two factor- three-level full factorial design was employed to optimize diverse critical material attributes influencing the response variables i.e. critical quality attributes. Amount of Explotab (X<sub>1</sub>) and Amount of Pearlitol SD 100 (X<sub>2</sub>) were selected as an independent critical material attributes and varied at three different levels i.e. low, medium and high on the basis of literature review (Table 1). The disintegration

**Design-Expert® Software**

Factor Coding: Actual

**Disintegration time (Minute)**

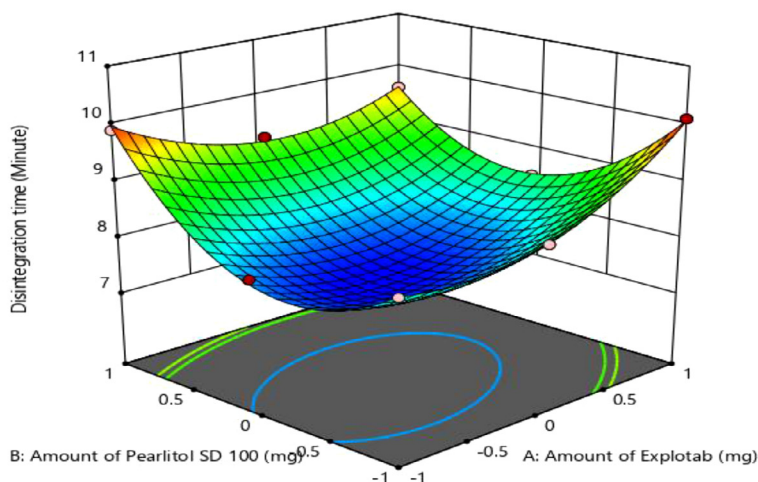
● Design points above predicted value

○ Design points below predicted value

7.5  10.1

X1 = A: Amount of Explotab

X2 = B: Amount of Pearlitol SD 100

**Design-Expert® Software**

Factor Coding: Actual

**Cumulative drug released at 30 minutes (%)**

● Design points above predicted value

○ Design points below predicted value

47.315  87.264

X1 = A: Amount of Explotab

X2 = B: Amount of Pearlitol SD 100

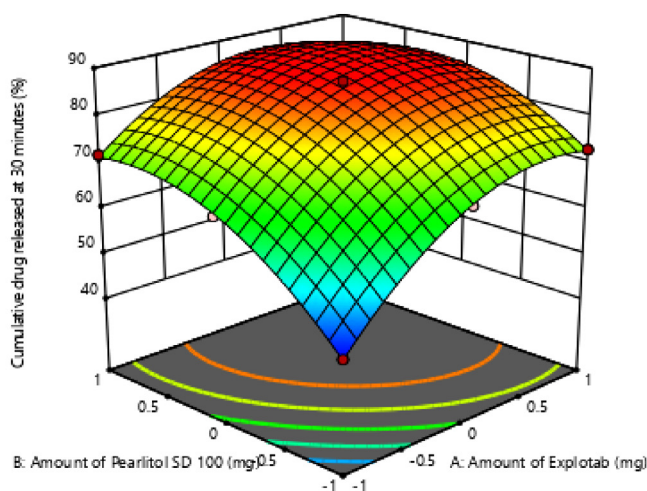


Fig. 5. 3D Response surface plots for evaluating the influence of CMAs on CQAs (a) Y<sub>1</sub> (Disintegration time) and (b) Y<sub>2</sub> (% Cumulative drug released at 30 min).

time (in a minute) (Y<sub>1</sub>) and cumulative drug released after 30 min (%) (Y<sub>2</sub>) were evaluated as a dependant response variable. Total 9 batches were prepared and evaluated. The obtained data were fitted into Design-Expert software (V.11, Stat-Ease Inc., and Minneapolis, USA). Response surface analysis was carried out and contour plots and 3D response surface plots were constructed to establish the understanding of the relationship of variables and their interaction. Polynomial equations were constructed and fitted into a multiple linear regression model. Check-point analysis & overlay plot were done by setting constraints for quality attributes at target levels to validate the design (Beg et al., 2018).

### 2.2.6. Characterization of formulation

**2.2.6.1. Differential scanning calorimetry (dsc).** DSC is a method for the detection of any physicochemical interaction between drug and excipients and it was done as outside analysis at Dharmsinh Desai University, Nadiad, Gujarat, India. The pure drug, Bosentan: Captisol inclusion complex and the optimized formulation were subjected to thermal analysis using a differential scanning calorimeter for drug-excipient compatibility study over a temperature range of 0 °C–450 °C with a heating

rate of 10 °C/min. The atmosphere around the sample cell was purged with nitrogen 200 mL/min. 4.0–6.0 mg sample amount was used for DSC testing. The instrument was calibrated by using indium and zinc as a standard and empty pan was used as a reference.

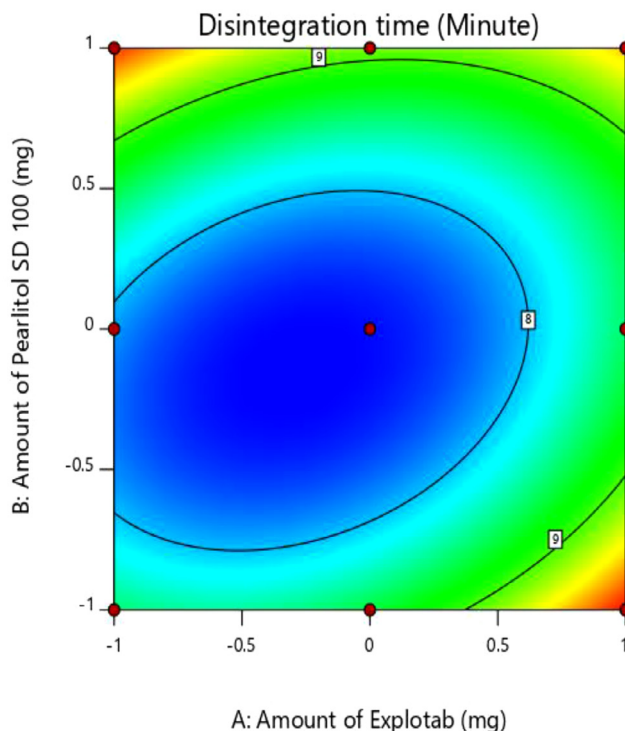
**2.2.6.2. Fourier transform infrared spectroscopy (FTIR).** FTIR spectroscopy helps in the determination of any kind of chemical interactions among drug and excipients used in the formulation and study was done as outside analysis at Dharmsinh Desai University, Nadiad, Gujarat, India. The FTIR spectra of pure drug, Bosentan: Captisol inclusion complex and optimized bosentan formulation were obtained in the frequency range of 4000–500 cm<sup>-1</sup> and resolution of 4 cm<sup>-1</sup>.

**2.2.6.3. X-Ray diffraction (XRD).** For the identification of crystalline structure after complexation, the XRD study of the pure drug and Bosentan: Captisol inclusion complex was carried out as outside analysis at Dharmsinh Desai University, Nadiad, Gujarat, India with Cu- target X-ray tube and Xe-fill detector over a scope of 5–85 (2θ). The conditions were 40 kV voltage and 20 mA current (Dangre et al., 2017).

**Design-Expert® Software**  
 Factor Coding: Actual

**Disintegration time (Minute)**  
 ● Design Points  
 7.5 10.1

X1 = A: Amount of Explotab  
 X2 = B: Amount of Pearlitol SD 100



**Design-Expert® Software**  
 Factor Coding: Actual

**Cumulative drug released at 30 minutes (%)**  
 ● Design Points  
 47.315 87.264

X1 = A: Amount of Explotab  
 X2 = B: Amount of Pearlitol SD 100

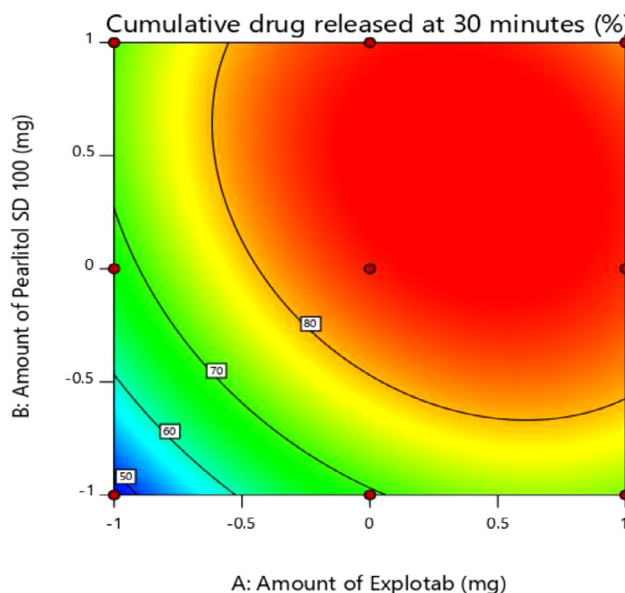


Fig. 6. Contour plots for evaluating the influence of CMAs on CQAs (a) Y1 (Disintegration time) and (b) Y2 (% Cumulative drug released at 30 min).

2.2.7. Evaluation of physicochemical characteristics of optimized bosentan formulation

2.2.7.1. Pre & post-compression parameters. The pre-compression parameters of formulations such as tapped density, bulk density, angle of repose, Carr’s index and Hausner’s ratio were performed to check the feasibility of powdered complex for compression (Khan & Agrawal, 2018).

The post-compression parameters of formulations such as weight variation, thickness, hardness, friability and content uniformity were carried out as per standard methods of pharmacopeia for evaluation of tablets prepared from powdered complex (Uddin et al., 2016).

2.2.7.2. Disintegration test. The disintegration test was carried out in 6.8 pH phosphate buffer at 37 °C ± 0.5 °C and the time taken for the disintegration of tablets were noted. Experiments were performed in triplicate (Uddin et al., 2016)

2.2.7.3. Dissolution studies. USP dissolution test apparatus type II (Paddle) was used for dissolution studies. A dissolution test was carried out using 900 ml of phosphate buffer 6.8 pH at 37 ± 0.5 °C temperature and 75 RPM. 5 ml sample solutions were collected at a precise time interval of 5, 10, 15, 20, 25, and 30 min and an equivalent volume of fresh solution was added to maintain the sink condition. The sample solution was

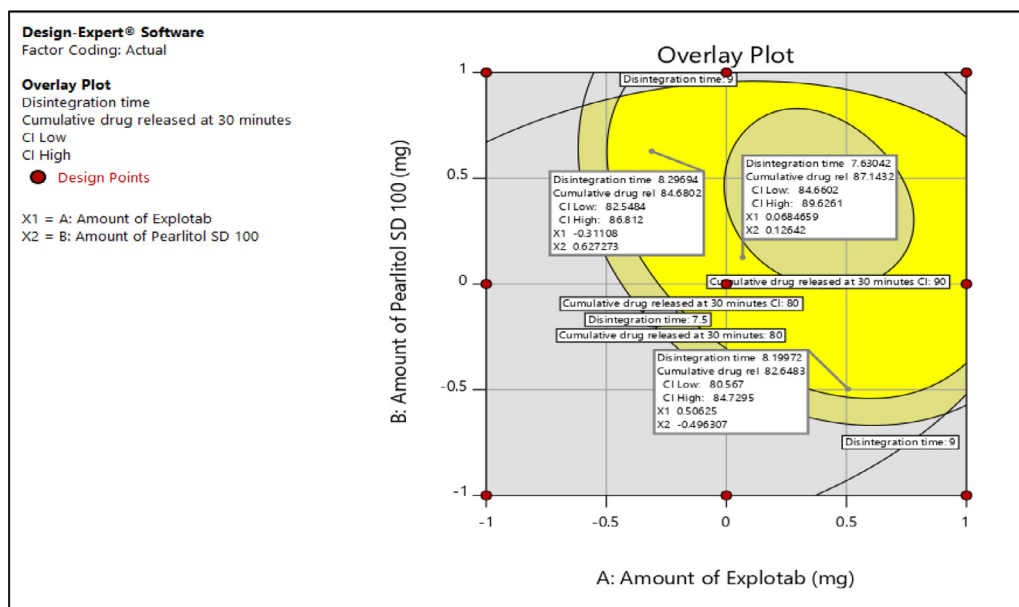


Fig. 7. Overlay plot.

analysed at 272 nm using a spectrophotometer against a suitable blank (Dangre et al., 2017).

**2.2.7.4. Comparison of dissolution profile with marketed product.** A comparison of the dissolution profile of the Bosentan formulation of Novel hybridization technique with the pure drug, marketed formulation (BOSENTAS 62.5), tablets prepared by complexation and liquisolid techniques was carried out as per dissolution studies specified in Section 2.2.7.3.

### 2.2.8. Stability study

Optimized formulation was subjected to stability study at  $40 \pm 2^\circ \text{C}$  and  $75 \pm 5\%$  RH conditions for 1 month to evaluate storage condition. After 10, 20 and 30 days the formulation was analysed for content uniformity and dissolution rate as per the procedures reported in Section 2.2.7 (Dangre et al., 2017).

## Results & discussion

### 3.1. Phase solubility study

The phase solubility study shows that a 1:1 molar ratio of Bosentan-Captisol exhibits the highest solubility (~28fold) as compared to 1:2 and 1:3 molar ratios. Inclusion complexation may attribute to form electrostatic/hydrogen bond interaction and salt formation. The phase solubility diagram corresponded to the  $A_L$ -type of the solubility curve shown in Fig. 2. Each result are in triplicate. The thermodynamics of the process of transfer of Bosentan from an aqueous solution of Captisol was anticipated from values of Gibbs free energy change ( $\Delta G^\circ$ ). The  $\Delta G^\circ$  value was shown in Table 2. The highest negative value of  $\Delta G^\circ$  value was found for 1:1 molar ratio indicating the spontaneous nature of Bosentan solubilization (Maulvi et al., 2011).

### 3.2. Screening of non-volatile solvent

Higher solubility in solvents indicates more drug solubility. Non-volatile solvent is an integral component of liquisolid system. So, The non-volatile solvent selection was based upon saturation solubility of bosentan as shown in Fig. 3. The highest solubility of bosentan was

found in PEG-600, this may be attributed to -OH groups for bond formation between drug and solvent (Kharwade et al., 2012). Several hydroxyl groups of PEG encapsulate the drug molecule and make them hydrophilic. The solubility of Bosentan: Captisol inclusion complex in PEG-600 was ~12 mg/ml.

### 3.3. Screening of carrier and coating materials

For the selection of best carrier and coating materials, Avicel pH 101 as a carrier and Syloid 244 FP (SA-380  $\text{m}^2/\text{g}$ , pore size-17 nm), Sylsisa 350 F CP (SA-300  $\text{m}^2/\text{g}$ , pore size-21 nm), Syloid XDP 3150 (SA-320  $\text{m}^2/\text{g}$ ) and Parateck SLC 500(SA-500  $\text{m}^2/\text{g}$ , pore size-6 nm), as coating materials were chosen for evaluation (Table 3). Because of higher surface area and pore size, Parateck SLC 500 showed the highest liquid loading capacity (~1.59) of 1:1 ratio with carrier and also showed better flowability as compared to other coating materials. Liquid retention potential ( $1.557 \pm 0.024$ ) was also highest for Parateck SLC 500 among all four coating materials.

### 3.4. Bosentan formulation by complexation, liquisolid & novel hybridization technique

Fig. 4 shows formulations of Bosentan by complexation, liquisolid & Novel hybridization technique.

### 3.5. Optimization of formulation by $3^2$ factorial experimental design

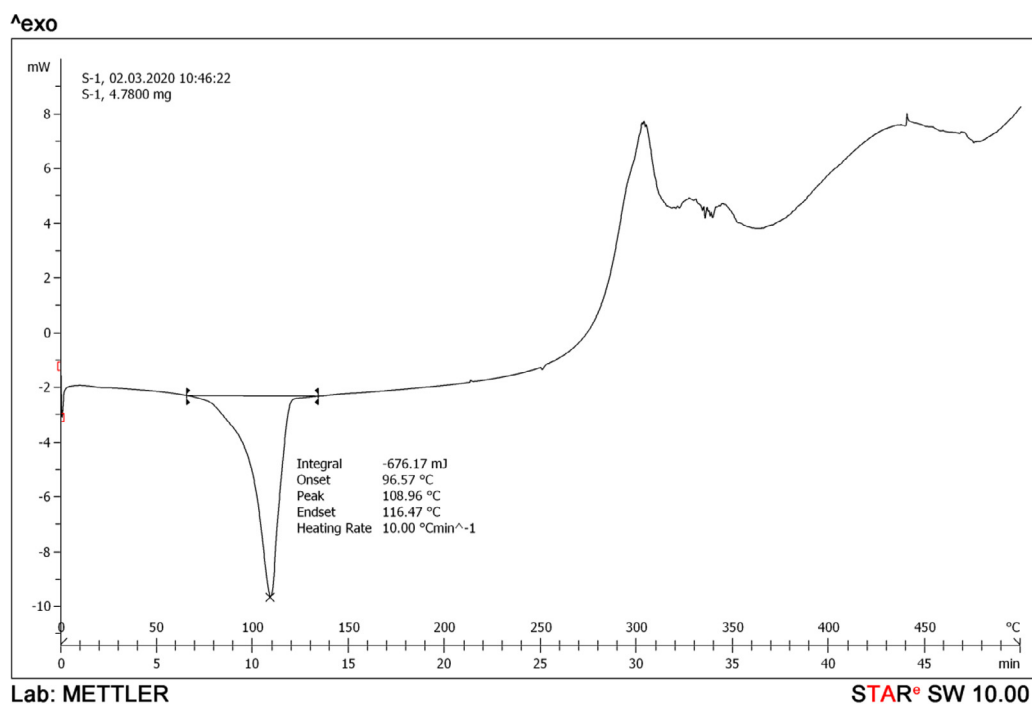
A two-factor, three-level full factorial design was employed to optimize various CMAs influencing the response variables i.e. CQAs as shown in Table 1. Total 9 batches were prepared. The design matrix formed via Design-Expert software (V.11, Stat-Ease Inc., and Minneapolis, USA) with response data for all experimental runs are shown in Table 4. The ranges of Y1 and Y2 for all batches were 6.6–10.1 min and 47.31%–87.26% respectively. The

Response values were fitted to various models by software and the best-fitted model was determined. From statistical analysis, quadratic was noted as the best-fitted model for all the responses.

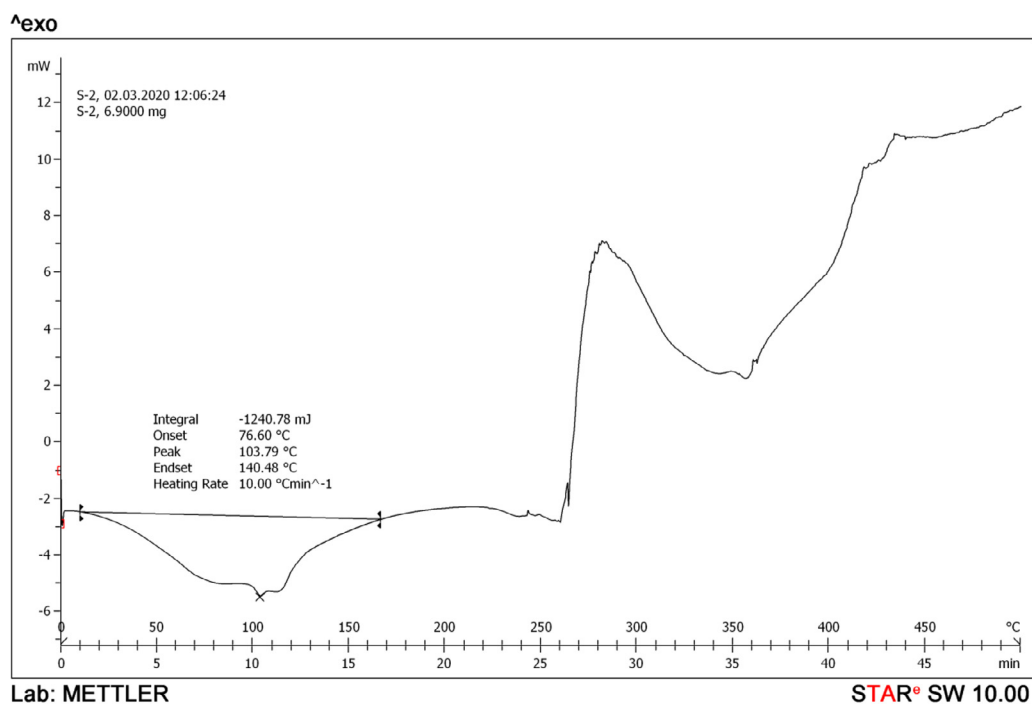
3.5.1. Response ( $Y_1$ ) disintegration time

The design expert software suggested a quadratic model with an  $R^2$  value equal to 0.9964. The p-value of the model was 0.0007 indicating the model was significant. As depicted in 3D and 2D plots (Fig. 5a &

6a), it was indicated that at higher levels of explotab, an increase in the levels of perlitol SD 100 showed a positive influence on Disintegration time, but it decreased up to a certain level, followed by its increases. On the contrary, increasing the level of explotab, at constant perlitol SD



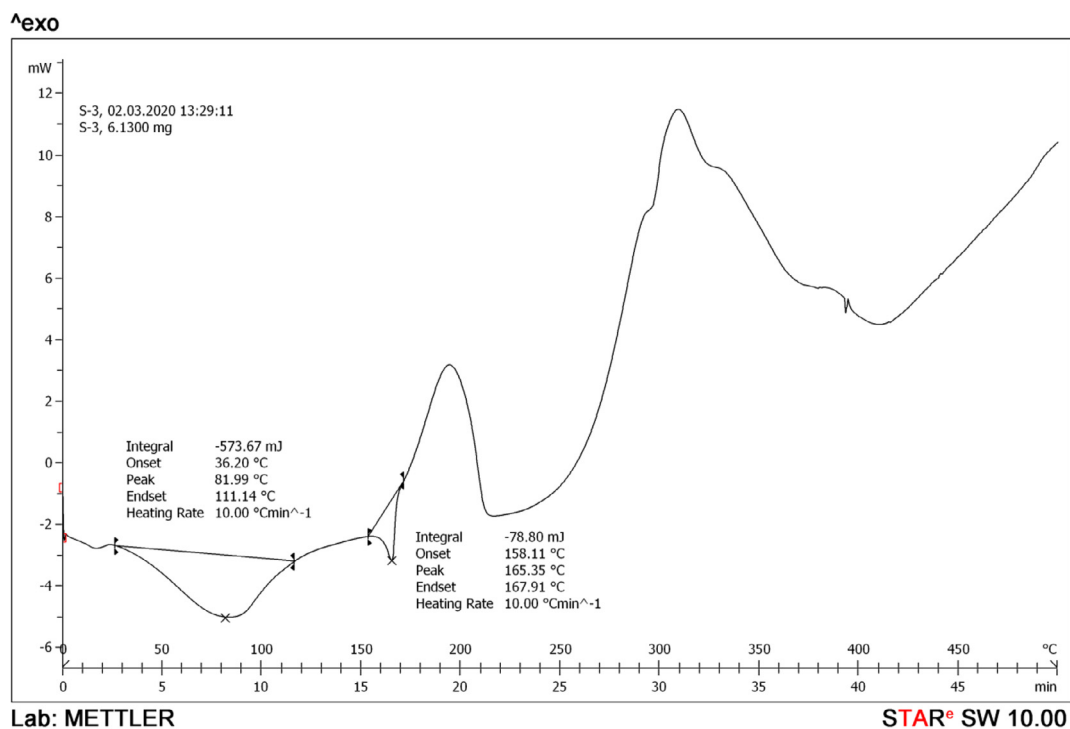
(A)



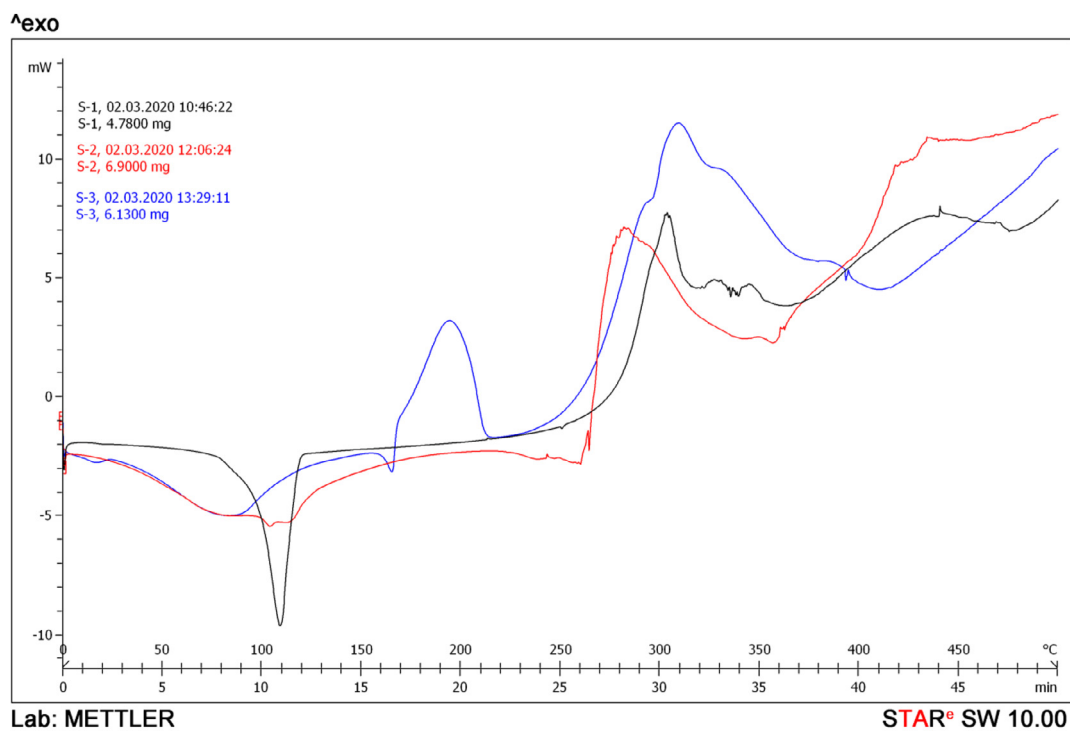
(B)

Fig. 8. DSC thermograms of the Bosentan (S1)(A), Bosentan: Captisol inclusion complex (S2)(B), Optimized formulation (S3)(C) & Overlay DSC spectra (D).





(C)



(D)

Fig. 8. Continued

100, an increase in disintegration time was observed. Thus, the lowest level of explotab while the highest level of Perlitol SD 100, resulted in maximum disintegration time. This can be attributed to the intermolecular van der Waals forces between molecules which can be easily bro-

ken up by perlitol SD1 100 as it is reported as fast-melting disintegrant (Martino, Martelli & Wehrlé, 200AD).

The final mathematical model for coded factors determined by the Design-Expert software is shown below in Eq. (4) for Disintegration

Time.

$$Y_1 = 7.56 + 0.303X_1 + 0.253X_2 - 0.480X_1X_2 + 0.663X_1^2 + 1.31X_2^2 \quad (4)$$

The Eq. (4) represented that the amount of Explotab for Pearlitol SD 100 have higher influence on disintegration time.

### 3.5.2. Response ( $Y_2$ ) cumulative drug released (CDR) at 30 min

The design expert software suggested a quadratic model with an  $R^2$  value equal to 0.9951. The p-value of the model was 0.0011 indicating the model was significant.

As depicted by the 2D contour plot (Fig. 6b), cumulative drug release at 30 min from the drug is positively correlated with both  $X_1$  &  $X_2$ . The quadratic equation indicated that on increasing  $X_1$  from low to intermediate levels, CDR increases gradually while, increasing beyond it up to higher levels, the extent of increase in CDR was found to be less. This can be attributed to the facilitation of breaking up tablets into smaller fragments in presence of disintegrants. Exlotab acts as a pore-forming agent and increasing the affinity of water towards tablets. This process resulted in faster drug release from formulation (El-Maradny, 2008). A similar positive influence of perlitol SD 100 on CDR was observed due to high wettability and pore size that promoted better penetration of dissolution media thus improved drug dissolution (Desai & Prabhakar, 2015; Paul et al., 2019). The final mathematical model to coded factors as de-

termined by the Design-Expert software is shown below in Eq. (5) for CDR.

$$Y_2 = 85.70 + 8.96X_1 + 8.26X_2 - 3.72X_1X_2 - 9.36X_1^2 - 8.20X_2^2 \quad (5)$$

### 3.6.3. Overlay plot and multiple response optimizations through checkpoint analysis

The search for the optimized formulation composition was carried out through post-analysis point prediction using the overlay plot approach with Design expert software, the criterion being one having the maximum desirability value. The optimization process was performed by setting the CQAs to achieve the desired goals. CMA's were set to target constraints like disintegration time ( $Y_2$ ) 6–9 min and CDR ( $Y_2$ ) maximum.

The overlay plot was built by superimposing the contour plot of all regions as shown in Fig. 7. The common region that was acquired was characterized as design space and within this design space-optimized and checkpoint batches (II and III) were prepared and results were compared with predicated values as shown in table 5. According to USFDA standards, the range chose for the overlay plot was  $\pm 10$ .

The optimized formulation was obtained at the amount of explotab ( $X_1$ )= 40.657 mg and the amount of perlitol SD 100 ( $X_2$ )= 56.35 mg. Predicted values of the responses by software at this factor level combi-

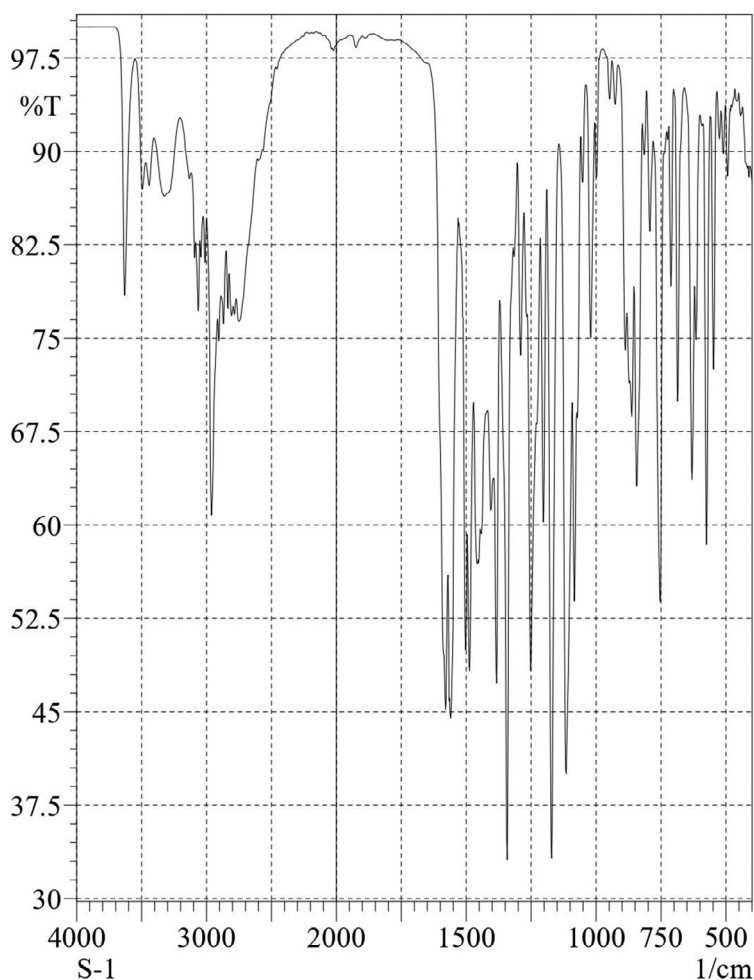


Fig. 9. FTIR spectra of (A) Bosentan (S1), (B) Bosentan: Captisol inclusion complex (S2) and (C) Optimized formulation (S3).

Comment;  
S-1

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(A)

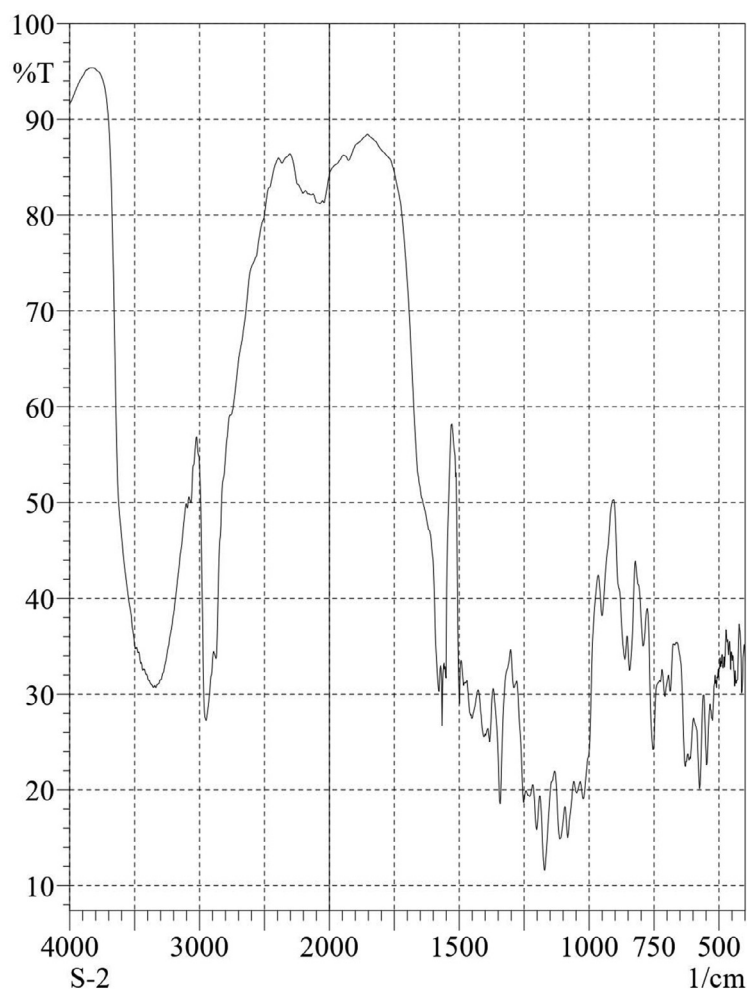


Fig. 9. Continued

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(B)

nation were found to be as  $Y1=7.63$  min and  $Y2=87.14\%$ , while experimentally observed values at this factor level combination were found to be as  $Y1=7.500\pm 0.163$  min and  $Y2=86.690\pm 0.901\%$ . Checkpoint analysis was carried out in which the predicted and experimental values were compared. Results showed a high degree of predictive ability with an acceptable percentage of prediction error, indicating the accuracy and validity of the design for the evaluation and optimization of Bosentan formulation as indicated in Table 5. The close resemblance acquired between observed and predicted values assessed the robustness of prediction which shows the validity of the produced model.

### 3.6. Characterization of physicochemical characteristics of optimized bosentan formulation

#### 3.6.1. Differential scanning calorimetry

The DSC thermographs obtained for pure drug (Fig 8A) & Bosentan: Captisol inclusion complex (Fig 8B) represents endothermic peaks at  $\sim 109$  °C &  $103.79$  °C with  $-676.17$  mJ &  $1240.78$  mJ respectively. For the optimized formulation (Fig 8C) endothermic peaks were observed at  $81$  °C &  $167.91$  °C with the latent heat of fusion  $-573.67$  mJ &  $-78.80$  mJ. The presence of the melting peak indicates that Bosentan

was present in crystalline form. The change in intensity of peak as well as temperature of the peak and decrease in latent heat of fusion indicates the transformation of the drug from crystalline to amorphous and the formation of a complex (Fig 8D). No change in peak value of the temperature of DSC spectra indicated that there was no physical interaction between drug and excipients (Kumar Panda, Das & Panigrahi, 2016).

#### 3.6.2. Fourier transform infrared absorption spectroscopy

FTIR spectra of pure drug (S1) showed characteristic peak at  $3630$   $\text{cm}^{-1}$  (OH Stretching),  $3062$   $\text{cm}^{-1}$  (NH-stretching),  $2962$   $\text{cm}^{-1}$  (CH-stretching),  $1579$   $\text{cm}^{-1}$  (C = C stretching),  $1342$   $\text{cm}^{-1}$  (S = O stretching),  $1290$   $\text{cm}^{-1}$  (C≡N stretching), and  $1170$   $\text{cm}^{-1}$  (C = O stretching). FTIR spectra of Bosentan: captisol inclusion complex (S2) and optimized formulation (S3) in Fig. 9 showed a slight broadening of the peak which indicated the formation of a stable hydrogen bond. This may be responsible for complex formation and increase of solubility. Disappearance of some peak was also observed due to amorphization of the formulation. The characteristic peak of pure drug bosentan was retained indicating chemical interaction between the drug and excipients.

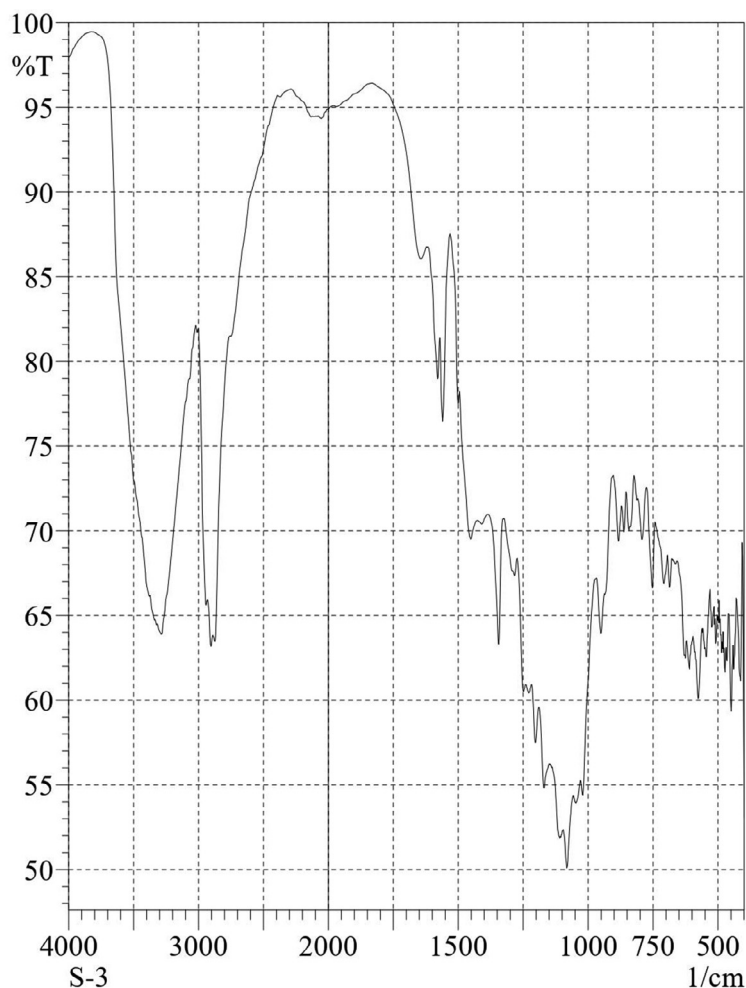


Fig. 9. Continued

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Resolution;

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### 3.6.3. X-Ray diffraction

The XRD spectra of Bosentan (S1) indicated crystalline nature of the drug and relative degree of crystallinity (RDC) at peak height of 2-Theta scale at  $9.18^\circ$ ,  $15.22^\circ$ ,  $16.66^\circ$ , and  $18.56^\circ$  and it was found to be 2646, 1331, 1296 & 2530 respectively as shown in Fig. 10A, whereas peak height for Bosentan: Captisol inclusion complex (S2) was found 293, 231, 341 & 456 (Fig. 10B). A decrease in intensity of peak indicated conversion of crystalline to amorphous form due to the formation of a complex. The higher degree of crystallinity results in greater solubility of the drug [16]. The following Eq. (7) was used to calculate the RDC:

$$RDC = I_c/I_d \quad (7)$$

Where,

$I_c$  = Peak height of complex under investigation

$I_d$  = Peak height of drug at the same angle with the highest intensity.

## 3.7. Evaluation of physicochemical characteristics of optimized bosentan formulation

### 3.7.1. Pre-Compressional parameters

Pre-compressional parameters of all formulations were evaluated. Carr's index was found to be <23% (18–23%) which indicated fair to

passable compression properties. Hausner's ratio was found to be < 1.3 (1.233 to 1.3) indicated good flow properties and the angle of repose was found to be < 31 (25.475–30.440) which indicated excellent flow properties (Patil, Pande & Sonawane, 2015).

### 3.7.2. Post-Compressional parameters

Post-compressional parameters of all formulations were evaluated. Weight variation was found to be within the acceptable limits. Hardness was found to be within the range of 4–4.5 kg/cm<sup>2</sup> which showed good mechanical strength. Friability was <1% indicating good mechanical resistance. Disintegration time was found to be between 6.5–10 min. All the formulations passed the content uniformity test and drug content was found to be between the range of 95–99.9% (Patil et al., 2015).

### 3.7.3. Dissolution study

A dissolution study of all factorial batches was carried out in 6.8 pH phosphate buffer as shown in Fig. 11. The result revealed that F5 showed the highest drug release ~87.26% among all batches within 30 min and so it was identified as an optimized formulation.

### 3.7.4. Comparison of dissolution profiles

The dissolution profile of the marketed tablet of Bosentan was also compared with tablets prepared by various techniques such as a com-

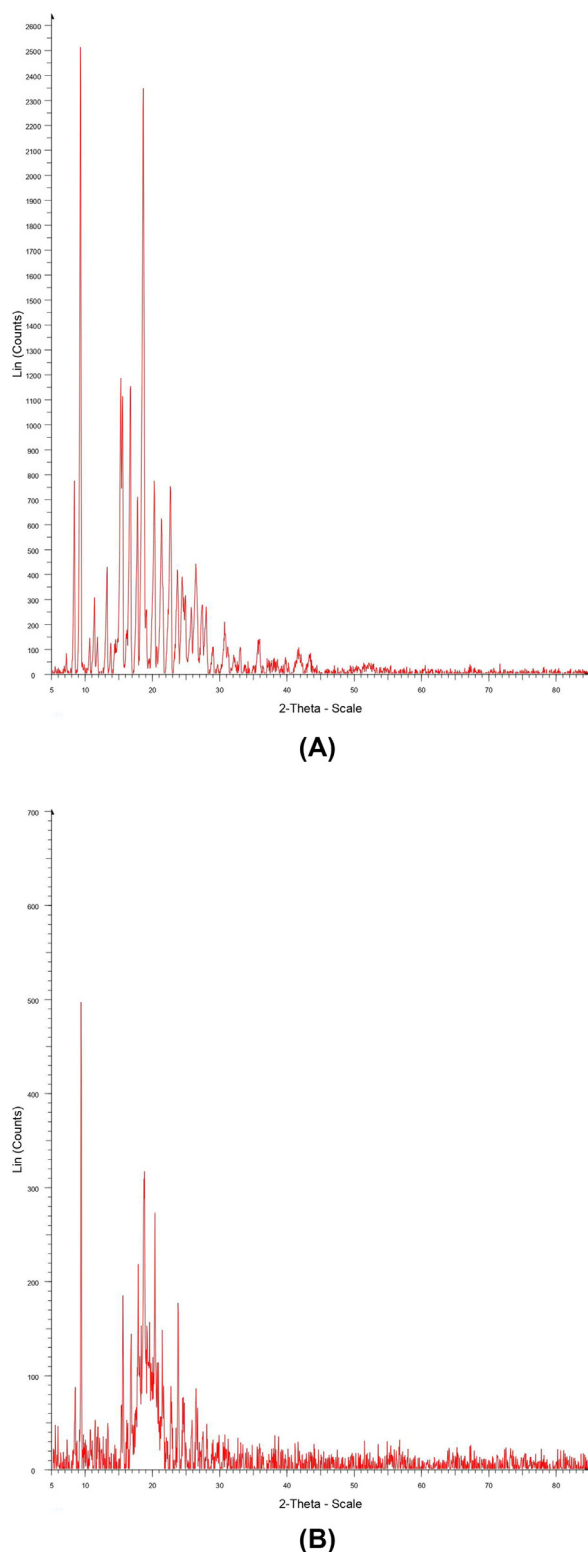


Fig. 10. XRD spectra of (A) Bosentan (S1) and (B) Bosentan: Captisol inclusion complex (S2).

plexation (L1), Liquisolid (L2), and optimized formulation of Novel hybridization (F5) (Fig. 12). Developed formulation by Novel hybridization technique (~87.26% in 30 min) showed better drug release as compared marketed formulation (~61.75% in 30 min). This is because of cyclodextrin derivatives facilitates faster dissolution due to conversion of particles from crystalline to amorphous status, which was proved by XRD study.

### 3.8. Stability study

The stability studies were performed on the optimized formulation (F5) at  $40 \pm 2$  °C and  $75 \pm 5\%$  RH conditions for 1 month to assess its storage conditions. The content uniformity and %CDR results were found satisfactory (Table 6) with no significant difference. Hence, the optimized formulation was said to be stable.

## Conclusion

The research work of this project focused on solubility and dissolution enhancement of Bosentan by using different techniques. Bosentan formulation was prepared using a novel hybridization technique by a combination of complexation and liquisolid techniques. Bosentan and Captisol complex enhance dissolution by converting drug from crystalline to amorphous form which as proven in XRD study. The Liquisolid system increased the solubility and dissolution of the drug in a non-volatile solvent. Parreck SLC-500 was found to provide high drug loading capacity, thereby converting liquid to free-flowing powder. The dissolution study showed that the optimized formulation (F5) of novel hybridization techniques exhibited higher solubility and dissolution (~87.26% in 30 min) as compared to a pure drug, marketed formulation, complexation and liquisolid techniques. Based on the results, it can be concluded that novel bosentan formulation will give immediate drug release with reduced dose frequency.

## Declaration of Competing Interest

The authors declare no conflict of interest.

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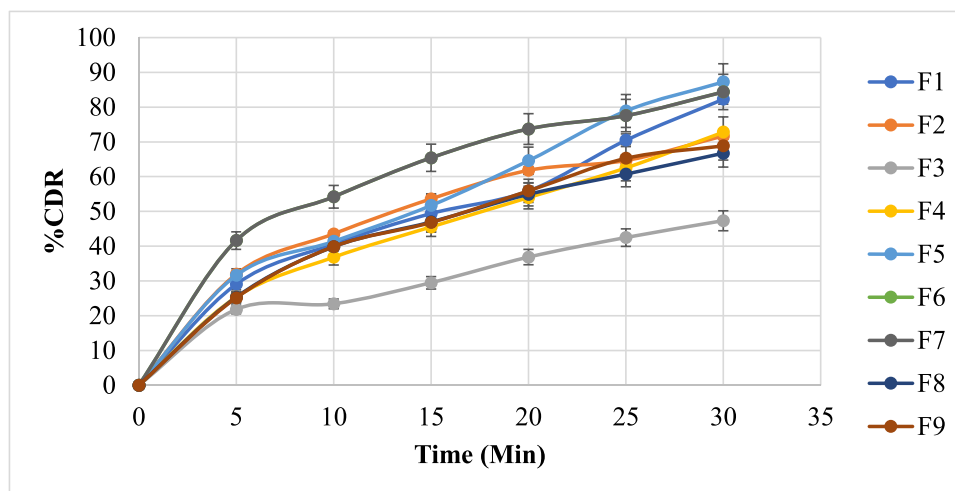


Fig. 11. Dissolution study of F1 to F9 Batches.

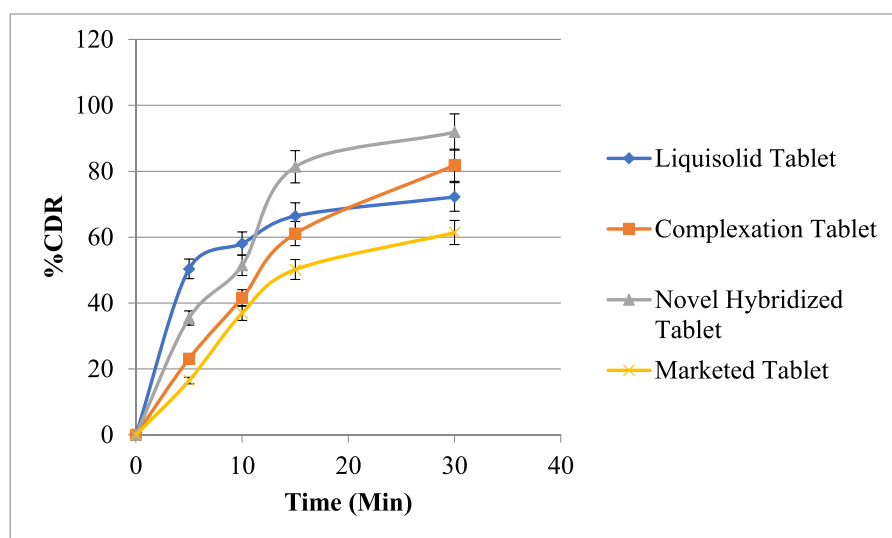


Fig. 12. Comparison of Dissolution Profiles.

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