Annals of R.S.C.B., ISSN: 1583-6258, Vol. 24, Issue 2, 2020, Pages. 1333 - 1343
Received 24 October 2020; Accepted 15 December 2020

Solubility and Dissolution Enhancement Bosentan by Third Generation Solid Dispersion

Rahul Radke* and Neetesh K Jain

Department of Pharmacy, Oriental University, Indore, M.P. India

*Corresponding author's E-mail: rahul.radke@rediffmail.com

ABSTRACT

The objectives of present investigation was to enhance the solubility and dissolution rate of poorly water soluble drug bosentanby third generation solid dispersion using Gelucire 50/13 as a carrier. Solid dispersion of bosentan with hydrophilic carrier were prepared in various drug to carrier ratios(1:1, 1:2, 1:3, 1:4 and 1:5) by kneading technique. Prepared dispersions was evaluated forsaturation solubility, percentage yield, drug content and in vitro dissolution.Infrared (FTIR) spectroscopy studyshowed no interaction between drug and carrier. Differential scanning calorimetry (DSC)and X-ray diffraction (XRD)study confirmed the conversion of crystalline drug in to amorphous form. All solid dispersions prepared with hydrophilic carrier showed increase in drug solubility and dissolution rate as compare to pure drug. It was confirmed that the Gelucire 50/13based third generation solid dispersion is aneffective approach to enhance the solubility and dissolution rate of bosentan.

Keywords: Bosentan,Gelucire 50/13, Solid dispersion, DSC, XRDetc.

INTRODUCTION

Oral absorption of a drug involves dissolution of drug from the formulations into gastric or intestinal fluids followed by its permeation through GI membranes. Bioavailability of drugs from oral route depends on their solubility as well as permeability. Solubility is one of the important parameter to achieve desired concentration of drug in plasma for desired pharmacological response.[1-2] Poor solubility results in higher dose and repeated administration of dosage form. Hence to improve the oral bioavailability of poorly water-soluble drugs by improving their solubility, dissolution rate is the major challenge.[3]Solid dispersion technology has been successfully utilized in the development of formulations with an aimed to improve the solubility, dissolution and bioavailability of poorly water-soluble drugs.[4,5]

Third-generation solid dispersions was preparation that was developed using surfactants, self-emulsifiers, or mixtures of amorphous polymers and surfactants as third generation carriers like Gelucire. Use of this carrier in the solid dispersion can improve drug dissolution by increasing drug wettability and solubility. The third generation SD is intended to achieve a higher level of bioavailability of poorly soluble drugs by improving the physical stability of solid dispersions, reducing drug recrystallization and preventing drug precipitation in the aqueous medium.[6,7]

Bosentan is a dual endothelin receptor antagonist used in the treatment of pulmonary arterial hypertension (PAH). Bosentan is safe and improves exercise capacity over the short term in patients with Eisenmenger's physiology It is classified as a BCS Class II drug because of its poor aqueous

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 24, Issue 2, 2020, Pages. 1333 - 1343
Received 24 October 2020; Accepted 15 December 2020

solubility. It is insoluble in water and in aqueous buffer solutions with lower pH, the solubility of bosentan increases at higher pH.[8,9]

MATERIALS AND METHODS

Marerials

Bosentan was obtained as a gift sample from CiplaLtd, Mumbai India.Gelucire 50/13 was supplied by Gattefosse India as a gift sample. All other chemical and reagents used were of analytical grade.

Method

Saturation Solubility Study:

Saturation solubility study of drugbosentan was determined according to method reported by Higuchi and Connor in distilled water, acetate buffer pH 1.2, phosphate buffer pH 6.8 and phosphate buffer pH 7.4. Excess amount of drug was added to 10 ml study fluid in a glass vial. Samples were shaken on rotary shaker at constant speed at 25°C±2°C for 48 hr. The saturated solutions were then filtered through a whatman filter paper no 1. Filtrates were suitably diluted and estimated spectrophotometrically.[10]

Phase solubility study:

The phase solubility study of drug isan important parameter to determine the possible solubilizing effect of carrier on drug solubility. Phase solubility Study was performed according to method reported by Higuchi and Connors. An excess amount of drug (approx. 100 mg) were added to 10 ml glass vial containing 0.25%, 0.50%, 0.75%, 1% and 2% aqueous solution of carriers and shaken on rotary shaker for 48 hr at a controlled temperature at 25^0C±2^0C. The solutions were filtered through whatman filter paper no 1. Filtrate were analyzed by UV- spectrophotometer in order to determine the concentration of the dissolved drug.[11]

Gibbs-Free Energy Transfer

In order to determine the possible favorable or unfavorable effect of carrier on solubilisation of drug in aqueous medium. The Gibbs free energy of transfer (ΔG∘tr) values was calculated using the Gibbs–Helmholtz equation. Negative Gibbs-free energy values indicate improved dissolution. The $\Delta G\circ$tr values of drug were calculated using the following equation:

$$\Delta G0tr = \{-2.303RTLog (S0/Ss)$$

Where S0/Ss is the ratio of the molar solubility of drug before and after treatment with polymer. R is the value of gas constant 8.31 JK−1 mol−1 and T is temperature in degree kelvin.[12]

Preparation of Bosentan – Gelucire 50/13 physical mixture

A physical mixture of bosentan with Gelucire 50/13 in different ratio (1:1, 1:2, 1:3, 1:4, 1:5) was prepared by mixing of drug and carrier using mortar and pestle. This mixture was then passed through sieve no 40 and store in desiccators. The composition was shown in table 1.

Preparation of BosentanSolid Dispersion:

Solid dispersion of Bosentanwith Gelucire 50/13 in different weight ratio (1:1, 1:2, 1:3, 1:4, 1:5) was prepared by kneading method.A mixture of drug and carrier was placed in a mortar and was kneaded thoroughly with water and methanol (1:1) for 20 min. The kneaded mixtures were then air dried until get a constant weight, and then pulverized and screened through 80-mesh and stored in desiccator for further study.[13]Thecomposition for solid dispersion is shown in table 1.

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 24, Issue 2, 2020, Pages. 1333 - 1343
Received 24 October 2020; Accepted 15 December 2020

Table 1: Composition of bosentan solid dispersion

Formulation	Formulation Code	Bosentan : Gelucire 50/13
Physical Mixture	BPM1	1:1
	BPM2	1:2
	BPM3	1:3
	BPM4	1:4
	BPM5	1:5
Solid Dispersion	BSD1	1:1
	BSD2	1:2
	BSD3	1:3
	BSD4	1:4
	BSD5	1:5

Characterization of Solid Dispersion

Determination of solubilityof solid dispersion

The saturation solubility of solid dispersion was determined in distilled water and phosphare buffer 6.8 using shake flask method. Excess quantities of sample were added in 25 ml of distilled water and phosphate buffer in conical flask and shaken for 24 hours at room temperature on rotary flask shaker. After shaking resultant samples containing undissolved solid sus-pended in the test medium were centrifuged at 10,000 rpm for 5 min, the clear supernatants obtained were filtered using whatman filter paper. Further sample ware suitably diluted and analyzed by spectrophotometer at 272 nm.[14]

Determination of percent yield of solid dispersion

The percent yield of bosentan solid dispersions was determined by using the following formula:
percent yield = Weight of prepared solid dispersion / Weight of drug + carriers × 100

Determination of drug content

Bosentan solid dispersion equivalent to 62.5 mg of drug was accurately weighed and dissolved in 100 ml of methanol. The solution was shaken vigorously and filtered. After suitable dilution drug content was analyzed at 272 nm against blank by UV spectrometer.[15]

Fourier transform infra-red spectroscopy

Compatibility studies of bosentan with carrier was performed using FTIR spectroscopy (Shimadzu FTIR-8700). Spectrum of pure drug, physical mixture and solid dispersion was recorded over the frequency range of 400 to 2000 cm-1 at 4cm resolution.

Differential scanning calorimetry

The thermal analysis was carried out using Shimadzu Thermal analyzer DT 40 (Japan). The samples were placed in sealed aluminum pans and heated at a rate of 10°C per min in the temperature range of 20-300°C under a nitrogen flow rate of 40 ml/min.[16]

Powder X-ray diffraction

X-ray powder diffraction patterns of drug, carrier and solid dispersion was recorded on an X-ray powder diffraction system (Rigaku, Mini Flex 600). The scanning was done over range of 2º to 90º.

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 24, Issue 2, 2020, Pages. 1333 - 1343
Received 24 October 2020; Accepted 15 December 2020

The position and intensities of diffraction peaks were considered for the comparison of crystallinity.[17]

Scanning electron microscope analysis (SEM)

The surface morphology of pure drug and selected solid dispersion wasstudied using SEM. (Jeol-JSM-5300 scanning microscope, Tokyo, Japan). The samples were mounted on a sample stubwith double-sided adhesive tape and coated under vacuum with gold ion using sputtering device prior to study. SEM image at differentmagnifications were recorded to study the morphological and surfacecharacteristics of the solid dispersions.

In vitro dissolution study

In vitro dissolution study of pure bosentanand solid dispersions were determined using USP dissolution test apparatus II (Paddle type) (Esico International, Mumbai). Accurately weighted preparation equivalent to 62.5 mg of bosentan ware added to 900 ml of phosphate buffer pH 6.8 as dissolution medium, maintained at 37±0.5°C and stirred at 50 rpm.5 ml samples were withdrawn at predetermine time interval and same volume was replaced with fresh media in order to maintain the sink condition. After suitable dilution, collected samples were analyzed at 272 nm using UV-visible spectrophotometer against the blank.[18,19]

RESULTS AND DISCUSSION

Saturation Solubility

The solubility of bosentan in distilled water, acetate buffer pH 1.2, phosphate buffer pH 6.8 and phosphate buffer pH 7.4 at 25°C is shown in figure 1. The solubility of bosentan in distilled water was found to be 10.87µg/ml, indicating poor water solubility. Solubility of bosentan in acetate buffer pH 1.2, phosphate buffer pH 6.8 and phosphate buffer pH 7.4 was found to be 5.383µg/ml, 37.12 µg/ml and 41.35µg/ml respectively. Bosentan solubility increases with increasing pH. The highest solubility of bosentan was found in pH 7.4 phosphate buffer and least in pH 1.2 acetate buffer.

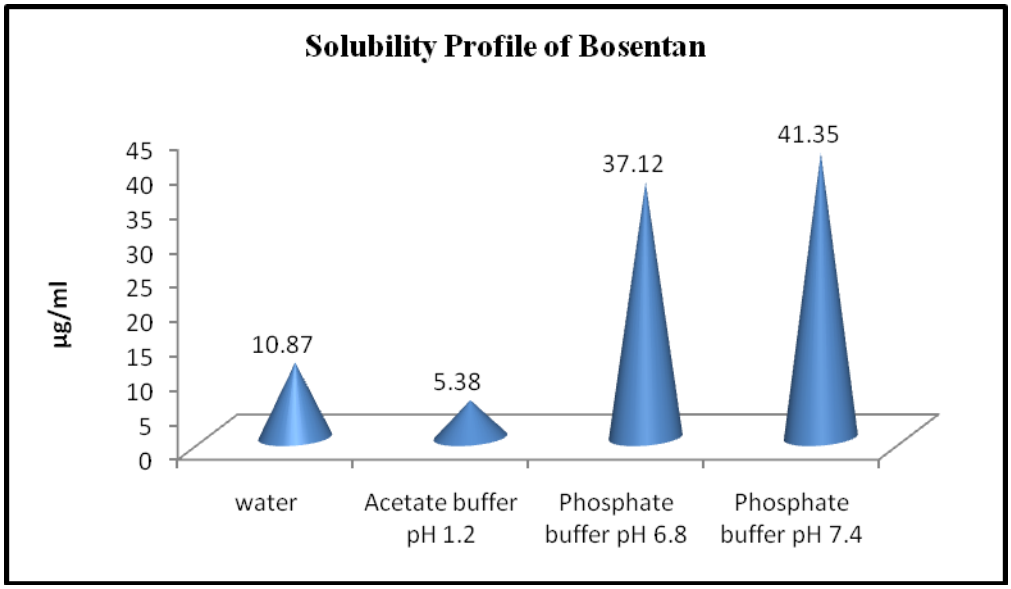

Figure 1: Solubility profile of bosentan in different pH

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 24, Issue 2, 2020, Pages. 1333 - 1343
Received 24 October 2020; Accepted 15 December 2020

Phase Solubility Study

Phase solubility study of bosentan was studied using increasing concentration of gelucire 50/13 solution.A gradual increase of solubility of bosentan was seen with an increasing concentration of carriers in water. The solubility of bosentan at 0.25, 0.5, 0.75, 1 and 2% aqueous solution of carrier was shown in phase solubility curve for bosentan in figure 2. At 2% w/v concentration Gelucire 50/13 the solubility of bosentanwas increased by 6.1 fold.Increased in solubility of drug may be due to solubilization effect of carriers that increased wettability of bosentan.

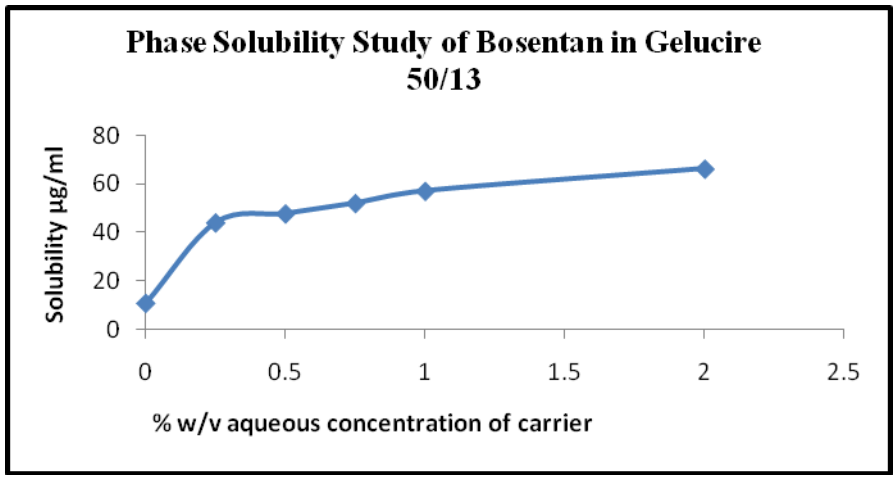

Figure 2: Phase solubility study of bosentanwith Gelucire 50/13.

Gibbs-Free Energy Transfer

Gibbs free energy transfer was studied for bosentan in aqueous solutions carrier. Gibbs free energy ($\Delta G^{o}tr$) values were negative at all levels of carriers. The obtained values were found to be increased with increase in concentration of carrier, indicating favorable drug carrier reaction and solubilization process in the aqueous carrier solution. The values of Gibbs-free energy ($\Delta G°tr$) with theGelucire 50/13 are presented in Table 2.

Table 2: Phase solubility and ΔG°tr of bosentan at different concentrations of Gelucire 50/13

Concentrartion of polymer (%)	Concentration of Bosentan (µg/ml) with Gelucire 50/13	$\Delta G^{0}tr$
0.25	44.11 ± 0.007	-3469.39
0.5	47.79 ± 0.005	-3667.95
0.75	52.09 ± 0.008	-3881.46
1	57.14 ± 0.012	-4110.76
2	66.30 ± 0.009	-4479.21

All values are mean ± SD, n = 3.

Characterization of Solid Dispersion

Determination of solubility of solid dispersion and physical mixture

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 24, Issue 2, 2020, Pages. 1333 - 1343
Received 24 October 2020; Accepted 15 December 2020

The solubility of bosentanphysical and solid dispersion was determined in distilled water and in phosphate buffer pH 6.8. Bosentan is poorly soluble in water, having a maximum solubilityof 10.87µg/ml in water. In the present study, a solid dispersion was prepared to increase the solubility and dissolution rate of bosentan.It was found that as the concentration of hydrophilic carrier increases, the solubility also increases. All SD formulation showed higher value of solubility as compare to pure and physical mixture. The value of solubility is more in phosphate buffer solution than distilled water. BSD4 with (1:4 drug to Gelucire 50/13 ratio) showed maximum solubility of 75.43 ± 0.54µg/mlin water and85.22± 0.71µg/mlin 6.8 pH buffer. The water-solubility of bosentan increasesalmost6.9fold in solid dispersionas compared with pure ambrientan.This may be due to conversion of drug in amorphous form or by increased wettability of drug. The solubility data for all the PMs and SDsformulations are presented in table 3.

Table 3: Solubility analysis of bosentansolid dispersion

Formulation Code	Solubility in distilled water (µg/ml)	Solubility in Phosphate buffer 6.8 (µg/ml)
BPM1	38.47 ± 0.18	42.22 ± 0.24
BPM2	41.12± 0.72	47.18 ± 0.64
BPM3	46.14 ± 0.42	51.62 ± 0.44
BPM4	51.18 ± 0.54	56.52 ± 0.55
BPM5	54.76 ± 0.62	59.57 ± 0.32
BSD1	44.13± 0.24	48.26± 0.42
BSD2	52.91± 0.37	57.15± 0.48
BSD3	61.52± 0.52	66.86 ± 0.54
BSD4	75.43± 0.54	85.22± 0.71
BSD5	77.28± 0.58	90.11 ± 0.48

All values are mean ± SD, n = 3.

Determination of percent yield and drug content

The percent yield of bosentan solid dispersions was fond in range of 80.56 to 82.6.All SD formulations gives low yield because of sticky nature of Gelucire 50/13.Drug content for all SD formulation was found within acceptable limit of 97.56% to 98.45%. Data of percent yield and drug content is shown in table 4.

Table 4: % Practical yield and Drug content of Bosentan SDs

Formulation Code	% Practical Yield	% Drug Content
BSD1	80.56	98.15
BSD2	81.34	97.86
BSD3	82.32	97.56
BSD4	82.50	98.45
BSD5	82.61	98.12

Fourier transform infra-red spectroscopy

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 24, Issue 2, 2020, Pages. 1333 - 1343
Received 24 October 2020; Accepted 15 December 2020

Sharp characteristic peaks showed in pure bosentan ware also appears in the spectra of SD indicating no interaction between the drug and the carrier. FTIR spectra of pure bosentan, Gelucire 50/13 and SD are shown in figure 3.

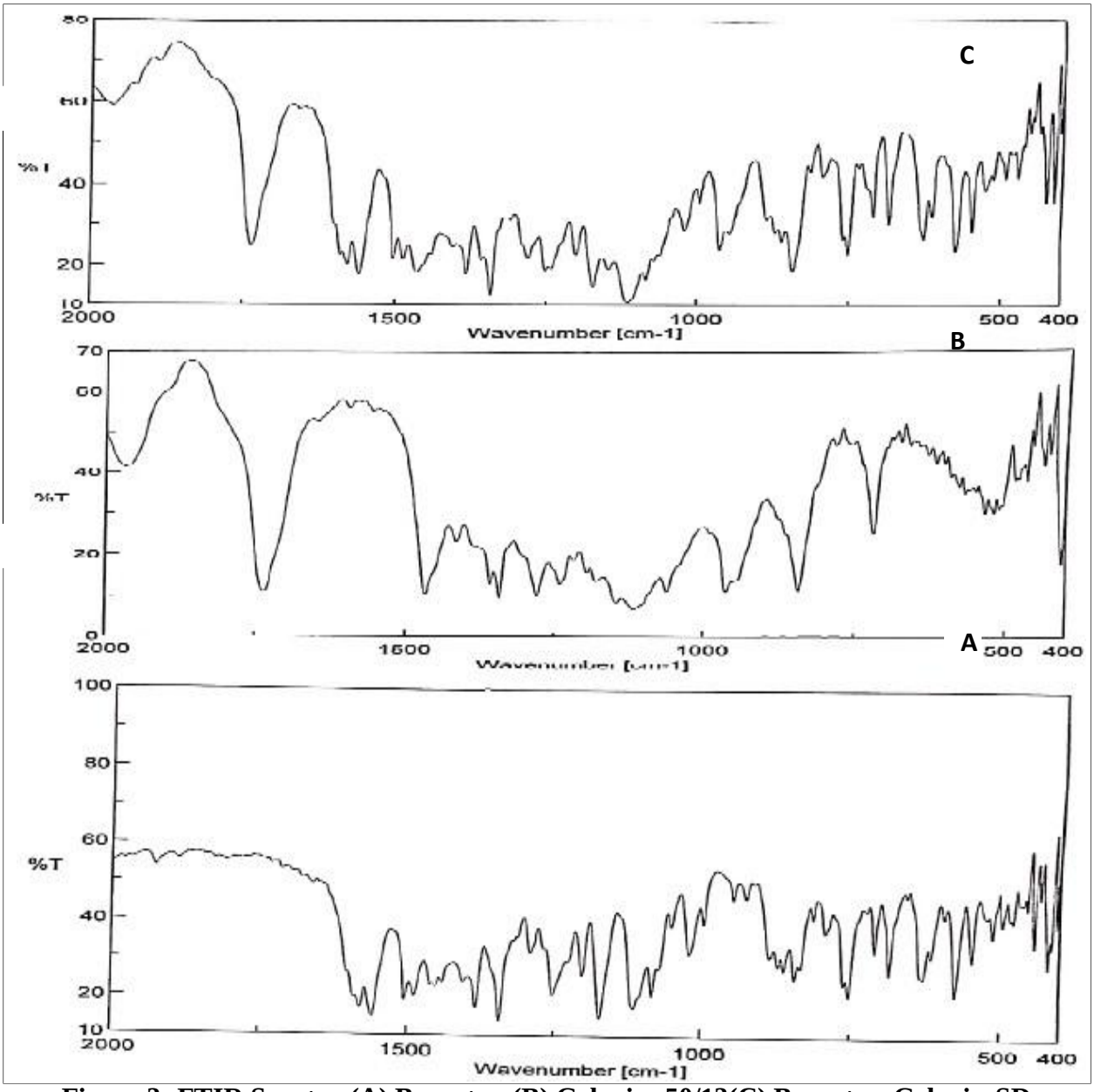

Figure 3: FTIR Spectra (A) Bosentan (B) Gelucire 50/13(C) Bosentan-GelucireSD

Differential scanning calorimetry

DSC thermogramof pure bosentan, Gelucire 50/13 and optimized solid dispersion BSD4 are shown in figure 4. Thermogram of pure drug showed a single sharp endothermic peak at 121.76^0C, corresponding to its melting point indicating the crystalline nature of drug. Gelucire 50/13 exhibited sharp endothermic peak at 56.76^0C. Optimized formulation BSD4 showed only single endothermic peak of Gelucire 50/13 at 53.58^0C, which confirmed the complete miscibility of drug in carrier and reduce crystallanity.

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 24, Issue 2, 2020, Pages. 1333 - 1343
Received 24 October 2020; Accepted 15 December 2020

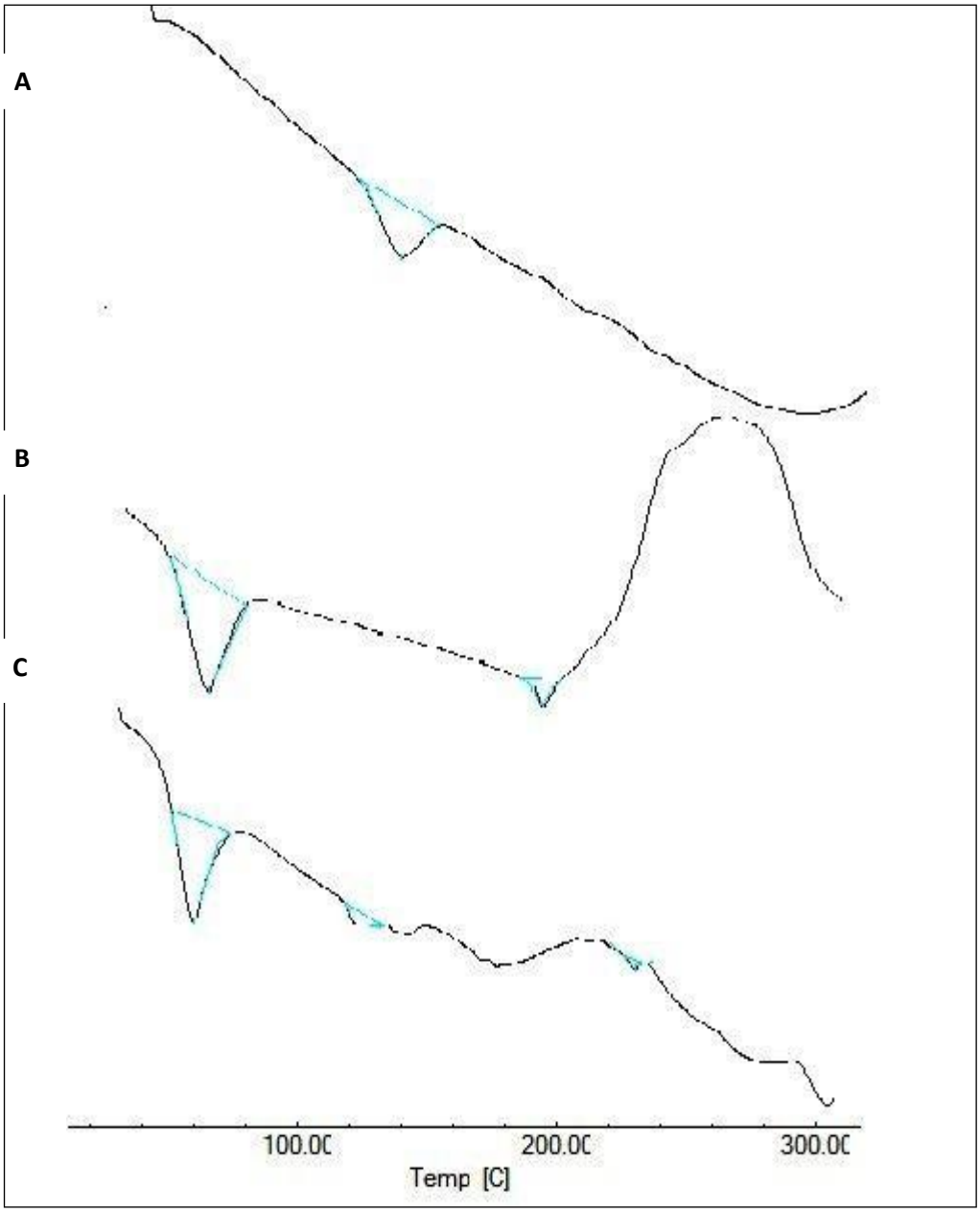

Figure 4: DSC thermogramofA. Pure bosentan, B. Gelucire 50/13and C. BosentanGelucireSD (BSD4)

Powder X-ray diffraction

XRD patterns of pure bosentan, Gelucire 50/13 and optimized solid dispersion of bosentanare shown in figure 5. The x-ray diffractograms of pure bosentan showed characteristic sharp high-intensity diffraction peaks, which indicates the high crystalline nature of bosentan. XRD pattern ofsolid dispersion with Gelucire showed less and low-intense peaks compare to pure drug, indicating conversion of highly crystalline bosentan to less crystalline or amorphous form.

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 24, Issue 2, 2020, Pages. 1333 - 1343
Received 24 October 2020; Accepted 15 December 2020

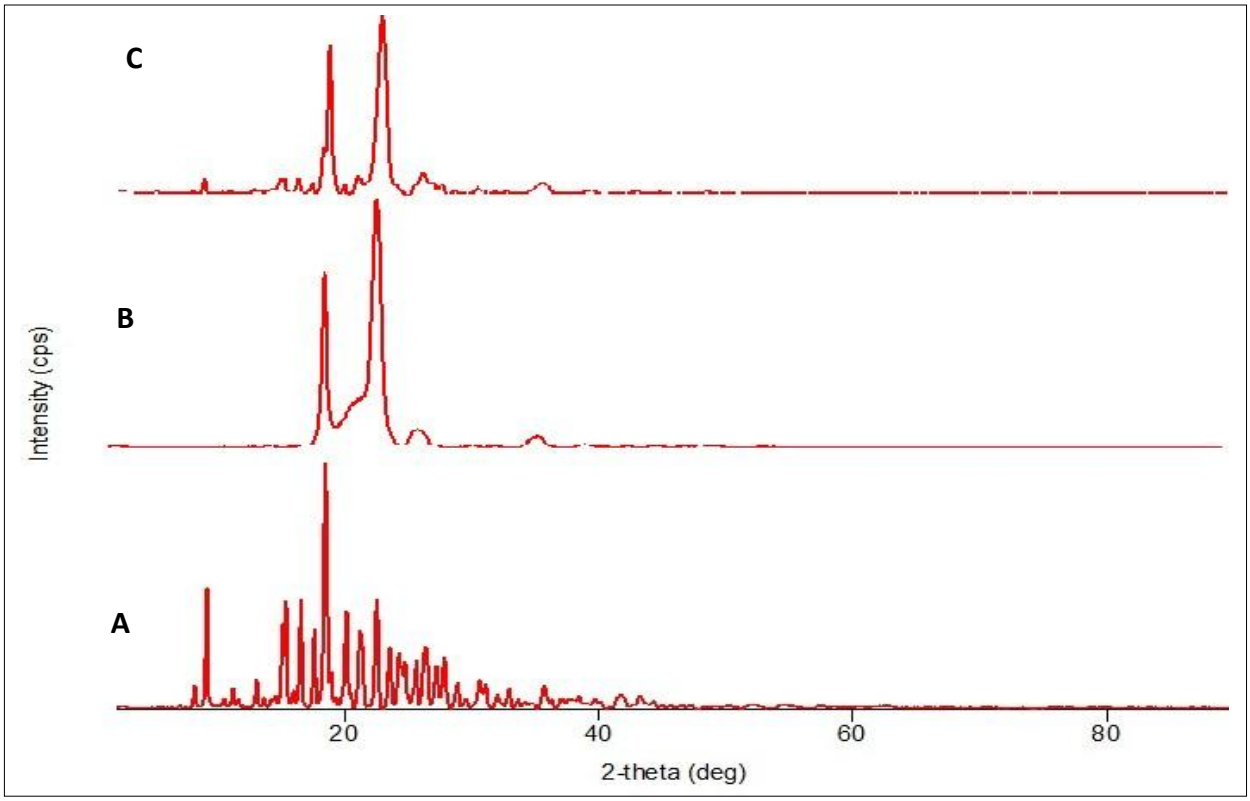

Figure 5: XRD spectra A) Bosentan B) Gelucire 50/13 C) BosentanGelucireSD

Scanning electron microscope analysis (SEM)

SEM images of pure bosentan and its selected optimized solid dispersion (BSD4)formulation were shown in figure 6.Fig. 6 a reveals that pure bosentan exists in irregular shape particles, while the prepared soliddispersion appeared as homogenous mixed mass inwhich the surface properties of the drug were disappear in solid dispersion(Fig. 6b). From SEM study, it is concluded that bosentanget converted in to fine amorphous form with reduced particle size.

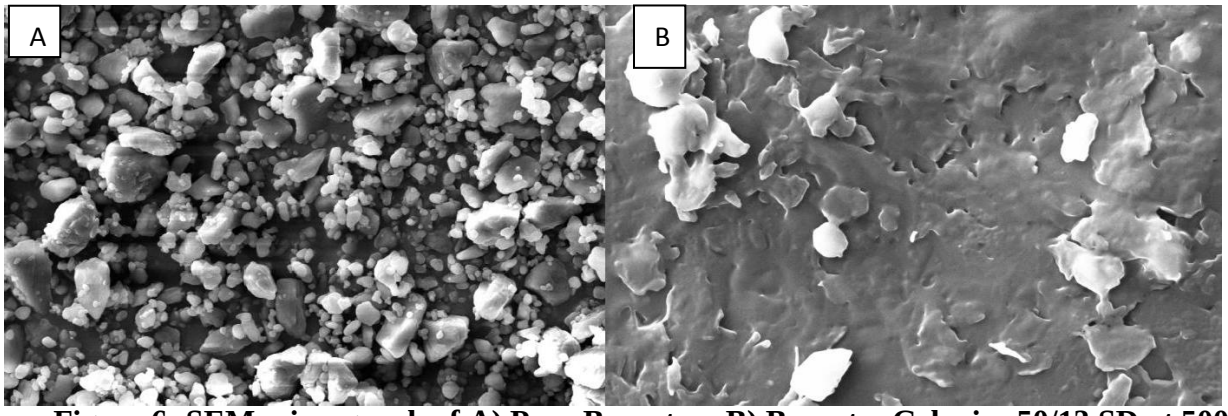

Figure 6: SEM micrograph of A) Pure Bosentan B) BosentanGelucire 50/13 SD at 500X

In vitro dissolution study

All the solid dispersion formulations and the pure bosentan were subjected to a dissolution study. The study was conducted in phosphate buffers pH 6.8 as the dissolution media. All SD formulation

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 24, Issue 2, 2020, Pages. 1333 - 1343
Received 24 October 2020; Accepted 15 December 2020

showed faster drug release and it was found that as concentration of polymer increased the rate of drug dissolution also increased. Among all formulation, BSD4containing drug and Gelucire 50/13 in 1:4 ratio showed highest drug release of 98.55% at the end of 60 min. The pure bosentan has shown 38.44% of releaseat the end of 60 min, indicating poor solubility and hence demand solubility enhancement.All solid dispersion formulation release more than 60% drug in first 10 min, which indicate increased solubility of drug in solid dispersion. The drug release profile of all SD formulation and pure bosentan are shown in figure 6.

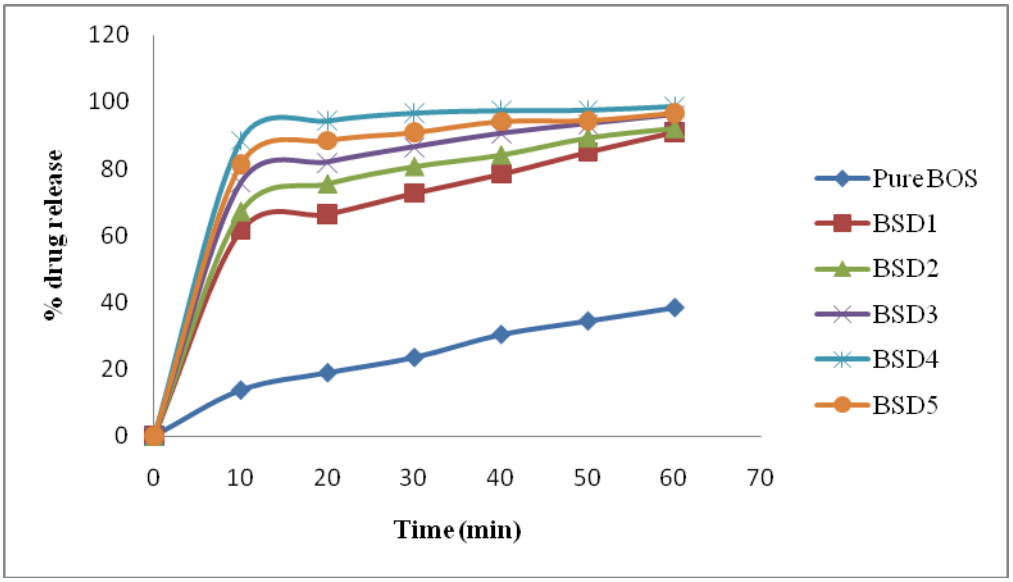

Figure 6: In-vitro drug dissolution of pure and SDs of bosentan

CONCLUSION

In the present investigation, an attempt was made to develop Gelucire 50/13 based third generation solid dispersion of poorly water soluble bosentan using kneading method. SDC and XRD study demonstrate the amorphous conversion of crystalline drug. Because of that the solubility and dissolution rate of bosentan was significantly improved in the solid dispersion. From this study, it was concluded that the solid dispersion prepared using Gelucire 50/13 is a good approach of enhancing the solubility, dissolution rate and bioavailability of poorly soluble drugs like bosentan.

ACKNOWLDGMENT

Authors are thankful to Alembic Pharmaceuticals Vadodara for providing gift sample of Bosentan to conduct this research.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

[1] Naseem A, Olliff CJ, Martini LG, & Lloyd AW. (2004). Effects of plasma irradiation on the wettability and dissolution of compacts of griseofluvin. Int J Pharm, 269(2): 443-50.
[2] Singh G, Kaur I, Gupta D, &Sharma S. (2017). Enhancement of the Solubility of Poorly Water Soluble Drugs through Solid Dispersion: A Comprehensive Review. Indian J Pharm Sci, 79(5): 674-687.

[3] Aulton M.E., (1996). Pharmaceutics: The science of dosage form design, 1st ed, London: Churchill Livingstone.
[4] Vo CL, Park C,& Lee B. (2013). "Current trends and future perspectives of solid dispersions containing poorly water-soluble drugs". European Journal of Pharmaceutics and Biopharmaceutics, (85)3: 799-813.
[5] Sareen S, Mathew G, &Joseph L. (2012). "Improvement in solubility of poor water-soluble drugs by solid dispersion", International Journal of Pharmaceutical Investigation, (2)1:12-17.
[6] Chaudhari SP,&Dugar RP. (2017).Application of surfactants in solid dispersion technology for improving solubility of poorly water soluble drugs, Journal of Drug Delivery Science and Technology, 41: 68-77.
[7] Yu DG, Yang GM, White CB, Lu P, Zhang L, &Zhu LM. (2010). Third generation solid dispersions of ferulic acid in electrospun composite nanofibers. International Journal of Pharmaceutics, 400:158–164,
[8] Channick RN, Simonneau G, Sitbon O, Robbins IM& Frost A. (2001). Effects of the dual endothelin-receptor antagonist bosentan in patients with pulmonary hypertension: a randomised placebo-controlled study. Lancet., 358: 1119-1123.
[9] Rubin LJ, Badesch DB, Barst RJ, Galie N, & Black CM. (2002). Bosentan therapy for pulmonary arterial hypertension. N Engl J Med., 346: 896-903.
[10] Higuchi T, &Connors KA. (1965). Phase-solubility techniques. Adv Anal ChemInstr, 4:117–212.
[11] Sharma A, &Jain CP. (2010). Preparation and characterization of solid dispersions of carvedilol with PVP K30. Res Pharm Sci, 5(1): 49–56.
[12] Maulvi FA, Dalwadi SJ, &Thakkar VT. (2010). Improvement of dissolution rate of aceclofenac by solid dispersion technique, Powder Technol, 207:47–54.
[13] Iqbal A, Hossain MS, Shamim MA, Islam M, &Siddique MAT. (2020). Formulation, in vitro evaluation and characterization of atorvastatin solid dispersion. Tropical Journal of Pharmaceutical Research, 19(6): 1131-1138.
[14] Sathali AAH, &Jayalakshmi J. (2013). Enhancement of solubility and dissolution rate of olmesartanmedoxomil by solid dispersion technique. J. Curr. Chem. Pharm. Sc.,: 3(2): 123-134.
[15] Yunoos M, Lavanya NSL, Sravani G, MadhuriRao P, &Krishna CH. (2014). Development of a Validated UV Spectrophotometric Method for the Estimation of Bosentan in Pure and Marketed Formulations. Sch. Acad. J. Pharm., 3(6): 427-431.
[16] Yang M, Wang P, Huang CY, Ku MS, Liu H, &Gogos C. (2010). Solid dispersion of acetaminophen and poly (ethylene oxide) prepared by hot-melt mixing. Int J Pharm., 395(1-2):53-61.
[17] Jain P, &Yalkowsky SH. (2007).Solubilization of poorly soluble compounds using 2 pyrrolidone, Int J of Pharmaceutics, 35:223-227.
[18] Varma MM, &Razia Begum SK. (2012). Formulation, Physicochemical Evaluation and Dissolution Studies of Carbamazepine solid dispersions. International Journal of Pharmaceutical Sciences, 5(3): 1790-1807.
[19] Avachat A, &Raut V. (2012). Solubility and dissolution enhancement of Nebivolol hydrochloride using hydrophilic carriers. Asian Journal of Pharmaceutical Sciences, 7(5): 337-345.