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DEVELOPMENT AND CHARACTERIZATION OF DRY POWDER FOR INHALATION TO IMPROVE DELIVERY OF POORLY WATER SOLUBLE DRUG

Vrushali Vijayrao Deshpande,*Vaibhav Vijayrao Deshpande, R. B. Wakade
Department of Pharmaceutics JSPM (PG) Sudhakarrao Naik Institute of Pharmacy,
Nagpur Road Pusad dist. Yavatmal, Maharastra-445204, India.

Corresponding author:

Vrushali V. Deshpande

Department of pharmaceutics,sudhakarro naik institute of pharmacy,pusad

Email- vrushalideshpande97@gmail.com

ABSTRACT: The newly synthesized active pharmaceutical ingredients(API) have problem related to their solubility.In this study, we have developed a alternative method to increase the solubility of poorly water soluble drug. Inhalable dry powder was formulated by using spray drying process which helps to enhance the solubility of drug as well as bioavailability. The bosentan microparticles were prepared by the spray drying method with bosentan hydrate and mannitol at different weight ratios. The bosentan microparticles were then characterized for their physicochemical properties, FTIR study, Spectroscopic studies, Morphology, Particle Size Distribution, in vitro dissolution behavior, Drug content. *In vitro* dissolution behavior were performed by Franz diffusion cell. The results were evaluated by comparing their dissolution rate compared to pure bosentan hydrate. The highest performance bosentan microparticles, named F1(1:1) had irregular shape and porous shape. The particle size of F1(1:1) also matches the range for inhalation route.In conclusion, F1 (1:1) showed promising *in vitro* results with the dry powder inhalation. The inhaled bosentan microparticles can be considered as a potential alternative to oral bosentan hydrate.
KEYWORDS: Dry powder Inhaler, Spray Drying, poorly water soluble, Microparticle, Inhalation route

1.Introduction

The respiratory tract is one of the oldest routes for the administration of drugs. Drug delivery to the lungs is an effective way for the treatment of respiratory diseases such as asthma, chronic obstructive pulmonary disorder (COPD), cystic fibrosis, lung cancer and pulmonary arterial hypertension. One major problem in drug delivery sciences and therapeutical applications is how to provide an appropriate dosage of the active pharmaceutical ingredient (API) at the right time to the right body location. Size is one determining factor for the successful drug delivery systems. Different micro- or nanostructured systems show great potential for stabilizing APIs and overcoming transport barriers.

Along with the size poor solubility of new active pharmaceutical ingredient is more promising challenge in delivery of drug to appropriate site. To overcome this spray drying method is used. Spray drying is the process in which the emulsion, solution or

any other pharmaceutical preparation get converted into powder form as in nanoparticle or microparticles and these nanoparticle or microparticle are further use to prepare tablet ,capsule or in powder form. This is viewed as a strong technological process since it brings feasibility to the development of free- flowing particles with obvious particle size.

Bosentan hydrate is a sulfonamide-based drug, competitive dual endothelin receptor antagonist poorly water soluble that is approved by the US Food and Drug Administration as the first oral drug for PAH with the brand name 'Tracleer®'. Even though it can alleviate the symptoms and improve the quality of life in patients with PAH, the drug can, after its hepatic metabolism and systemic exposure, produce adverse effect. Oral administration of bosentan hydrate leads to exposure-dependent liver injury, as well as a reduction in hemoglobin level, inhibition of spermatogenesis, and headache. In addition, bosentan-induced isoenzymes inhibit the effects of co-administrated drugs, such as warfarin, cyclosporine, oral estrogens, simvastatin, and sildenafil.

Alternatively, the pulmonary delivery system can release the drugs in the vicinity of the pulmonary circulation, and avoid hepatic first-pass metabolism. The alveoli in contact with the small pulmonary arteries are the target sites of bosentan hydrate. Hence, pulmonary delivery of bosentan hydrate is expected to demonstrate high local concentration and bioavailability, since it evades the hepatic first-pass metabolism. This result in a more effective pulmonary vasodilatation, than that produced after oral administration of the drug. In addition, it can improve ventilation and perfusion matching by vessels supplying the ventilated regions, thus improving gas exchange. The conventional treatment of PAH using pulmonary drug delivery has already been approved in some countries with other drugs, such as iloprost inhalation solution (Ventavis®), and treprostinil inhalation solution (Tyvaso®) . We believe that pulmonary delivery of bosentan hydrate can overcome the limitations of oral administration by improving the bioavailability of the drug, resulting in a reduction in the dosage.

In this study we have formulated Bosentan hydrate dry powder and evaluated its physicochemical properties, FTIR study, Spectroscopic studies, Morphology, Particle Size Distribution, in vitro dissolution behavior and Drug content. The DPIs contain a powder formulation, which most frequently consists of an ordered mixture of micronized drug (<5 μm in diameter) and larger carrier lactose particles that are required to improve powder flow properties. The patient's inhalation through the device is used to disperse the powder and to ensure that some of the dose is carried into the lungs. An alternative type of formulation used in some DPIs consists either of micronized drug particles alone loosely aggregated into small spherules or of cospheronized drug and lactose.

2. MATERIAL AND METHODS

Materials

Bosentan hydrate ($C_{27}H_{31}N_5O_7S$; molecular weight (MW), 569.63 g/mol) was obtained from MSN Labs, Hyderabad. D mannitol (MNT, $C_6H_{14}O_6$; MW, 182.17 g/mol) was obtained from Fine chem. Industry, Mumbai. Ethanol (HPLC grade, Fine chem.Industry, Mumbai), and all other reagents were of analytical grade.

3. EXPERIMENTAL WORK

Physical Characters of Bosentan hydrate.

Melting point analysis

Melting point of the drug sample was determined because it is a good first indication of purity of the sample since the presence of relatively small amount of impurity can be detected by a lowering as well as widening in the melting point range. Melting point of the drug was determined by taking a small amount of drug in a capillary tube closed at one end and it was placed in melting point apparatus-veego (VMP) and the temperature, at which the drug melts, was noted. Average of triplicate readings was taken.

solubility analysis

Solubility of bosentan in ethanol, water and phosphate buffer was experimentally determined at temperature, the drug was taken in excess amount and added to ethanol and then take supernatant from that and check the absorbance. Same procedure for water and phosphate buffer was done.

FTIR study of Bosentan hydrate

FTIR study is another identification test for Bosentan hydrate. The FTIR spectra of pure drug and mannitol were determined. In this study, we prepared the bosentan microparticles by the co spray-drying method with bosentan hydrate and mannitol the compatibility of drug and carrier in defined ratio were determined.

Spectroscopic studies

UV Spectroscopy- Determination of λ max:

The λmax (wavelength of maximum absorption) of bosentan was determined by scanning 10 µg/ml solution of the drug (ethanol used as diluent) using UV-Visible spectrophotometer within the wavelength region of 220 to 400 nm against ethanol as blank. The absorption curve showed characteristic absorption maxima at 270 nm for Bosentan.

Standard calibration curve of Bosentan hydrate

i) Preparation of standard solution:
Accurately 10 mg of bosentan was weighed and transferred into 100 ml volumetric flask and dissolved in ethanol. Then the volume was made up to the mark with ethanol to produce 100 µg/ml concentration stock solution.

ii) Preparation of working solution:
From the standard stock solution 1, 2, 3, 4 and 5 ml aliquots were pipetted into separate 10 ml volumetric flasks and the volume was made up to the mark with ethanol to get working standard solutions with concentrations of 10, 20, 30, 40 and 50 µg/ml respectively.

iii) Procedure:
Measure the absorbance of prepared dilutions of 10, 20,30,40,50 ug/ml at 270 nm. Plot graph between conc. Vs absorbance and calculate the coefficient of correlation and slope.

Preparation of spray dried bosentan hydrate
To prepare the bosentan microparticles, the co-spray-drying process was performed using a laboratory scale spray dryer(SD-111 Technosearch,Mumbai) .The feeding solution was prepared by completely dissolving bosentan hydrate and D-mannitol in 70% ethanol (*v*/v) [each ratio of 1:1,2:1,1:2(w/w)], to obtain a total powder concentration of 1% (*w*/*v*). The following parameters were used during spray-drying: inlet temperature, 100 °C;nozzle size, 0.4 mm; feed rate, 2 mL/min; atomization air pressure, 200 kPa; and drying air flow rate, 0.30 m3/min. The designations of the spray-dried bosentan microparticles (SDBMs) were F1 (1:1), depending on the ratio of bosentan hydrate to mannitol, respectively. The bosentan microparticles were kept in a glass vial containing silica gel at −20 °C, until used.

Table No. 1: Formulation table of spray dried bosentan

Formulation ratio	Amount of drug bosentan (in mg)	Amount of D-mannitol (in mg)	Final weight (in mg)	Inlet temperature	Feed rate (ml/min)	Atomization air Pressure (kpa)
F1 (1:1)	500	500	1000	100°C	2	200
F2(2:1)	1000	500	1500	100°C	2	200
F3(1:2)	500	1000	1500	100°C	2	200

Post spray drying parameter of powder:

1. Morphology

The morphology was examined by scanning electron microscopy .The particle morphology examined by scanning across the surface of powder, an electron beam with low energy is radiated to the material and scans the surface of the sample.

2. Particle Size Distribution

Particle size distribution is important for understanding the physical and chemical properties of powder. The particle size distribution was carried out using electron microscopy.

3. Zeta Potential Determination

The zeta potential was determined using a Zeta potential analyzer. The sample weight to 10mg is dissolve in ethanol and the solution is placed in analyzer for further analysis.

4. True Density

A pycnometer (Dolphin, Mumbai) was used to determine the true density of the particles. Weight empty pyconometer then fill ethanol and measure the weight of bottle then add 2gm of bosentan in empty bottle and weight the bottle after that add ethanol to this powder and weight the bottle. Calculate the true volume of drug.

True density =weight of powder/True volume of powder

5. Moisture Content

The water content of bosentan particles was analytically quantified via drying sample and determining loss on drying.

6. Cumulative percent drug release

The *in vitro* dissolution behavior of the SDBMs was evaluated using a Franz diffusion cell system (Electro lab. Mumbai). The receptor compartment of the Franz diffusion cell was filled with 12 mL of phosphate-buffered saline solution, adjusted to a pH of 7.4 with Tween 80 (5% *w*/w), which was maintained in a sink condition for the experiment. A cellulose membrane filter (pore size, 0.45 µm) was used as a barrier, and was placed on the receptor. The membrane was in contact with the receptor medium, which allowed the air–liquid interface. The receptor medium was maintained at 37 ± 1 °C, and continuously stirred to ensure homogeneity.

Raw bosentan hydrate and SDBMs equivalent to 10 mg of the drug was uniformly spread on the surface of the membrane at the air–liquid interface. At a defined time, a volume of 200 µL of the medium was taken, and the same volume of fresh buffer was added. The content of drug was quantified using UV.

7. X Ray Diffraction

X-ray Diffractometry was used to investigate the solid-state of the particle. The sample was sent to analytical lab for X-ray diffractometery.

8. Drug content

Drug content was used to determine the amount of drug present in formulation. Solid dispersion equivalent to 10 mg drug were taken and dissolve in 20 ml of ethanol from this solution 1ml was taken and again diluted with 10ml ethanol. The solution was analyze using uv at 270nm.

Accelerated Stability Study

In any rationale design and evaluation of dosage forms for drugs, the stability of the active component must be major criteria in determining their acceptance or rejection.

During the stability studies the product is exposed to normal conditions of temperature and humidity. However the studies will take a longer time and hence it would be convenient to carry out the accelerated stability studies where the product is stored under extreme conditions of temperature. In the present study, stability studies were carried out on optimized formulation. The powder were stored at 40 ± 2^0 C/75 ± 5 % RH for duration of 45 days, sample was withdrawn and tested for drug entrapment, and drug release study.

The reasons for stability studies:

- There may be chemical degradation of the active drug leading to a substantial lowering of the quantity of the therapeutic agent in the dosage form.
- Although chemical degradation of the active drug may not be extensive, a toxic product may be formed in the decomposition procedure.
- Instability of a drug product can lead to a decrease in its bioavailability. This can lead to a substantial lowering in the therapeutic efficiency of the dosage form.

4. RESULTS AND DISCUSSION

1. Physical characters of Bosentan hydrate.

Table No. 2: Physical Characters of drug.

Sr.No.	Characters	Inference
1.	Nature	Crystalline powder
2.	Color	White
3.	Odor	Odorless
4.	Taste	Tasteless

The Physical characters was found to be complacent with Standard, so drug used in formulation was found to be pure according to I.P specifications.

2. Melting Point Determination

Table no.3: Melting point of bosentan hydrate

Sr.No	Drug	Melting point
1.	Bosentan	107-110 ^{O}C

The melting point of pure bosentan hydrate was found to be 107^{O}C– 110^{O}C. so drug used in formulation was found to be pure according to I.P specifications.

3. Solubility Determination

Table No 4: Solubility profile of Bosentan Hydrate

Sr. No	Solvent	Solubility(mg/ml) (Mean± S.D.)
1	Water	1± 0.03
2	Buffer solution 7.4	3± 0.07
3	Ethanol	48±0.14

n=5

The solubility of pure drug in 10 mg/ml of solvent was carried out and it reveals that it is soluble in ethanol and sparingly soluble in water, soluble in phosphate buffer pH7.4

Spectroscopic studies.

1. UV Spectroscopy- Determination of λ max

Bosentan Hydrate showed the maximum wavelength at 270 nm, which matches with the standard. Hence drug used in formulation was found to be pure according to I.P specification.

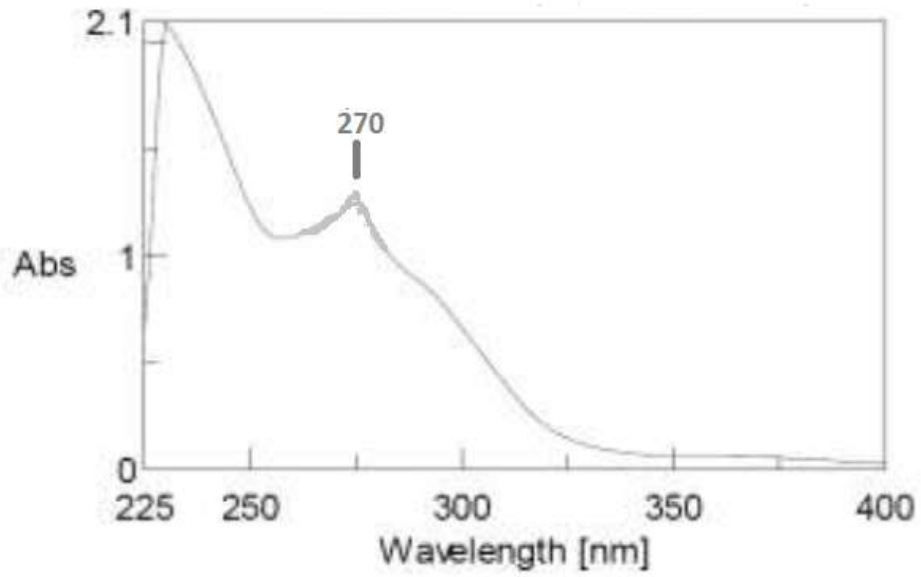
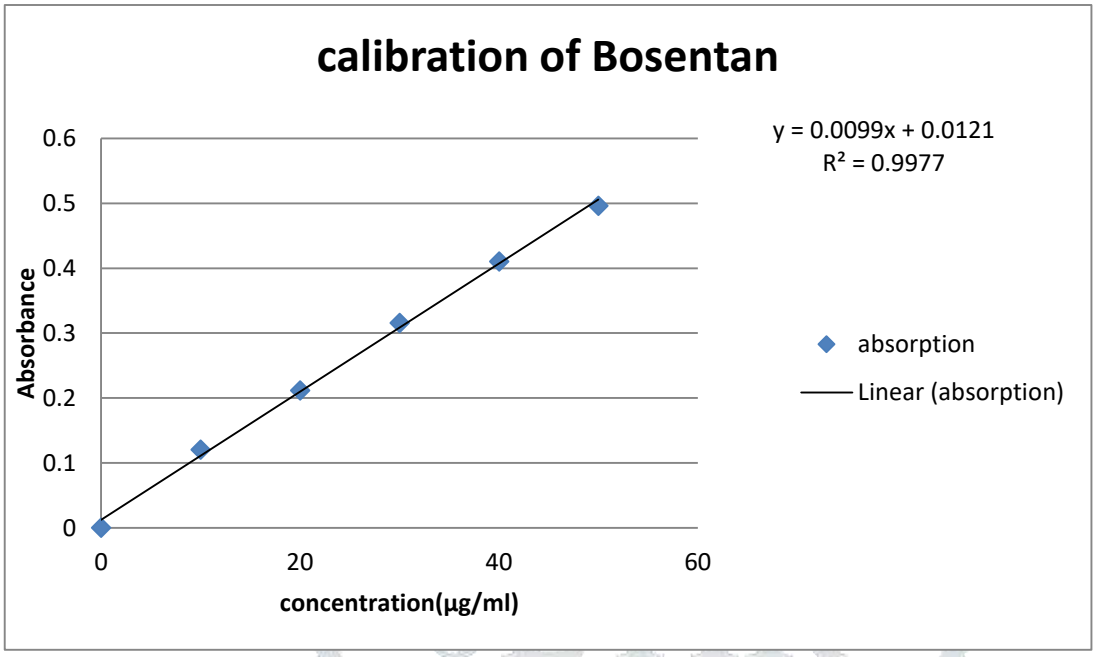


Figure No.1: λ max of Bosentan hydrate

2. Standard Calibration curve of Bosentan hydrate in ethanol

Table No. 5: Calibration curve for bosentan hydrate

Sr. No.	Concentration (µg/ml)	Absorbance at 270 nm
1.	0	0
2.	10	0.1201
3.	20	0.2116
4.	30	0.3153
5.	40	0.4100
6.	50	0.4961



Correlation of coefficient (R^2) = 0.997

Equation of regressed line; Y= 0.009x +0.012

Bosentan hydrate follows Beers and Lamberts law. In the conc. range of 0-5ug/ml at 270 nm against ethanol and correlation of coefficient was found to be 0.997and equation of regression found to be Y=0.009x+0.012.

3. FTIR study of bosentan Hydrate

Figure 2. presents the results of the Fourier-transform infrared spectroscopy (FT-IR). When compared to the raw bosentan hydrate, the FTIR spectra of the spray-dried bosentan showed a disappearance in the peaks at 3650–3600 cm−1, which corresponded to the OeH stretching. The FT-IR spectra distinguishing mannitol was present at 3400–3300 cm−1 corresponding to the OeH absorption, and 3000–2850 cm−1 corresponding to the CeH stretching.

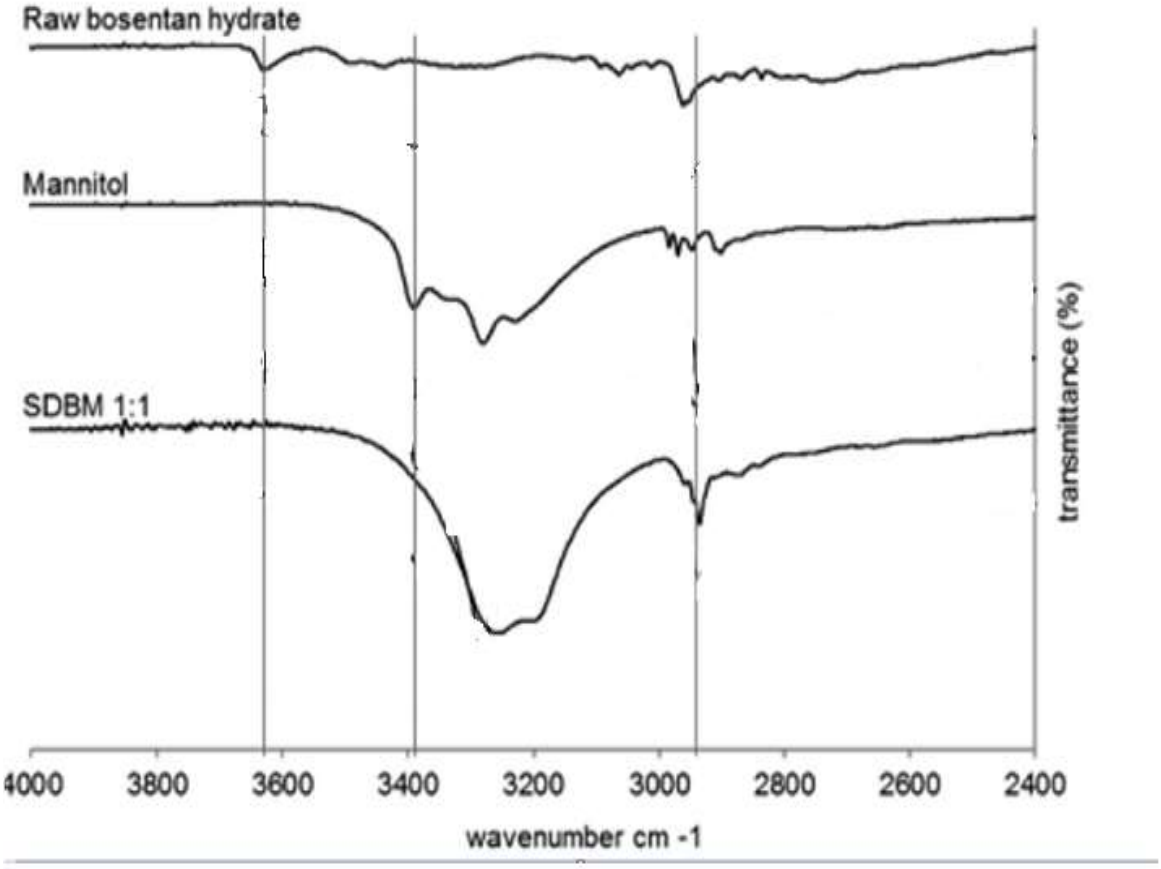


Figure 2: FT-IR Spectra

When mannitol and spray-dried powder were compared, no additional or absent peaks were observed, and the peak intensity was increased as a result of spray-drying. In the SDBMs, it was found that there were no major shifts in the positions of the peaks, as compared to those of the bosentan and spray-dried powder. The main intensity of the peak increased with a higher mannitol amount.

3. Evaluation of spray dried powder

Powder prepared by spray drying were evaluated for different parameter such as particle morphology, particle

Size, zeta potential, true density, moisture content, drug content and results are shown in table no.6

Table No.6 Particle size distribution, zeta potential, true density, Moisture content and drug content of SDBMs (mean ± standard deviation)

Formulation	Particle size distribution (µm)	Zeta potential (mV)	True density (g/ cm3)	Moisture content (%)	Drug content
Pure bosentan hydrate	19.0 ±2.5	−41.03±2.80	1.32 ± 0.00	3.51	98.5
F1 1:1	6.17± 1.5	−34.37±1.33	1.34±0.00	2.22	91.5
F2 2:1	5.12 ±0.2	−30.13± 1.59	1.25±0.00	1.99	86.2
F3 1:2	4.96 ±1.2	−22.20± 1.37	1.40± 0.00	2.56	88.26

3.1 Particle size

The particle size of raw bosentan hydrate was 19.0± 2.5µm . The F1 (1:1) showed 6.17± 1.5µm of size, F2 (2:1) showed 5.12±0.2µm, F3 (1:2) showed 4.96±1.5µm of size. The particle size distribution of SDBMs decreased relative to the raw bosentan hydrate.

3.2 Zeta potential

The zeta potential was −41.03±2.80, −34.37±1.33,−30.13± 1.59 ,−22.20± 1.37 for raw bosentan hydrate, F1 (1:1),F2 (2:1),F3 (1:2) respectively, The absolute values of SDBMs were significantly lower than that of the raw bosentan hydrate.

3.3 True density

The values of true density of raw bosentan hydrate, F1 (1:1), F2 (2:1),F3 (1:2) were 1.32, 1.34, 1.25, 1.40, respectively. The true density slightly increased as the mannitol ratio increased, as the true density of mannitol (1.51 g/cm3) is larger than that of bosentan hydrate (1.32 g/cm3).

3.4 Moisture content

The Moisture content of bosentan particles was analytically quantified *via* loss on drying. raw bosentan hydrate,F1 (1:1), F2 (2:1),F3 (1:2) had water content of 3.51, 2.22 , 1.99,2.56 %, respectively. The water content of the SDBMs was lower than that of raw bosentan hydrate, indicating that during the spray-drying process, water was removed. In addition, as the mannitol ratio increased in the formulations, the water content of the SDBMs was slightly increased.

3.5 Drug content

The drug loading was found to be uniform along all formulations and ranged from 91.5± 0.40 to 96.4±0.64 as per pharmacopoeial standards.

3.6 Particle morphology

The morphology was examined by scanning electron microscopy Fig.3 shows the scanning electron micrographs of pure bosentan hydrate and co-spray-dried particles of bosentan hydrate and mannitol (SDBMs), respectively. The raw bosentan hydrate had irregular and non-spherical shape, and polydispersed size range. When we observed the SDBMs, the macroscopic images (left columns) showed that all the

SDBMs had a similar shape, and were micro-size. They were nonspherical, and had raisin-shaped morphology. However, the micro morphologies of the SDBMs (middle and right columns) were different, depending on the formulation. F1 (1:1) had a rough surface with small pores.

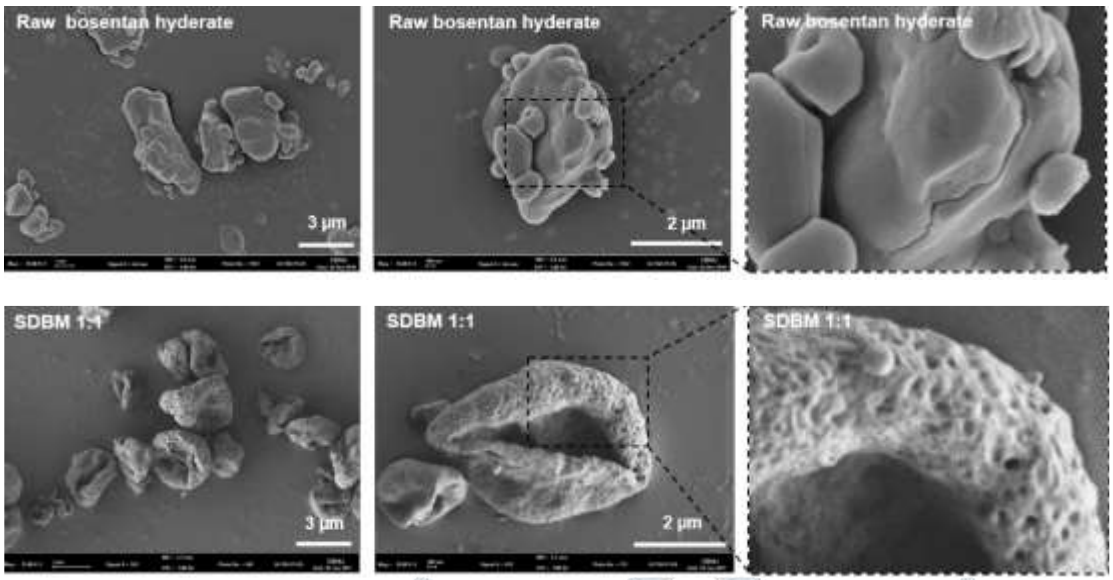


Figure 3. SEM micrographs of SDBMs (Magnifications for samples were 15 K, 35 K, and 350 K).

3.7 Powder x- ray diffraction

Figure 4 shows the powder X-ray diffraction (PXRD) patterns. The diffractograms of the spray-dried bosentan hydrate had no specific diffraction peaks, due to complete phase transforming to an amorphous solid-state, whereas the pure bosentan hydrate had approximately identical diffractograms with sharp and strong diffraction peaks at the main angles (2θ) of 9.28°, 15.55°. This indicated that the raw bosentan had high crystallinity, and that spray-drying influenced the solid-state of the drug to the amorphous state. SDBMs had diffraction peaks at angles (2θ) typically at 9.47°. The peak intensity was proportional to the amount of mannitol in the formulations.

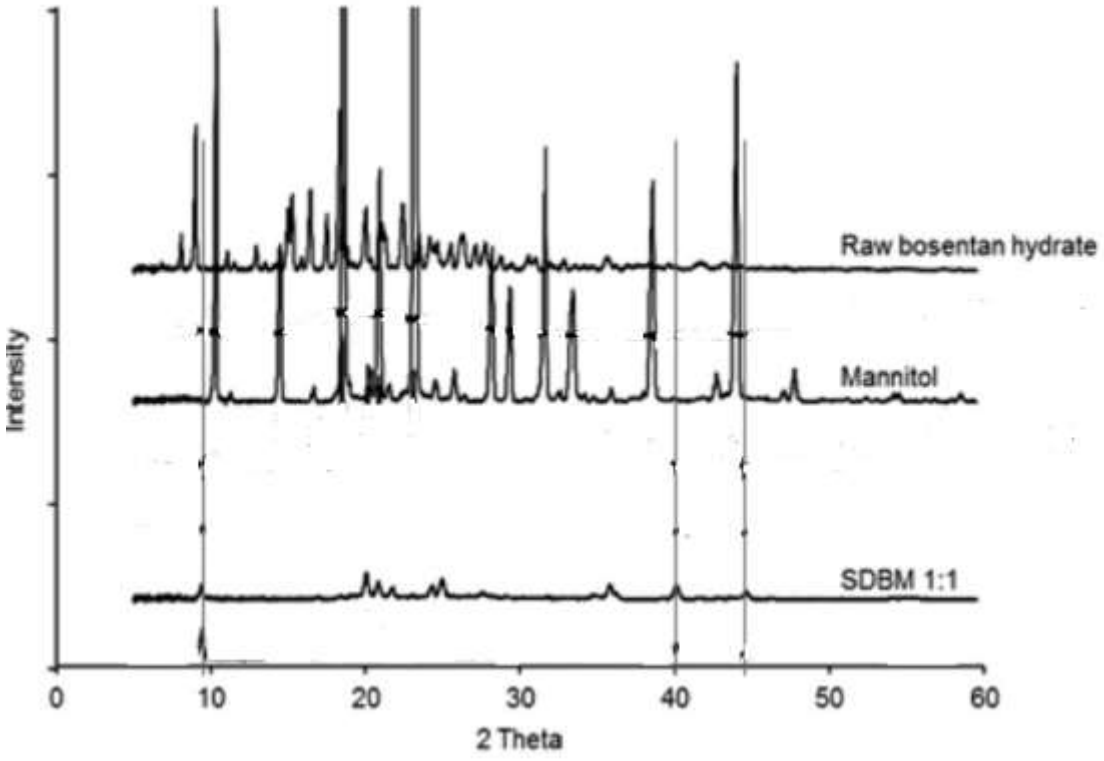


Figure 4. PXRD patterns

In this study, we prepared the bosentan microparticles by the co spray- drying method with bosentan hydrate and mannitol at different weight ratios. They have significantly different physicochemical properties. The results of PXRD and FT-IR of the bosentan particles show that the SDBMs consisted of amorphous bosentan hydrate and crystalline mannitol, and the crystallinity of mannitol was proportional to the mannitol ratio in the formulations. The spray drying conditions that control the evaporation rate of droplets, such as drying temperatures, and composition; concentration; and solvent types of the feeding solutions offer the

opportunity of changing the crystallinity and polymorphs of bosentan and mannitol, as shown in the results of PXRD, and FT-IR. Have significant effects on the physicochemical properties of each spray-dried particle formulations, such as surface morphology and zeta potential.

3.8 In vitro dissolution study

Table No 7: In vitro dissolution data in different ratio

Time (min)	Cumulative percent drug release			
	Pure bosentan hydrate	F1 (1:1)	F2 (2:1)	F30 (1:2)
10	25.14	48.25	44.27	42.56
20	36.71	80.55	57.24	66.56
30	41.18	81.41	61.35	70.56
40	50.71	87.83	66.24	71.54
50	55.44	94.02	71.98	81.56
60	60.91	96.80	89.78	85.77

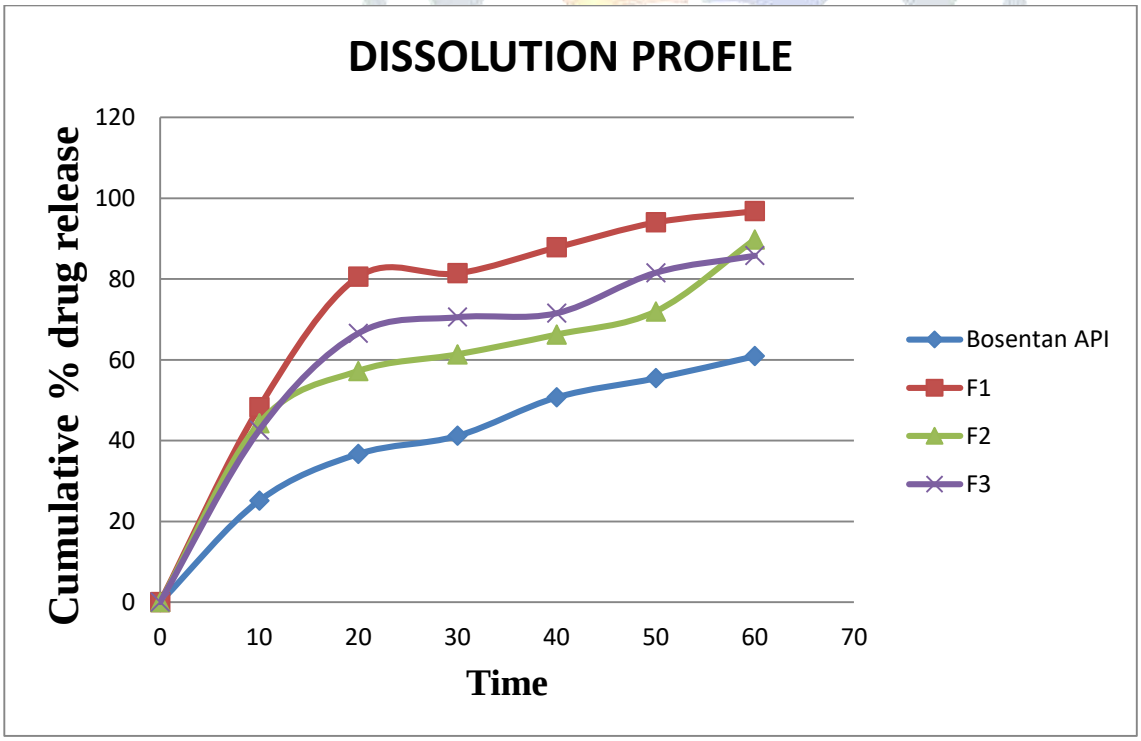


Figure 5: Dissolution profile of pure drug and batches F1, F2,F3

Fig.5 shows the dissolution profiles of the bosentan particles obtained by a Franz diffusion cell. When comparing the dissolution behavior of pure bosentan hydrate that showed a constant drug dissolution rate during the 60 min period, the SDBMs showed a

significantly higher cumulative dissolution % during 60 min. In addition, the drug dissolution rate of the SDBMs significantly increased as the mannitol ratio increased in the formulations. F1 (1:1) showed a rapidly reduced dissolution rate after 30 min.

The release profiles of all samples fit well into each kinetic model, where the dissolution constants of SDBMs increased as the mannitol ratio increased in the formulations. The presence of mannitol as a hydrophilic agent can increase the wet ability, thus enhancing the dissolution profile of bosentan. There are many physicochemical factors that can influence the *in vitro* dissolution behavior of DPI, such as crystallinity, polymorphism, particle size, water solubility, and dose. Pure bosentan hydrate is extremely insoluble in water (< 0.001 mg/mL), and is highly crystalline in the solid-state. This property reduces the affinity between the solid particles and the dissolution medium, resulting in a low dissolution rate.

4. Stability Study

The stability studies were carried out for optimized formulation F1 1:1. The formulations were stored at 40 ± 2^0C/75 ± 5 % RH for 45 days. The samples were withdrawn and retested for % drug release studies. It indicates that irrespective of different ratio of carrier and drug, these formulations were found to retain their stability for 45 days under the above conditions.

Table No8: Parameters studies on optimized F1 (1:1) before and after stability study.

Sr.No.	Parameters	Observation	
		Before	After
1	Drug content	70.52	69.52

Table No9:Cumulative percent drug release of optimized formulation before and after stability study

Time in (min)	Observation			
	Before stability study	After 15 Days	After 30 Days	After 45 Days
0	0	0	0	0
10	48.25	46.22	43.21	42.80
20	80.55	70.15	68.03	66.80
30	81.65	80.65	79.60	78.34
40	87.83	83.07	81.33	80.20
50	94.02	92.65	92.06	91.50
60	96.80	96.80	95.62	95.61

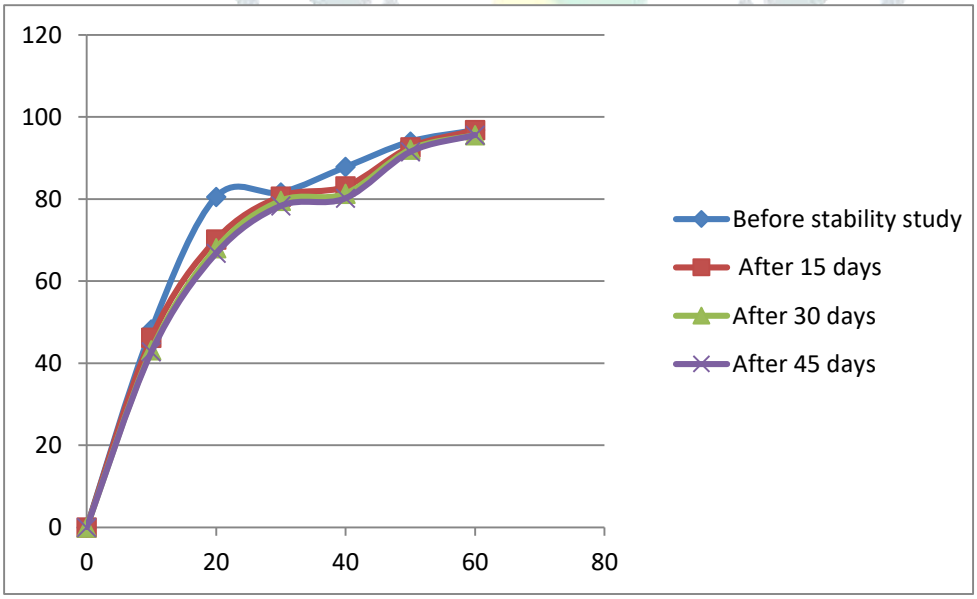


Figure No. 6: In vitro Dissolution profile of formulation F1 Before and after stability study.

The stability study was carried out on optimized formulation F1(1:1). The formulation was stored at 40 ± 2^0C/75 ± 5 % for 45 days. After 15 ,30 and 45 days sample were withdrawn and retested for in vitro drug release studies, other parameters were checked after 45 days.
There were no considerable changes in physical parameters of powder such as particle size, water content, drug content of formulation F1 (1:1) after accelerated study.

SUMMARY & CONCLUSION

Summary

The aim was to design dry powder for inhalation of Bosentan hydrate. The dry powder for inhalation was prepared by spray drying by using D mannitol as carrier and bosentan these spray dried bosentan microparticles shows more water solubility and release in short time than that of raw bosentan. The particle were prepared by spray drying process, during process different parameter were studied such as inlet temperature, atomization pressure, feed rate.

Various drug and carrier ratio were prepared and there parameters were studied. The batches of Dry powder of bosentan hydrate containing D Mannitol as carrier by spray drying method were developed and evaluate their physical parameter such as particle size, , zeta potential, true density, drug content, moisture content,% Drug release.

The particle size of prepared powder was in range of 4.96-6.17µm. Zeta potential was found to be 34.37-22.20mv. The dissolution rate was found to be in range of 85-96%. Dry powder for inhalation by spray drying method of formulation F1 before and after accelerated stability study. Hence dry powder for inhalation prepared was found to be stable and having better result.

Conclusion

The spray dried bosentan microparticle prepared by spray drying method the evaluation shows the formulation were lies within official standard limit.

The spray dried bosentan microparticle F1(1:1) prepared by spray drying is assessed increased water solubility and fast release of drug, so it can be conclude that The carrier d mannitol improves the drug release as that of raw drug.

Also more satisfactory results were shown by this microparticle prepared by spray drying.

Hence, the objective of this study i.e.:

i) To overcome the poor solubility of some active pharmaceutical ingredient (API).

ii) To study effect of concentrations of careers on performance of system.

Had been substantiate.

Future Prospects:

1. In-vivo study.
2. Use of different carriers.
3. To determine aerosol performance of dry powder for inhalation.
4. To study the effects of process parameter on spray drying.

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