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FORMULATION AND EVALUATION OF BOSENTAN NANOSUSPENSIONS BY NANOPRECIPITATION METHOD

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ABSTRACT

In the present study, an attempt was made to formulate and evaluate Bosentan Nanosuspension by Nanoprecipitation method and to reduce symptoms in patients suffering with pulmonary arterial hypertension. Nanosuspension containing the drug were prepared by Nanoprecipitation method using combinations of polymers such as Sodium lauryl sulphate (SLS), TWEEN-80, Poloxamer 188, Urea and methanol. Estimation of Bosentan was carried out spectrophotometrically at 274nm. The Oral Nanosuspension were evaluated for drug content uniformity, particle size analysis, zeta potential, in-vitro drug release, short-term stability, drug- excipient interactions (FTIR). IR spectroscopic studies indicated that there are no drug-excipient interactions. Of all the formulations (PF1 – PF6) PF3 formulation containing SLS 1.25%, Tween-80 0.2% and Poloxamer 1% were found to be promising which showed 99.55±0.84% release at the end of 30min & it follows first order drug release kinetics. For optimized formulation (PF3) particle size, polydispersible index & zeta potential was found to be 38.1nm, 2.88 & -4.49mV respectively. The optimized formulation was stable at 40^0C/75 % RH for 3 months.

Keywords: Bosentan, oral Nanosuspension, Urea, SLS, Poloxamer (188), TWEEN-80 and Methanol.

INTRODUCTION

Poor solubility of drug substance has always been a challenging problem faced by pharmaceutical scientists and it is increased now because more than 40% of new chemical entities are poorly water soluble. One of the most persistent problems faced by drugs with poor aqueous solubility is that their oral delivery is frequently associated with implication of low bioavailability and lack of dose proportionality. A novel technology that can used to overcome problems associated with this method is nanosuspension, which is based on size reduction mechanism.

In the present research work an attempt was made to improve the solubility and dissolution rate of Bosentan. Bosentan is a dual endothelin receptor antagonist important in the treatment of pulmonary artery hypertension (PAH). It is licensed in the United States, the European Union and other countries by Actelion Pharmaceuticals for the management of PAH under the trade name Tracleer®. Bosentan is used to treat pulmonary hypertension by blocking the action of endothelin molecules that would otherwise promote narrowing of the blood vessels and lead to high blood pressure.

MATERIALS AND METHODS

Bosentan obtained as a gift sample from Dr Reddy's, Sodium lauryl sulphate, TWEEN- 80, Poloxamer (188), Urea and all other chemicals and solvents used are from Rankem, Mumbai.

Preparation of Bosentan Nanosuspension by nanoprecipitation: Nanosuspension was prepared by the nanoprecipitation technique. Bosentan was

dissolved in a methanol at room temperature (organic phase). This was poured into water containing different combinations of Poloxamer 188 and SLS, UREA, TWEEN 80 solutions maintained at room temperature and subsequently stirred on magnetic stirrer which is stirred at rpm 2000-3000 for 15 min to allow the volatile solvent to evaporate. Addition of organic solvents by means of a syringe positioned with the needle directly into stabilizer/surfactant containing water. Organic solvents were left to evaporate off under a slow magnetic stirring of the nanosuspension at room temperature for 1 hour followed by sonication for 1 hour as shown in Table 1.

Evaluation parameters of Bosentan Nanosuspensions:

Drug content uniformity:

10 ml of each formulation was taken and dissolved in 10 ml isotonic solution and kept overnight. 10 mg (similar as in formulation) of drug was taken and dilution was made to 10µ g/ml. The dilutions were filtered and analyzed using UV for their content uniformity. The absorbance of the formulations was read using one cm cell in a UV-Vis spectrophotometer. The instrument was set at drugs wavelength (nm). The drug content in each formulation was calculated based on the absorbance values of known standard solutions.

Entrapment efficiency:

The freshly prepared nanosuspension was centrifuged at 20,000 rpm for 20 min at 5°C temperature using cool ultracentrifuge. The amount of unincorporated drug was measured by taking the absorbance of the appropriately diluted 25 ml of supernatant solution at required wavelength (nm) using UV spectrophotometer against blank/control nanosuspensions. DEE was calculated by subtracting the amount of free drug in the supernatant from the initial amount of drug taken. The experiment was performed in triplicate for each batch and the average was calculated.

The entrapment efficiency (EE %) could be achieved by the following equation:

%Entrapment efficiency= Drug content *100/Drug added in each formulation.

Particle size and shape:

Average particle size and shape of the formulated nanosuspensions was determined by using Malvern Zetasizer ZS using water as dispersions medium. The sample was scanned 100 times for determination of particle size.

***Invitro* drug release study:**

In-vitro dissolution studies were performed in USP apparatus-II (LAB INDIA DS 8000), employing paddle stirrer at rotation speed of 50 rpm and 500 ml of pH 6.8 phosphate buffer as dissolution medium. Accurately weighed bulk drug and nanosuspensions were dispersed in dissolution medium. The release study is performed at 37 ± 0.5^{o}C as shown in Table 4. Samples of 5 ml are withdrawn at predetermined time intervals and replaced with fresh medium to maintain sink condition. The samples were filtered through 0.22 µm membrane filter disc (Millipore Corporation) and analyzed for Bosentan after appropriate dilution by measuring the absorbance at 274 nm .

RESULTS AND DISCUSSION

Flow properties of Bosentan:

The flow properties like bulk density, tapped density, angle of repose, compressibility index and Hausner's ratio was found to be 0.47 g/cc, 0.54g/cc, 25.65o,12.96% and 1.14 respectively shown in Table 2, which indicates that flow of API, was good as per I.P limits.

The solubility of Bosentan was very poor in water (0.09 mg/ml) when compared to 0.1N HCl & 6.8 pH. From the results it indicated that, as the pH of the buffer increased, the solubility increased from 0.09 to 0.380 mg/ml. Hence the solubility of Bosentan was pH dependent. Determination of Bosentan λ-max was done in pH 6.8 phosphate buffer medium for accurate quantitative assessment of drug dissolution rate as shown in Fig 1. The λ-max was found to be 274 nm, i.e., at its absorption maxima.

Compatibility studies were performed using IR spectrophotometer. The IR spectrum of pure drug and physical mixture of drug and excipients were studied as shown in Fig 2.

The drug content of all the formulations (PF1-PF6) prepared by this method was found in the range between 85.42±0.22% to 96.12±0.97% respectively.

The entrapment efficiency of all the formulations prepared by this method was found in the range between 94.12±1.33% - 99.71±1.10% respectively.

Optimized formulations (PF3) evaluations like scanning electron microscopy, particle size analysis, & zeta potential analysis were shown in below at Fig 3,4,5 respectively.

In vitro release studies were carried out in USP

dissolution test apparatus-II employing paddle stirrer at 50 rpm and 500 ml of pH 6.8 buffer as dissolution medium. The in vitro dissolution data of all the designed formulations (nanoprecipitation method) are shown and dissolution profiles depicted in figures (6-7). The formulation PF1, PF2& PF3 the drug release was found to be 97.54±0.02% at the end of 45 min, 95.04±0.19% at the end of 40 min and 99.55±0.84% at the end of 30 min respectively. The formulation PF4, PF5& PF6 the drug release was found to be 93.47±0.23% at the end of 45 min, 97.65±0.40% at the end of 45 min and 98.77±0.09% at the end of 40 min respectively as shown in Fig 6,7. Among all the formulations (PF1-PF6) PF3 was selected as optimized formulation based on drug release studies, which containing SLS 1.25%, Tween-80 0.2% and Poloxamer 1%.

The drug release from the Nanosuspension was explained by the using mathematical model equations such as zero order, first order, and equation methods. Based on the regression values it was concluded that the optimized formulation PF3 follows first order kinetics.

CONCLUSION

Bosentan nanosuspensions were prepared by (Nanoprecipitation method) using polymers such as SLS, Urea, TWEEN-80, and Poloxamer. Total of six (PF1- PF6) formulations were prepared and of that PF3 was found to be the best formulation containing (SLS 1.25%, Tween-80 0.2% and Poloxamer 1%). The results obtained from these studies indicated that the powder had good flow properties. The prepared nanosuspensions were evaluated for drug content & entrapment efficiency. For optimized formulation (PF3) particle size, polydispersible index & zeta potential was found to be 38.1nm, 2.88 & -4.49mV respectively. The optimized formula PF3 (SLS 1.25%, Tween-80 0.2% and Poloxamer 1%) has 99.55±0.84% drug release by the end of 30 min. The release kinetics of the optimized formulation showed zero order (R2=0.980) drug release. The optimized formulation was stable at 400C/75 % RH for 3 months.

Table-1: Composition of Nanosuspension of Bosentan

Ingredients	PF1	PF2	PF3	PF4	PF5	PF6
Bosentan (mg)	62.5	62.5	62.5	62.5	62.5	62.5
SLS	0.75%	1.0%	1.25%	--	--	--
Urea	--	--	--	0.75%	1.0%	1.25%
Tween-80	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%
Poloxamer	1	1	1	1	1	1
Methanol (ml)	2	2	2	2	2	2
Water (ml)	40	40	40	40	40	40

Table-2: Flow properties

1	Bulk Density (g/cc)	0.47
2	Tap Density(g/cc)	0.54
3	Compressibility Index	12.96
4	Hausner's ratio	1.14
5	Angle of repose	25.650

Table-3: Standard graph of Bosentan in pH 6.8 (λmax 274 nm)

Concentration (µg/ml)	Absorbance (λmax 274 nm)
0	0
2	0.158±0.040
4	0.312±0.024
6	0.471±0.015
8	0.655±0.074
10	0.815±0.005
12	0.967±0.033

TABLE-4: *IN-VITRO* drug release data of formulation PF1 – PF6

TIME	PF1	PF2	PF3	PF4	PF5	PF6
0	0	0	0	0	0	0
5	25.44±0.23	29.04±0.11	31.45±0.05	13.32±0.26	16.47±0.13	23.26±0.20
10	32.29±0.36	36.41±0.56	50.20±0.97	27.74±0.77	29.40±0.04	35.33±0.31
15	45.77±0.15	48.11±0.33	69.88±0.24	36.52±0.20	40.23±0.17	45.27±0.78
20	58.15±0.78	61.12±0.21	78.41±0.31	45.79±0.78	48.85±0.44	59.84±0.33
25	67.02±0.45	70.45±0.03	89.78±0.66	59.22±0.65	62.77±0.62	73.69±0.12
30	79.16±0.41	81.14±0.14	99.55±0.84	68.02±0.74	70.64±0.11	82.04±0.02
35	85.51±0.56	87.46±0.40		78.39±0.85	81.61±0.89	91.08±0.54
40	92.12±0.15	95.04±0.19		86.11±0.88	90.78±0.65	98.77±0.09
45	97.54±0.02			93.47±0.23	97.65±0.40	

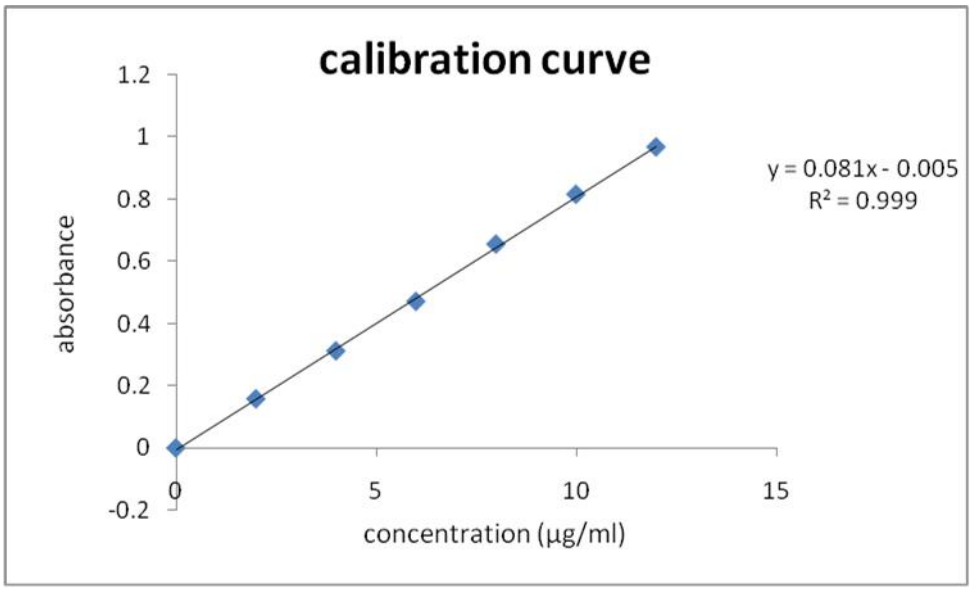

Fig-1: **Standard calibration curve of Bosentan in pH 6.8**

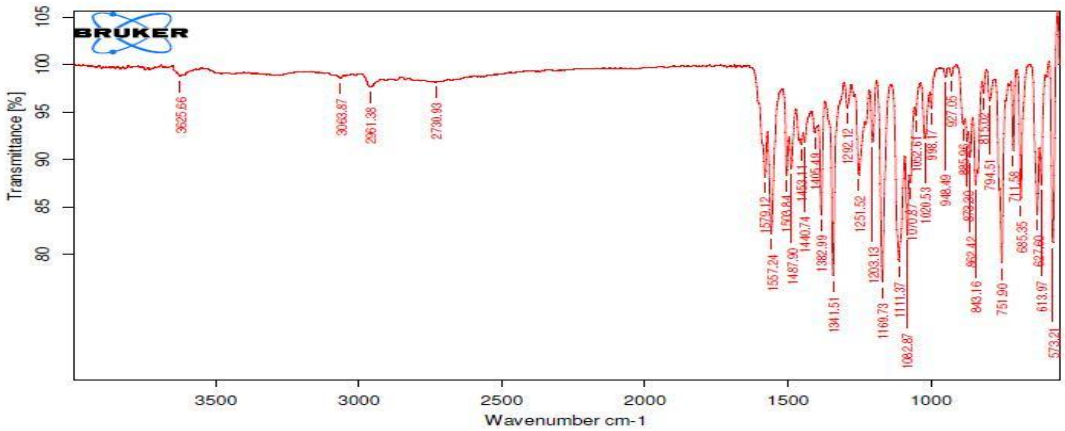

Figure-2: IR spectrum of Bosentan pure

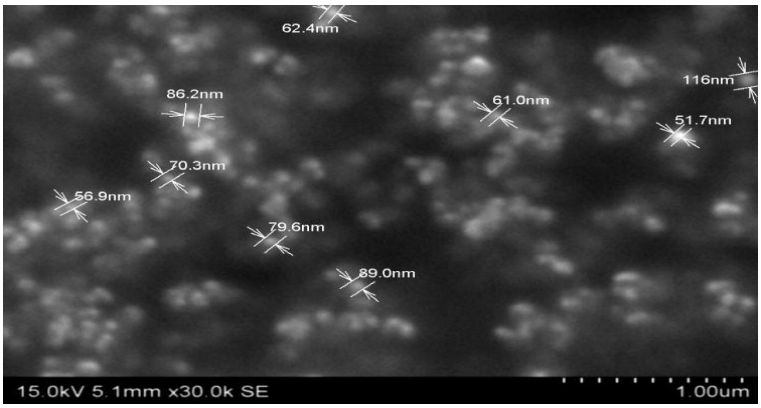

Fig-3: **SEM image by nanoprecipitation method optimized (PF3)**

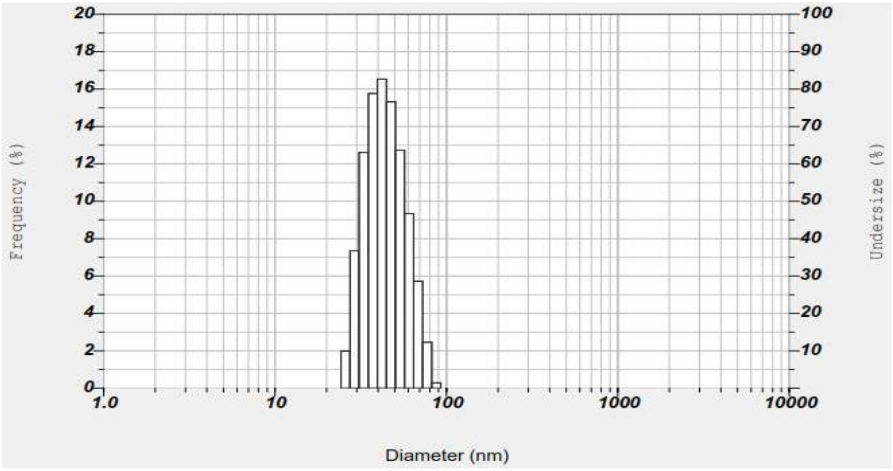

Fig-4: **Particle size analysis by nanoprecipitation method optimized (PF3)**

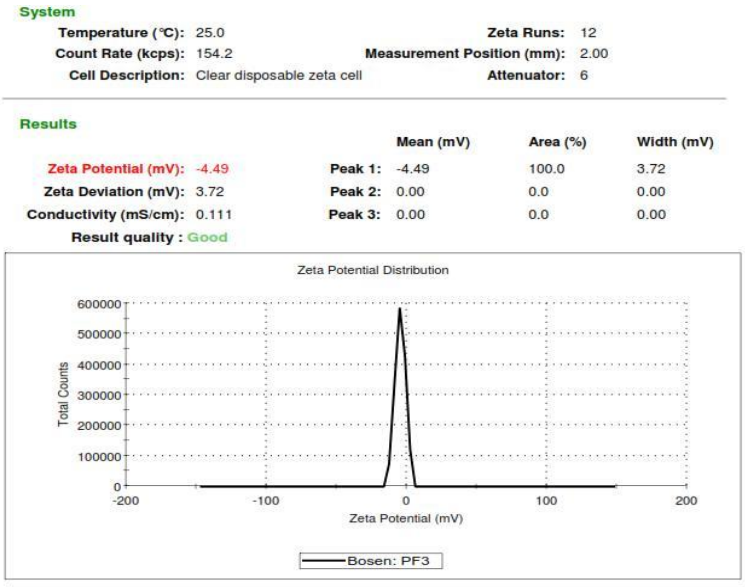

Fig-5: **Zeta potential analysis by nanoprecipitation method optimized (PF3)**

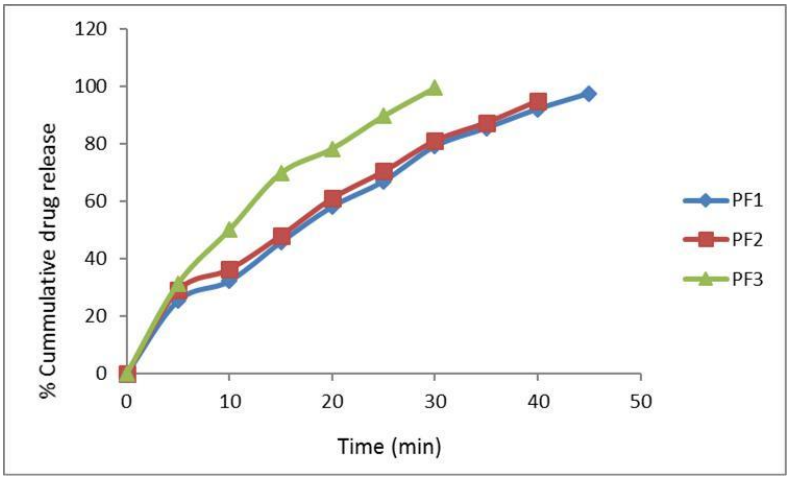

Fig-6: *invitro* dissolution profile of PF1-PF3

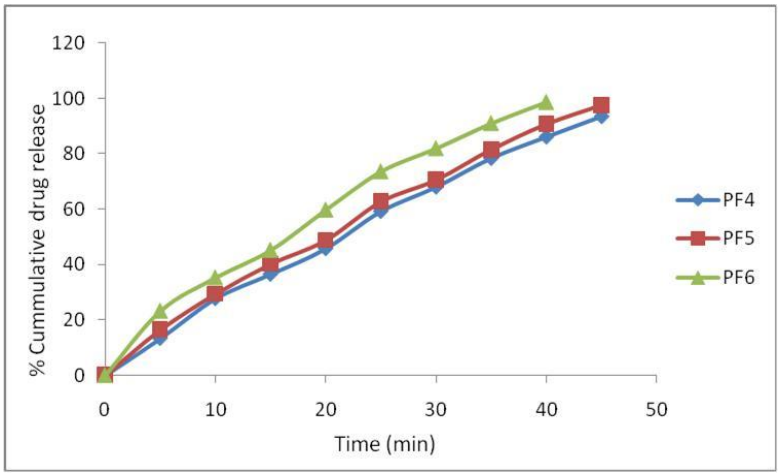

Fig-7: *invitro* dissolution profile of PF4-PF6

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