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ENHANCEMENT OF SOLUBILITY AND DISSOLUTION PROPERTIES OF BOSENTAN BY INCLUSION COMPLEXATION

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ABSTRACT

The cyclodextrins have a wide range of applications in different areas of drug delivery and pharmaceutical industry due to their complexation ability and other versatile characteristics. The most common pharmaceutical application of cyclodextrin is to enhance the solubility, stability, safety and bio availability of drug molecules. The major aim of the study was to enhance the solubility and dissolution rate of Bosentan by complexation with Cyclodextrins (β-CD). The solid complexes of bosentan and β-CD were prepared in 1:1 and 1:2 molar ratios by two methods like kneading and co-solvent evaporation. The binary complexes 1:1 molar ratio of kneading method heighted of drug release compared to reaming method of ratios. Further the selected cyclodextrin complexes are designed to formulate dispersible tablet using selected for different concentration of super disintegrate like sodium starch glycol late, dicalcium phosphate by direct compression method. The optimized formulation showed more than 80% drug release with in 1hr.

KEYWORDS: Bosentan, Cyclodextrins, inclusion complexation, solubility enhancement, dissolution rate.

INTRODUCTION

The aqueous solubility and permeability of drug(s) through biological membranes are the main physicochemical properties that limit the bioavailability of a new drug molecule. Various methods are proposed to enhance aqueous solubility of poorly soluble drugs that includes: chemical modifications (e.g., pro drugs or salt derivatives), physical modifications (e.g., solid dispersions, size reduction, loading on porous carriers, co crystals), alteration of solvent compositions (e.g., pH adjustment, use of co solvents, addition of surfactants) and use of carrier systems (e.g., cyclodextrins, micelles, liposomes).

A simple and convenient solubilizing technique is the use of water-soluble cyclodextrins (CD) complexes. Among various approaches to improve solubility, inclusion complexation with cyclodextrins is a successful approach to improve dissolution rate and bioavailability of biopharmaceutical classification scheme (BCS) class II/ class IV drugs. Cyclodextrins are pharmaceutical solubilizing excipients that have the ability to temporarily camouflage undesirable Physicochemical properties and offer desired therapeutic and drugable properties. Cyclodextrins can enhance oral dose availability, drug stability and affect permeability through biological membranes under certain circumstances.

Bosentan is a competitive antagonist of endothelin-1 at the endothelin-A (ET-A) and endothelin-B (ET-B) receptors. Important in the treatment of Pulmonary Artery Hypertension (PAH). It can be considered as a drug with low solubility and high permeability, therefore Bosentan belongs to BCS class II. Practically insoluble in water and especially in low pH and solubility is slightly higher above pH 7, Hence, poor aqueous soluble drug (s) (i.e., solubility<1 mg/ml) typically exhibit dissolution rate limited absorption. The dissolution study is important particularly for insoluble or low-soluble drugs, where absorption is a dissolution rate limited process for BCS class II and class IV drugs. Drugs those do not release completely in the GIT shows limited bioavailability which results in wastage of a large portion of an oral dose and adds cost of therapy.

Improvement of solubility in such case is an important and challenging objective to improve therapeutic efficacy. Inclusion complexation of drugs with CDs may improve stability, solubility and dissolution.

MATERIALS AND METHODS

Bosentan(Heterolabs), Beta-cyclodextrins (Rouettepharma), Sodium hydroxide (Merck chemicals), Mumbai, Potassium dihydrogen phosphate(Merckchemicals), Lactose (Merck chemicals), Talc-(Otto chem-biochem), Mumbai. Magnesium stearate (Sd fine chemicals Ltd), Microcrystalline cellulose (PH 101 Indian research products), Methanol (Sd fine chemicals Ltd), Sodium starch glycolate (Loba chemicals), Dicalcium phosphate (Yarrow chemical products), Mumbai.

METHODS

Preparation of Standard Stock solution

10 mg of bosentan was accurately weighed and dissolved in 10 ml volumetric flask containing methanol. The volume was made up to 10 ml with the methanol to get concentration of (1 µg/ml).

Calibration curve in 7.4 Ph phosphate buffer

The present analytical method obeyed Beer's law in the concentration range 2-10µg/mL and is suitable for the estimation of Bosentan. The value of R^2 (correlation coefficient) for the linear regression equation was found to be 0.9968. The results are reported in Table.

Solubility studies

Solubility profiles studies were performed with different solvents like Distilled water, Ph 1.2 Buffer, Ph 4.6 Buffer, Ph 6.8 Buffer and Ph 7.4 Buffers in order to determine the aqueous medium that offer good solubility condition for bosentan. The solubility of bosentan was also determined in Ph 7.4 phosphate buffer more solubility compared to other solvents.

Preparation of complexation

Solvent Evaporation Method

Inclusion complexes of Bosentan were prepared by dissolving carriers β-Cyclodextrin and Bosentan at their corresponding ratio in common volatile solvent like methanol using a glass mortar. They were mixed by slight pressure for 15 min. Then the solvent was allowed to evaporate in hot air oven at 45 0C for 2h. The dried mass were passed through 100 # mesh and stored in desiccators at room temperature until further use. The complexes were made in different ratios with respect to drug and polymers.

Kneading Method

Bosentan and carrier β-Cyclodextrin were weighed according to their corresponding molar ratio. Bosentan and carrier were transferred to a mortar pestle. The mixture was reduced the size by continuous stirring with pestle. Water-methanol mixture (1:1 v/v) ratio was added to the above physical mixture and continuously stirred until the slurry mass was formed. Slurry mass was collected and dried in a hot air oven for 2 hrs at 50°C, dried mass was collected and further dried in desiccators over for 24 hrs. The dried mass were collected and passed through 100 # mesh, and packed it in a closed container. The complexes were made in different ratios with respect to drug and β-CDs as shown in Table.

EVALUATION OF FLOW PROPERTIES

Bulk density

It is the ratio of total mass of powder to the bulk volume of powder. It was measured by pouring the weighed powder into a measuring cylinder and the volume was noted. It is expressed in gm/mL and is given by,

$$D_b = M/V_0$$

Where, M is the mass of powder

V_0 is the Bulk volume of the powder.

Tapped density

Tapped density was determined by using graduated cylinder. An accurately weighed sample was carefully added to the graduated cylinder with the aid of funnel. The initial volume was noted and the sample was tapped on a horizontal base. Tapping was continued until no further reduction in sample volume was observed. Volume was noted and tapped density is calculated by using the following formula.

$$D_t = M/V_t$$

Where, M is the mass of powder

V_t is the Bulk volume of the powder.

Compressibility Index(CI) and Hausner's ratio (H)

In recent years the compressibility index and the closely related Hausner's ratio have become the simple, fast and popular methods of predicting powder flow characteristics. The compressibility index has been proposed as an indirect measure of the bulk density, size, shape, surface area, moisture content and cohesiveness of the materials. Both the compressibility index and the Hausner's ratio were determined by using bulk density and the tapped density of the powder.

$$\text{Carr's index} = \frac{\rho t - \rho b}{\rho t} \times 100 \quad --- \quad (6)$$

$$\text{Hausner's ratio} = \frac{\rho t}{\rho b} \quad --- \quad (7)$$

Angle of repose

The angle of repose has been used to characterize the flow properties of solids. Angle of repose is a characteristic related to inter particulate friction or resistance to movement

between particles. This is the maximum angle possible between surface of pile of powder or granules and horizontal plane. The angle of repose was determined by funnel method suggested by Newman.

Fixed funnel method was adopted for measuring the angle of repose. In this method, powder was passed through a funnel (8 cm diameter at top and 1.7cm diameter at efflux tube) that is fixed at predetermined height (2cm) and allowed to pass with or without shaking to get precise vibration. Following equation was used for the calculation of angle of repose value.

$$\text{Angle of repose} = \tan^{-1}\left(\frac{h}{r}\right)$$

Where 'r' is the radius of pile and 'h' is height of pile measured.

Table-20 lists flow characterization of powders based on values of CI, HR and Angle of repose.

Table-: Reference ranges to assess flow properties of powders.

S.No	Flow character	Compressibility index	Hausner's ratio	Angle of repose
1	Excellent	≤10	1.00-1.11	25-30
2	Good	11-15	1.12-1.18	31-35
3	Fair	16-20	1.19-1.25	36-40
4	Passable	21-25	1.26-1.34	41-45
5	Poor	26-31	1.35-1.45	46-55
6	Very poor	32-37	1.46-1.59	56-65
7	Very very poor	>38	>1.6	>66

Formulation and Evaluation of Bosentan Tablets

Formulation of Bosentan tablets

Tablets contain Bosentan (62.5mg) and inclusion complexes prepared by kneading method (equivalent to 62.5 mg of pure Bosentan) using beta cyclodextrins were formulated. The formulae of Bosentan tablets were given in Table**.** Direct compression method was used for the preparation of tablets. In this, Sodium Starch Glycolate (SSG) was used as super disintegrant, Dicalcium phosphate (DCP) was used as direct compressible vehicle, lactose used as diluent and talc, magnesium stearate as lubricant and glidants respectively.

Formulae for Tablets of Kneaded Complexes of Bosentan with Different Carriers.

Ingredients (mg/tablet)	Formulation Code		
	F1	F2	F3
Dispersion	203	203	203
Lactose	46	46	46
Magnesium stearate	3	3	3
Talc	3	3	3
MCC 101	30	-	-
Croscarmellose sodium	15	15	-
Dicalcium Phosphate	-	30	30
Sodium Starch Glycolate	-	-	15
TOTAL	300	300	300

IN-VITRO EVALUATION TESTS

Estimation of drug content

An accurately weighed quantity of Solid Dispersions equivalent to 10mg of bosentan, was taken into a 10 mL volumetric flask and dissolved in methanol and filtered through a Whatman No.1 filter paper (0.45µ). The filtrates were diluted suitably 7.4 phosphate buffer solution. The content of BOSENTAN was determined spectrophotometrically at 273 nm against suitable blank using UV-visible Spectrophotometer (UV-3000, LABINDIA).

Weight variation test

10 individual tablets of each formulation were weighed and their weights were recorded. Percent deviation from average weight was calculated for each formulation. The limits of deviation allowed as per IP were listed in table: Specifications for uniformity of weight of capsules.

Average weight	Percent deviation allowed
Less than 130mg	10
More than 130mg but less than 324mg	7.5
324mg or more	5

Hardness

The Hardness of the tablets was measured with a Monsanto hardness tester (M/s Campbell Electronics, model EIC-66, India). The results reported were average value with standard deviation of 10 tablets for each formulation.

Friability

For each formulation 10 tabs were weighed, placed in Friabilator (M/S Cambell Electronics, India) and were subjected to 100 rotations in 4 min. The tablets were reweighed and Friability was calculated along with mean and the standard deviation. The results are given in,

$$Friability = \frac{W_1 - W_2}{W_1} \times 100$$

Where "W_1" is the initial weight and "W_2" is the final weight of the tablets.

***In-vitro* disintegration test**

One tablet in to each tube was introduced and disc was added. The assembly was suspended in a beaker containing 1000mL of water and the apparatus was operated for 30 minutes. The time taken for complete disintegration of each tablet was noted. The tablets pass the test if all of them have disintegrated within the time (30 min).

***In-vitro* dissolution test**

The quantity of Inclusion complexes equivalent to 62.5 mg of Bosentan was placed in dissolution medium. The dissolution study of dispersion was conducted using dissolution testing apparatus II (paddle method) in 900 ml of 7.4pH phosphate buffer solution at 37±0.5°C and at speed of 50 rpm. Aliquots of 5 ml was withdrawn at predetermined time interval (0,5,10,15,20,30,45,60) and equivalent amount of fresh medium was replaced to maintain volume after each sampling and analyzed Spectrophotometrically at 273 nm against suitable blank using UV-visible Spectrophotometer (Elico SL150).

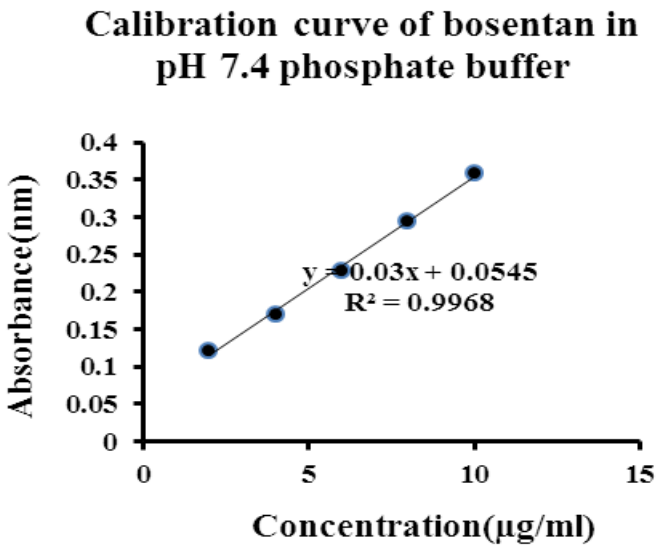
RESULTS

Calibration curve data of Bosentan.

SI No.	Concentration (µg/mL)	Absorbance (at 273 nm)
1	2	0.121
2	4	0.169
3	6	0.229
4	8	0.294
5	10	0.358

Solubility data of various Buffers.

S no	Solution	Concentration (mg/mL)
1	Distilled water	0.005
2	Ph 1.2 Buffer	0.042
3	Ph 4.6 Buffer	0.053
4	Ph 6.8 Buffer	0.065
5	Ph 7.4 Buffer	1.54



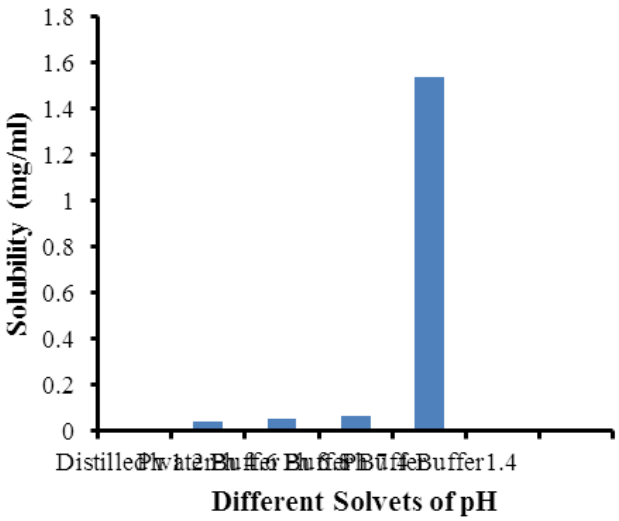


Table-: Flow properties of formulations.

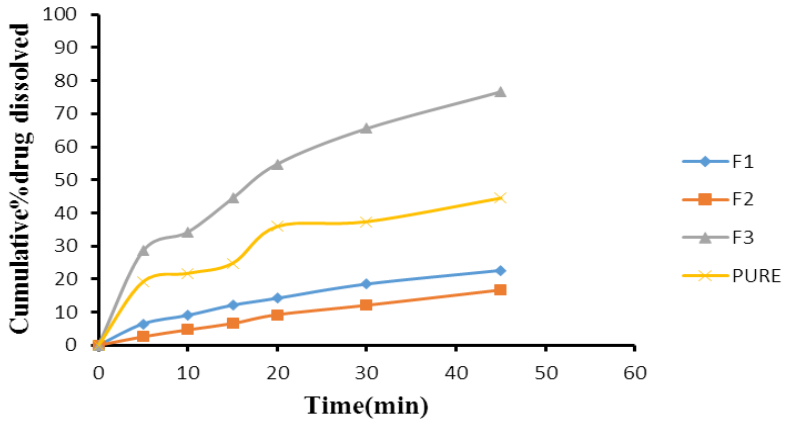
Formulations	Bulk density	Tapped density	Carr's index	Hausner's ratio	Angle of repose	Flow comment
F1 CSE 1:1	21±0.65	0.71 ±0.84	0.91±0.10	17.54	1.156	Good
F2 CSE 1:2	22±1.4	0.73 ±0.24	0.92±0.52	17.09	1.14	Good
F3 KM 1:1	25±3.5	0.82 ±0.56	0.94±0.21	9.59	1.042	Excellent
F4 KM 1:2	24±0.9	0.84 ±0.24	0.965±0.05	9.61	1.021	Excellent

Post compression parameters

Formulations	Thickness (mm)	Hardness (Kg/cm^2)	Weight variation (mg)	Percentage Drug Content (%)
F1	21±0.65	0.71 ±0.84	0.91±0.10	17.54
F2	22±1.4	0.73 ±0.24	0.92±0.52	17.09
F3	25±3.5	0.82 ±0.56	0.94±0.21	9.59

Invtro dissolution profile of formulae

Time (min)	Pure drug	F1	F2	F3
0	0	0	0	0
5	19.20 ±2.66	6.51±1.96	2.60±1.45	28.53±2.26
10	21.77 ±3.66	9.09±2.13	4.69±1.45	34.22±1.18
15	24.79 ±5.66	12.13±2.93	6.59±1.77	44.44±1.87
20	35.94 ±4.06	14.24±2.07	9.25±2.97	54.79±2.18
30	37.37 ±6.46	18.53±2.68	12.12±2.29	65.52±3.05
45	44.49 ±6.31	22.66±3.68	16.74±1.78	76.65±4.26
60	48.25 ±4.66	26.98±2.08	21.67±0.91	81.82±1.53



Comparative *In-vitro*dissolution profiles of Bosentan Inclusion Complexes [F1 (CS+MCC), F2 (CS+DCP), F3 (SSG+DCP)] and pure drug.

DISCUSSION

Calibration Curve of Bosentan

The calibration curve of Bosentan was obtained in the range of 2-10 µg/mL at the wave length of 273 nm. It has shown good linearity with a regression coefficient of 0.9968(r^2 value).

Solubility studies

In the present work the drug is added to Buffers like pH 1.2 HCL Buffer, pH 4.6 Acetate Buffer, pH 6.8 Phosphate buffer, pH 7.4 Phosphate Buffer and the following data was

obtained. The results of solubility studies revealed that Bosentan is more soluble in 7.4pH buffer solution. The order of solubility is 7.4 pH >DW>6.8 pH>4.6 pH> 1.2pH.

Characterization of Bosentan Inclusion complexes

Fourier Transform Infrared spectroscopy

Pure Bosentan

The FT-IR spectrum of Bosentan is shown in Figure**.** Important vibrations detected in the spectrum of Bosentan are attributed to the stretching of different bonds vibrations:3394.29 cm^{-1} is stretching of N-H bond, 1077.21cm^{-1} is stretching of C-O bond, 1555.67cm^{-1} is stretching vibrations of C=C bond,1203.08 cm^{-1} is stretching of C-N bond and 712.47 cm^{-1} is C-H stretching,1109.34cm^{-1} is stretching of S=O, 3666.75cm^{-1} is stretching of O-H bond. Therefore no reaction has observed between drug and carrier when compared to Pure drug and the data were given in Table.

Pre compression Parameters

The values for angle of repose were found to be in the range of 31.72° to 32.74º. Bulk densities and tapped densities of various formulations were found to be in the range of 0.47±0.001 to 0.49±0.001 (g/cc) and 0.56±0.006 to 0.59±0.004 (g/cc) respectively. Carr's Index of the prepared blends fall in the range of 15.38% to 18.45%. and Hauser's Ratio of the powder blends was in the range of 1.12 to 1.19. From the result it was concluded that the powder blends had good flow properties and compressibility, so these can be used for tablet manufacture. The values are given in Table**.**

Post compression Parameters

(a) Thickness

Thickness of the three tablets (Prepared tablets) of each batch were checked by using dial thickness gauge and dial Vernier calipers respectively. The data's are shown in Table**.** The results showed the Thickness of the tablets prepared by 1:1 (KM) Inclusion Complexes were in the range of 4.39±0.03 mm to 4.45±0.02respectively.

(b) Hardness test

Hardness of three tablets (Prepared tablets) of each batch was checked by using Monsanto hardness tester The results showed that the hardness of the tablets prepared by 1:1 (KM) of Inclusion Complexes were found to be in the range of 4.04±0.10 to 4.70±0.08 Kg/cm^2.

(c) Weight variation test

Tablets of each batch (Prepared tablets) were subjected to Weight variation test, difference in weight and percent deviation was calculated for each tablet, shown in the Table. The average weight of the tablet is approximately 300mg; so the permissible limit is ±5. The results of the test showed that, the tablet weights were within Pharmacoepial limit.

(d) Drug content uniformity

Drug content uniformity study data is shown in the table**.** The content uniformity for all the formulations prepared by using of 1:1 (KM) of Inclusion Complexes were found to be in range of 98.65±1.65% to 99.45±1.32% which showed that there was uniform distribution of the drug in tablets of all formulations.

(e) Friability

Tablets of each batch prepared by using of 1:1 (KM) of Inclusion Complexes were evaluated for percentage friability and the data is shown in the Table**.** The test for Friability of all the tablets formulation lies in the range of 0.765% to 0.931% (less than 1%) indicating good mechanical strength of tablets.

(f) *In-vitro* disintegration time

Tablets of each batch were evaluated for *In-vitro* disintegration time. The formulation F1 contain Beta cyclodextrin (KM 1:1) had shown maximum disintegration time of 28±2.9 min. The average disintegration time of all the formulations prepared by using of 1:1 (KM) of Croscarmellose was obtained in the range of 19±3.5 to 28±2.9 min and by using Sodium Starch Glycolate 07±1.3 min was obtained as shown in the **Table.**

(h) *In-vitro* dissolution studies

Tablets contain Bosentan (62.5mg) and inclusion complexes prepared by kneading method (equivalent to 62.5 mg of pure Bosentan) using beta cyclodextrins were formulated. The formulae of Bosentan tablets were given in table. Direct compression method was used for the preparation of tablets. In this, Sodium Starch Glycolate (SSG) was used as super disintegrant, Dicalcium phosphate (DCP) was used as direct compressible vehicle, lactose used as diluent and talc, magnesium stearate as lubricant and glidants respectively.

The release of Bosentan from β-CD dispersion (KM1:1) (F1, F2, F3) based IR tablets was determined using USP paddle type Dissolution Tester at 50 rpm. Dissolution was examined

using 900 mL of 7.4pH phosphate buffer. The temperature was maintained at 37 ± 0.5°C. Samples each containing 5 mL were withdrawn at 5, 10, 15, 20, 30, 45 and 60 min intervals, filtered through a Whatman filter of 0.45 μm and replaced with an equal amount of fresh dissolution medium to maintain sink condition. Samples were then suitably diluted and analyzed spectrophotometrically at 273 nm. The dissolution studies were conducted in triplicate**.** The dissolution profiles were evaluated for amount of drug released in initial 15 min and time taken to release 50% of the drug (T 50).

Finally, the tablets were evaluated for *In-vitro* dissolution studies in 7.4pHbuffer Solution. The average dissolution study data of all the formulations were shown in the Table**.** Among all the formulations F3 prepared with β-CD dispersion (KM 1:1) and Sodium Starch Glycolate showed about 81.82±1.53% i.e. highest drug release within 60 min when compared to other formulations (F1 and F2) because Sodium starch glycolate absorbs water rapidly, resulting in swelling which leads to rapid disintegration of tablets. Pure drug showed drug release about 91.62±0.92% within 60 min. Formulation F3 is having superior drug release properties. Hence F3 is optimized choice of formulation out of all formulations.

SUMMARY AND CONCLUSION

The enhancement of dissolution rate and oral bioavailability of poorly soluble drugs remains one of the most challenging aspects of drug development. Among the various methods of enhancement of the dissolution rate and oral bioavailability. Inclusion Complexes technologies were found to be more successful with a number of drugs. In the present investigation studies were carried out on enhancement of dissolution rate of Bosentan by Inclusion Complexes technology (Kneading method) employing β Cyclodextrins as carriers.

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