



in silico and Physico-Chemical Assessment of Effectiveness of Hydroxy/Amino Acids as Auxiliary Substances to Improve the Complexation Efficiency of β -Cyclodextrin Towards Bosentan

YOGESH V. PORE and PRIYANKA H. JADHAV*

Department of Pharmaceutical Chemistry, Government College of Pharmacy, Karad-415 124, India

*Corresponding author: Fax: +91 2164 271196; E-mail: priyagcopk90@gmail.com

Received: 7 June 2017;

Accepted: 5 August 2017;

Published online: 29 September 2017;

AJC-18585

The effectiveness of certain hydroxy and amino acids as an auxiliary substance to improve the complexation efficiency of β -cyclodextrin towards bosentan, a poorly soluble endothelin receptor antagonist, were assessed to enhance its physico-chemical performance. The phase solubility studies conducted in distilled water demonstrated a better choice of L-arginine as an auxiliary substance to improve complexation efficiency and association constant (K_s) of β -cyclodextrin. As a complementary evidence, *in silico* calculations were performed to foresee the stability, possible interactions and geometry of bosentan monohydrate inside β -cyclodextrin cavity, optimizing L-arginine again as an auxiliary substance. Subsequently, the solution state thermodynamic investigations revealed entropy driven complexation process. The lyophilized complexes were characterized by FTIR, DSC, XRPD and SEM. The performance of ternary complex was found to be appreciating in overall assessment, indicating better effectiveness of L-arginine as an auxiliary substance in improving complexation efficiency of β -cyclodextrin towards bosentan monohydrate.

Keywords: Bosentan, β -Cyclodextrin, Hydroxy acids, Amino acids, Lyophilization, *in silico*.

INTRODUCTION

Bosentan monohydrate (BOS), 4-*tert*-butyl-N-[6-(2-hydroxyethoxy)-5-(2-methoxyphenoxy)-2-(pyrimidin-2-yl)pyrimidin-4-yl]benzene-1-sulfonamide (Fig. 1) is a white crystalline powder acting as non-peptide antagonist of human endothelial-I receptor [1]. It is mainly recommended in the treatment of pulmonary arterial hypertension [2]. Basically, it has been classified as a BCS class-II drug, thus, exhibiting poor aqueous solubility, resulting in low bioavailability [3]. Inadequate oral bioavailability often limits desired therapeutic outcome and patient compliance. Therefore, improving both physico-chemical and biopharmaceutical characteristics of BCS class II drugs has always attracted the attention of most of the researchers to improve the therapeutic efficacy of such molecules.

Inclusion complexation with β -cyclodextrins (β -CDs) is an industrially feasible approach to modify the physico-chemical properties of poorly water soluble drugs [4]. β -cyclodextrins act as a powerful complexing and solubilizing agent resulting in an improvement in dissolution, bioavailability and stability of a drug molecule without affecting its pharmacological properties and hence is more suitable for oral drug delivery [5,6]. Despite the wide applications, unfortunately, β -cyclodextrin suffers from lower complexation efficiency, allowing its large amount

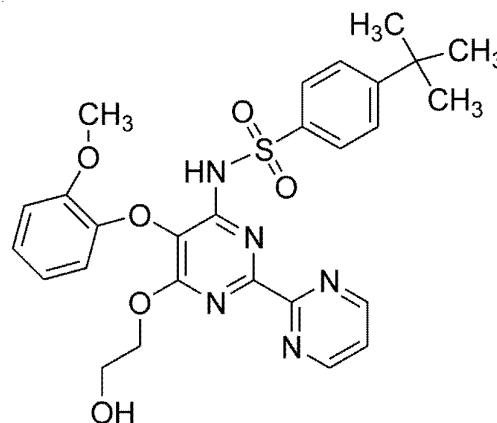


Fig. 1. Chemical structure of bosentan

to be incorporated during formulation, thus, adversely affecting its practical usefulness [7,8]. However, the complexation efficiency can be improved by the use of certain water-soluble auxiliary substances such as hydrophilic polymers [7,8] hydroxy acids [9] and/or amino acids [10-13] to the complexation media. Therefore, it was decided to investigate the effect of various auxiliary substances, theoretically as well as practically, on the complexation efficiency of β -cyclodextrin towards bosentan monohydrate.

In continuation of previous work [14], the present investigation was aimed to describe the assessment of *in silico* and physico-chemical effect of ternary auxiliary substances such as citric acid (CA), tartaric acids (TA) and L-arginine (Arg) on complexation efficiency of β -cyclodextrin towards bosentan monohydrate. The optimization of ternary component was based on phase solubility, thermodynamic and molecular modeling studies. Phase solubility studies were performed for analyzing the solubility of bosentan monohydrate in aqueous solution of β -cyclodextrin in the presence and/or absence of ternary components and for calculating corresponding thermodynamic parameters involved in inclusion complexation. Molecular modeling studies were carried out to predict the possible orientations of pure bosentan monohydrate inside the β -cyclodextrin cavity and for the optimization of possible stoichiometry, geometry and stability of inclusion complexes [15]. The solid state inclusion complexes were prepared by lyophilization technique and characterized by ultra-violet spectroscopy (UV), Fourier transformation-infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), X-ray powder diffractometry (XRPD), scanning electron microscopy (SEM), saturation solubility and partition coefficient (log P) determination. All the samples, including pure drug were further examined for dissolution profiles in 1 % sodium lauryl sulfate (SLS). The protein binding assay was carried out with the help of dialysis membrane.

EXPERIMENTAL

Bosentan monohydrate was kindly gifted by Sun Pharmaceutical Industries Ltd (Vadodara, India). β -Cyclodextrin (Molecular weight: 1135 g/mol, Purity 98 %) was procured from Himedia laboratories Pvt. Ltd (Mumbai, India). L-Arginine (Arg, Purity 99 %), citric acid (CA, Purity 98 %), tartaric acid (TA, Purity 98 %) and bovine albumin (Purity 98 %) were purchased from Loba Chemie Pvt. Ltd. (Mumbai, India). Analytical grade reagents and glass distilled water were used for all experimental procedures. All the chemicals were used without any further purification.

Phase solubility studies: The phase solubility studies in distilled water at 298 ± 2 K, were performed in triplicate according to the method described by Higuchi and Connors [16]. An excessive amount of pure bosentan monohydrate was added to 10 mL of aqueous solution containing different concentrations of β -cyclodextrin (0-0.005 M) with or without the addition of auxiliary substances such as citric acid (0.25 % w/v), tartaric acid (0.25 % w/v) and L-arginine (0.25 % w/v). The obtained suspensions were mechanically shaken on an incubator shaker (REMI-CIS 24 plus Incubator Shaker, Mumbai, India) for 72 h at 150 rpm. After achieving the equilibration, the samples were withdrawn and filtered through Whatman filter paper no. 41 and appropriately diluted if necessary. The concentrations of bosentan monohydrate were determined spectrophotometrically (Shimadzu UV-visible spectrophotometer 1800, Japan) at 271 nm. The apparent binding constant (K_s) and complexation efficiency of inclusion complexes were resulted using following equations [17].

$$K_s = \text{slope}/S_0 (1 - \text{Slope}) \quad (1)$$

S_0 is the solubility of bosentan monohydrate without β -cyclodextrins and slope is obtained from phase solubility diagram

prepared by plotting moles of bosentan monohydrate on Y axis and moles of β -cyclodextrin on X axis.

Complexation efficiency (CE) = $D_0 \cdot K_{1:1}$ = Slope/(1-Slope) (2)
 D_0 is the intrinsic solubility of the drug.

In silico studies: *In silico* studies were conducted using VLifeMDS 4.3 software (VLife Sciences and Technologies, Pune, India) on Intel i3 CORE processor with an operating system of Windows 7. The molecular structure of bosentan monohydrate, complexation efficiency, citric acid, tartaric acid and L-arginine were drawn on the workspace of VLife engine module. All structures were allowed for systematic conformational analysis with rms gradient 0.01 Kcal/mol and dielectric constant 1. The most stabilized bosentan monohydrate conformer was placed in complexation efficiency cavity with or without auxiliary substances at different orientations and reoptimized. The respective complexation energy (ΔE) was calculated by using the following equation:

$$\Delta E = E_{\text{IS:D}} - E_{\text{IS}} + E_{\text{ICD}} + E_{\text{fAx}} \quad (3)$$

where, $\Delta E_{\text{IS:D}}$ is a total energy of the complex after optimization, ΔE_{IS} is a total energy of the guest molecule after optimization, ΔE_{ICD} is a total energy of host molecule after optimization, ΔE_{fAx} is a total energy of the auxiliary molecule after optimization.

Thermodynamic studies: The phase solubility data and *in silico* calculations generated interesting evidences experimentally and theoretically, respectively, to support the better choice of L-arginine as an appropriate auxiliary substance effectively and continued further work by considering L-arginine to investigate thermodynamics of the complexation.

The phase solubility studies at different temperatures 298 ± 2 K, 310 ± 2 K, 315 ± 2 K was carried out for demonstrating different thermodynamic parameters in solution state. The integrated form of the van't Hoff equation is useful for the calculation of the enthalpy (ΔH) and of entropy (ΔS), depending on the alteration in stability constants with temperatures [18].

$$\ln K = -\Delta H/RT + \Delta S/R \quad (4)$$

where, ΔH is enthalpy of the complex, ΔS is the entropy of the complex, R is the gas constant, T is temperature.

An indication of the process of transfer of drug from pure water to an aqueous solution of β -cyclodextrin was determined from the values of Gibbs free energy of transfer (ΔG), which can be calculated by following equation [15]:

$$\Delta G_{\text{tr}}^0 = -2.303RT \log (S_0/S_0) \quad (5)$$

where, S_0/S_0 is the molar solubility of pure drug in aqueous solution of β -cyclodextrin in the presence or in the absence of L-arginine (0.25 % w/v) to that in distilled water without β -cyclodextrin.

Preparation of solid inclusion complexes: Equimolar quantities of pure drug and β -cyclodextrin with or without L-arginine (0.25 %, w/v) were dissolved in 15 mL of methanol and 85 mL of distilled water, respectively. These solutions were allowed for sonification for 15 min. The solutions were shifted to conical flask and stirred for 72 h at room temperature (25 ± 2) with 150 rpm by using a magnetic stirrer (REMI 2MLH, India). The obtained solutions were filtered and allowed for deep freezing in a deep freezer (ELCOLD, Denmark) at -80 °C for 24 h. After deep freezing the resultant solutions were freeze

dried (DELVAC-mini Lyodel, Chennai, India) at -80°C for 4 days. The lyophilized complexes were collected and stored in desiccators.

DSC: The thermal properties of pure drug and the complexes were examined using DSC analyzer (TA Instruments, SDT Q600 USA) under a nitrogen atmosphere (flow rate 100 mL/min) at a heating rate of $10^{\circ}\text{C}/\text{min}$ over the temperature range of 0 – 200°C .

FTIR: Attenuated total reflectance (ATR) is a sampling technique which is useful for both solid and liquid samples directly without any further preparations. Infrared spectra were obtained by using ATR (BRUKER-ECO-ATR-ALPHA, Germany) instrument. The samples were directly analyzed from 4000 – 600 cm^{-1} spectral range for 24 scans.

XRPD: The XRPD analysis of all the samples were performed using X-ray diffractometer (BRUKER- D_2 PHA-SER, Germany) with tube anode Cu over the interval 10 – $90^{\circ}/2$. The operational data were as follows: Generator tension (voltage) 30 kV, Generator current 10 mA.

SEM: The surface morphology of all samples was studied using scanning electron microscope operated at an acceleration voltage of 20 Kv. All samples were directly mounted on the aluminum stub and coated with a thin gold-ion layer by sputter coated unit (SEM-JOEL Instruments, JSM-6360, Japan).

Percentage drug content study: Prepared inclusion complexes equivalent to 5 mg of bosentan monohydrate were dissolved in 10 mL of methanol and the volume was adjusted up to 50 mL with distilled water. The final solution was filtered through Whatman filter paper no. 41, appropriately diluted if necessary and analyzed spectrophotometrically at 271 nm (Shimadzu UV-visible Spectrophotometer 1800, Japan).

Saturation solubility studies: The saturation solubility of all samples was determined as follows: Excess amount of bosentan monohydrate and prepared inclusion complexes were added to 10 mL of distilled water in solubility tubes and allowed for shaking on an incubator shaker (REMI-CIS 24 Plus Incubator Shaker, Mumbai, India) for 24 h at 150 rpm. After equilibration, samples were withdrawn, filtered with Whatman filter paper no. 41 and diluted with distilled water if required and analyzed. The amount of drug solubilized was analyzed by a UV spectrophotometer (Shimadzu UV-visible spectrophotometer 1800, Japan) at 271 nm. The observed data statistically analyzed by ANOVA (Instat, GraphPad software, Inc. Version 3.05).

In vitro dissolution studies: The dissolution studies of samples were conducted on dissolution test apparatus (ELECTROLAB-TST-06L/LX, New Mumbai, India) according to USP type II and US FDA guidelines (US FDA website) 62.5 mg of bosentan monohydrate or its equivalent amount of complexes were added in dissolution vessel containing 900 mL of 1 % SLS, maintained at $37 \pm 0.5^{\circ}\text{C}$ at 50 rpm [3]. 5 mL of samples were withdrawn at appropriate time intervals. The volume of dissolution media was adjusted to 900 mL by replacing each 5 mL aliquot withdrawn with 5 mL of fresh 1 % SLS. The solution was filtered and diluted if necessary and analyzed spectrophotometrically at 221.5 nm (Shimadzu UV-visible spectrophotometer 1800, Japan). The data obtained through dissolution studies were statistically analyzed by ANOVA (Instat, GraphPad software, Inc. Version 3.05).

Partition coefficient determination (log P): The partition coefficient was determined by adding excess amount (equivalent to 15 mg) of samples to the glass tubes containing 10 mL each of octanol and water, previously allowed to stand overnight for 24 h at room temperature. These tubes were shaken on an incubator shaker (REMI-CIS 24 Plus Incubator shaker, Mumbai, India) for 24 h at 25°C . After equilibration, solutions were transferred to the separating funnel and allowed to stand for 6 h. Both layers were separated and the aqueous layer was analyzed spectrophotometrically (Shimadzu 1800, Japan) at 271 nm [19]. log P value calculated by using the following equation:

$$\log P = \log (C_o/C_w) \quad (6)$$

C_o is the concentration of the drug in octanol and C_w is the concentration of drug in the water.

Protein binding assay: 62.5 mg of bosentan monohydrate or its equivalent amount of prepared complexes were added in a beaker containing 100 mL of phosphate buffer saline (PBS, pH 7.4). The dialysis membrane was pretreated with PBS and then it was tied at opening end of the test tube containing 6 % bovine albumin. The tied dialysis membrane was dipped in to a drug solution and total assembly was shifted on magnetic stirrer and switched at minimum rpm with temperature $37 \pm 2^{\circ}\text{C}$. After 1 h 5 mL of PBS containing drug sample was replaced with fresh PBS. Then the sample was filtered by Whatman filter paper no. 41, adequately diluted and estimated by spectrophotometrically (Shimadzu 1800, Japan) at 223.5 nm [20].

RESULTS AND DISCUSSION

Phase solubility studies: Fig. 2 shows the phase solubility curves of bosentan monohydrate in presence and/or in absence of all ternary auxiliary substances. The solubility of pure drug increased with increasing the concentration of β -cyclodextrin, indicating A_L type of phase solubility curve with remarkable enhancement in aqueous solubility of bosentan monohydrate [9,11]. The possible stoichiometries for all systems were found to be 1:1. The phase solubility parameters are displayed in Table-1 suggesting positive effect of addition of ternary substances in to the complexation media where, arginine has shown greater

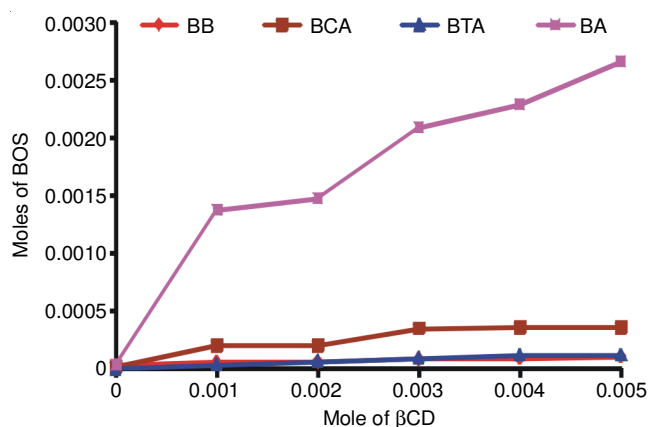


Fig. 2. Phase solubility diagram of BOS: β CD inclusion complexes in water at $298 \pm 2\text{ K}$. **BB:** β CD + BOS; **BCA:** β CD + BOS + CA; **BTA:** β CD + BOS + TA; **BA:** β CD + BOS + ARG; where BOS: bosentan; β CD: β -cyclodextrin; CA: citric acid; TA: tartaric acid; ARG: L-arginine

TABLE-1
PHASE SOLUBILITY DATA OF BINARY AND TERNARY INCLUSION
COMPLEXES OF PURE DRUG WITH β -CYCLODEXTRIN AT 25 °C

Systems	Slope	S_0	r^2	K_s (M^{-1})	K_{TS}/K_{BS}	CE
BB	0.013	0.000037	0.968	393 ± 27	—	0.0137
BCA	0.067	0.000080	0.842	888 ± 12	2.25	0.2718
BTA	0.024	0.000020	0.952	1203 ± 11	3.06	0.0245
BA	0.471	0.000477	0.894	1866 ± 10	4.74	0.8900

BB: β CD + BOS; BCA: β CD + BOS + CA; BTA: β CD + BOS + TA; BA: β CD + BOS + ARG; BOS: bosentan; β CD: β -cyclodextrin; CA: citric acid; TA: tartaric acid; ARG: L-arginine; S_0 : solubility of BOS in absence of β CD; r^2 : regression coefficient of phase solubility graph; K_s (M^{-1}): association constant of complex; K_{TS}/K_{BS} -Ratio of K_s for ternary and binary system; CE: complexation efficiency.

effectiveness as a ternary substance than others in terms of K_s and complexation efficiency [15,21].

in silico Studies: *in silico* Studies were performed for the prediction of most stabilized structure and to check out the involvement of certain non-bonded forces of interactions during complexation process. Table-2 shows data regarding complexation energies and different forces of interactions of all systems whereas Fig. 3 displayed all relevant images which are complementary to the Table-2. Various forces are involved in driving the complexation process such as hydrophobic interactions, van der Waals force of interaction, the hydrogen bond formation [17,22]. Thus it could be predicted that the ternary system with arginine was the most stable system with

TABLE-2 <i>in silico</i> DATA OF BINARY AND TERNARY SYSTEMS				
Systems	ΔE	Vdw	Elctr	OH bonds
BB	-76	167	0.00	01
BCA	-79	240.10	-67.49	08
BTA	-306	315.80	143.54	12
BA	-653	428.29	-519.65	09

BB: β CD + BOS; BCA: β CD + BOS + CA; BTA: β CD + BOS + TA; BA: β CD + BOS + ARG; BOS: bosentan; β CD: β -cyclodextrin; CA: citric acid; TA: tartaric acid; ARG: L-arginine; ΔE : Complexation energy (Kcal/mol); Vdw: van der Waals force of interactions; Elctr: Electrostatic force of interactions; OH bonds: number of hydrogen bonds.

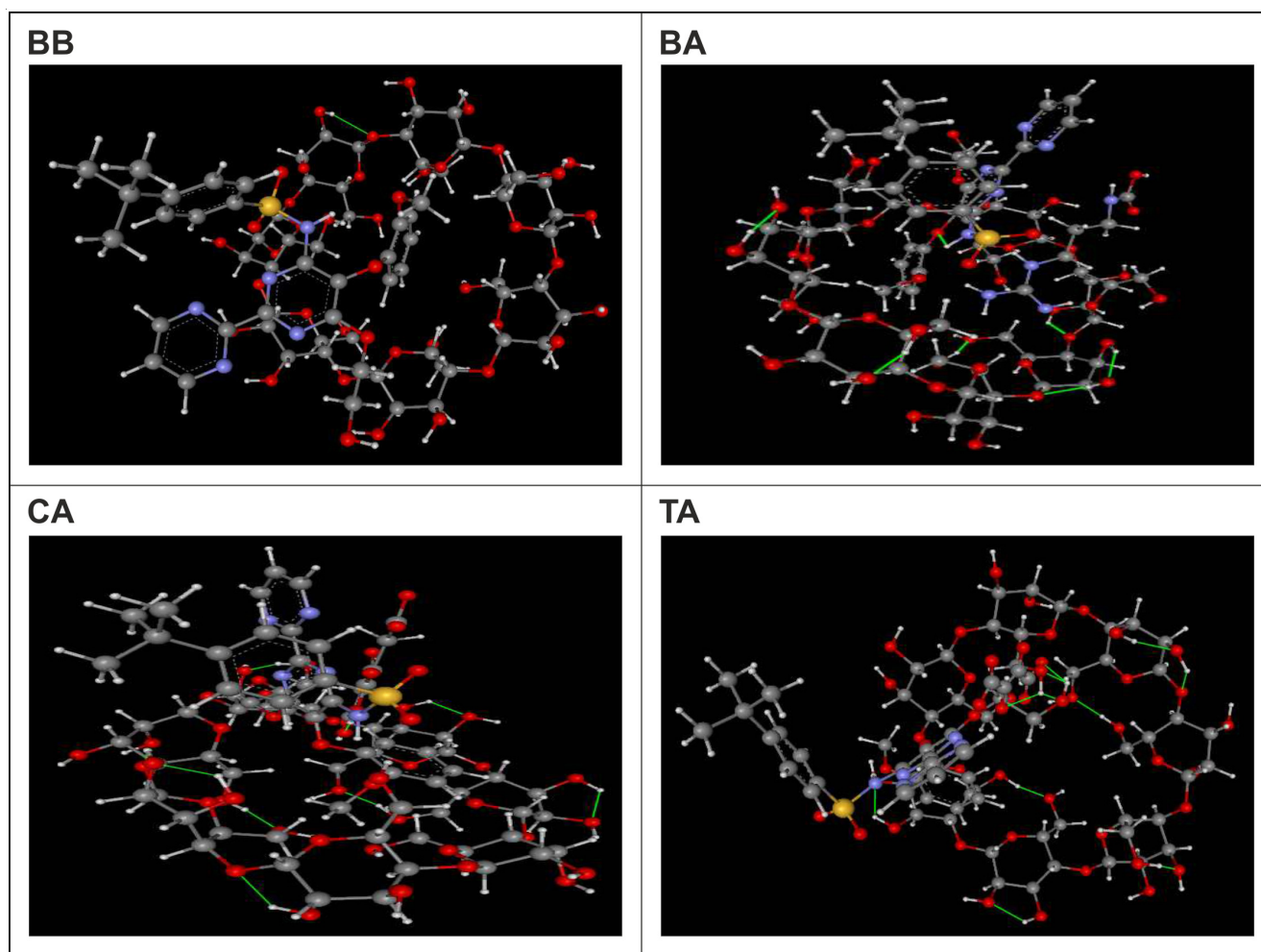


Fig. 3A. Optimized geometric models of **BB**: β CD + BOS; **CA**: β CD + BOS + CA; **TA**: β CD + BOS + TA; **BA**: β CD + BOS + ARG; where BOS: bosentan; β CD: β -cyclodextrin; CA: citric acid; TA: tartaric acid; ARG: L-arginine

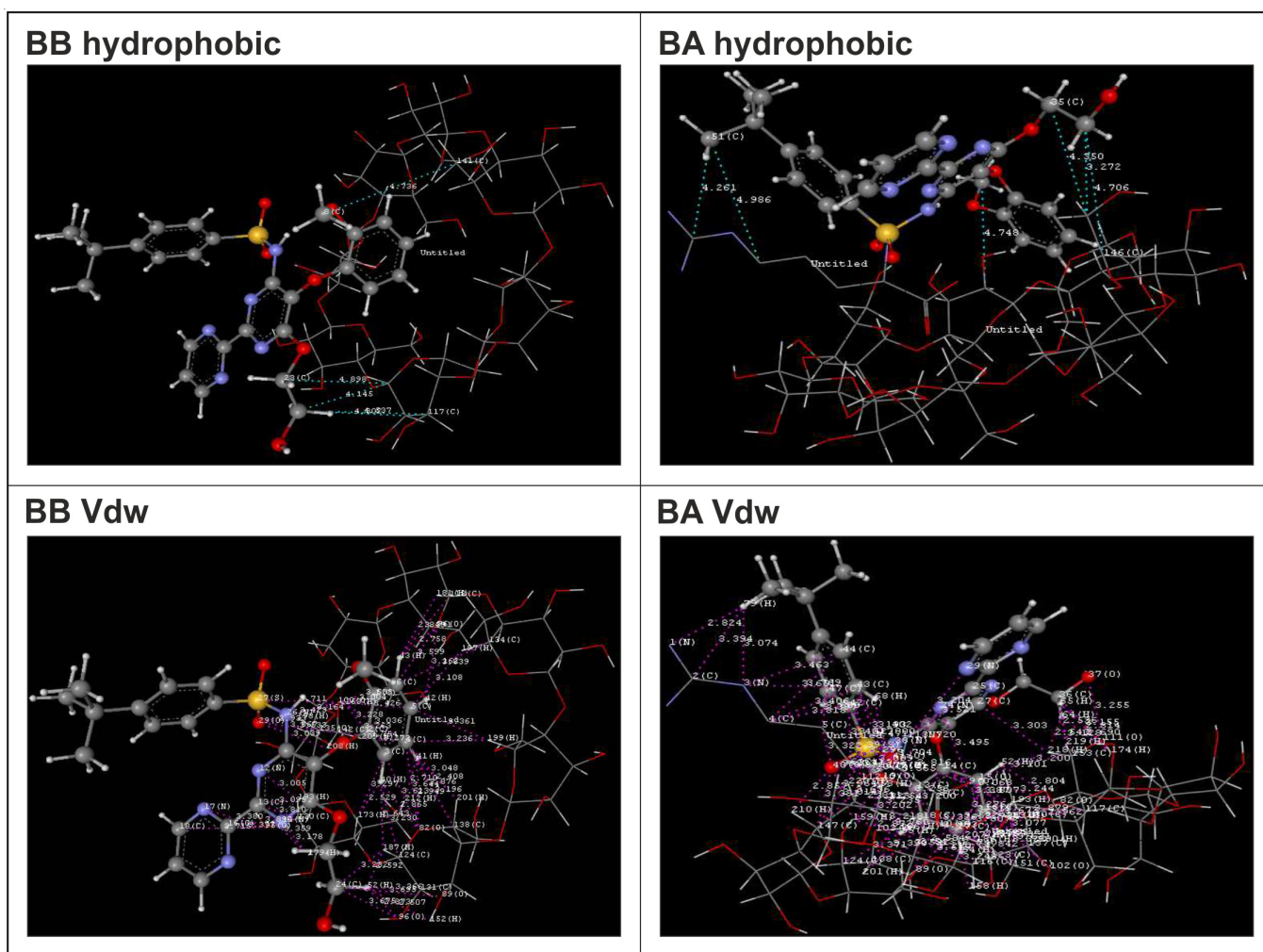


Fig. 3B. Hydrophobic and Van der Waals interactions of BOS with β CD in presence and absence of ARG. **BB**: β CD + BOS and **BA**: β CD + BOS + ARG; where BOS: bosentan; β CD: β -cyclodextrin; ARG: L-arginine; Vdw: van der Waals

lowest complexation energy and formation of 09 hydrogen bonds.

Thermodynamic studies: Fig. 4 shows van't Hoff's plot described as the function of $\log$ of K_s and inverse of absolute temperature whereas Table-3 shows different thermodynamic parameters involved in inclusion phenomenon. Accordingly, it was evident that there was decrease in the association constant with rise in the temperature. The value of enthalpy (ΔH) for binary and ternary complexes was observed to be positive indicating endothermic complexation process. Further, ΔH value for ternary system was found to be lower than that of binary system showing more favourable complexation than binary system. The entropy (ΔS) values were found to be positive showing highly disordered state of complexes and complexation process was without extensive desolvation.

Standard Gibbs free energy change (ΔG) given by the change in enthalpy and entropy (Table-3) indicated spontaneous complexation process in aqueous solution. For the concentration of β -cyclodextrin, 0.001, 0.002, 0.003, 0.004, 0.005, the values of ΔG_{tr}° (KJ/mol) for binary and ternary complexes were found to be: -1255, -1456, -2329, -2470, -2827 and -9216, -9377, -10227, -10456, -10827, respectively. The negative values of ΔG_{tr}° gave an idea about the spontaneity of pure drug solubilization and also indicated that complexation process became more favou-

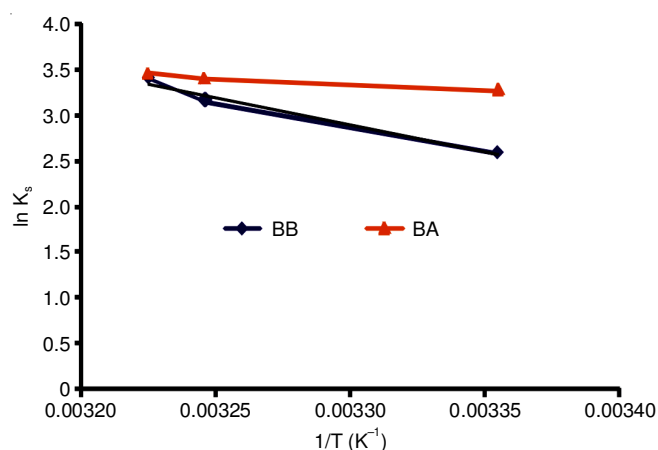


Fig. 4. van't Hoff plots ($\ln K$ vs. $1/T$) for BOS: β CD inclusion complexes, determined by solubility diagrams experiments. **BB**: β CD + BOS; **BA**: β CD + BOS + ARG; where BOS: bosentan; β CD: β -cyclodextrin; ARG: L-arginine

able towards increasing the concentration of cyclodextrin [15,23].

DSC: DSC has become a powerful tool to investigate the interactions between host and guest. When hydrophobic molecules are incorporated in β -cyclodextrin hydrophobic cavity,

TABLE-3
THERMODYNAMIC PARAMETERS INVOLVED IN INCLUSION COMPLEXATION

Systems	Temp. (K)	Slope	S_0	r^2	$K_s (M^{-1})$	ΔH (KJ/mol)	ΔS (J/mol/K)	ΔG (KJ/mol)
BB	298	0.013	0.000037	0.968	380 ± 5	114	412	-122662
	308	0.334	0.000350	0.902	1435 ± 15			
	310	0.077	0.000030	0.914	2522 ± 10			
BA	298	0.471	0.000477	0.894	1866 ± 10	25	147	-43781
	308	0.468	0.000355	0.901	2481 ± 7			
	310	0.155	0.000063	0.974	2870 ± 4			

BB: β CD + BOS; BA: β CD + BOS + ARG; BOS: bosentan; β CD: β -cyclodextrin; ARG: L-arginine; S_0 : solubility of BOS in absence of β CD; r^2 : regression coefficient of phase solubility graph; $K_s (M^{-1})$: association constant of complex; ΔH -Enthalpy, ΔS -Entropy, ΔG -Gibbs free energy.

their melting points shifts to different temperatures or disappears [24]. DSC thermograms of all systems are shown in Fig. 5.

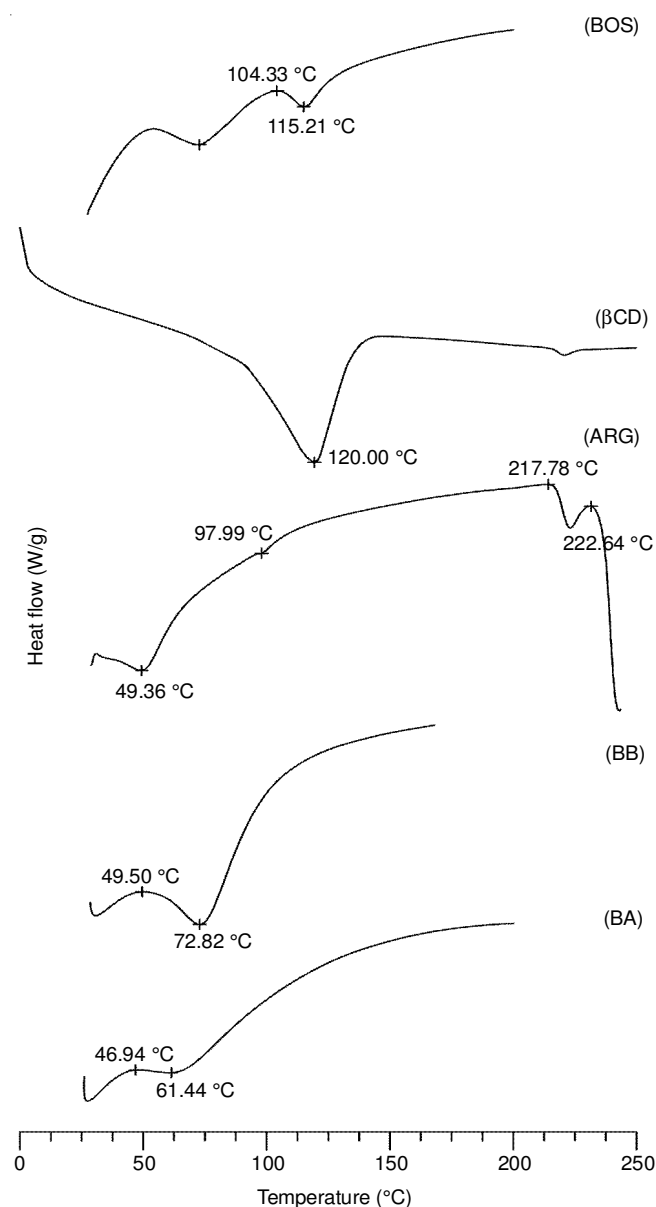


Fig. 5. DSC thermograms of BOS, β CD, ARG, **BB**: β CD + BOS and **BA**: β CD + BOS + ARG; where BOS: bosentan; β CD: β -cyclodextrin; ARG: L-arginine

The crystalline nature of bosentan monohydrate and β CD was clearly visible from their respective thermograms with sharp melting endotherm at 115 and 120 °C, respectively. DSC

thermogram of L-arginine indicated broad endothermic peaks at 49.36 °C, 99.97 °C and sharp endothermic peak at 222.64 °C due to dehydration of L-arginine and crystalline nature of it respectively [14,21]. In case of binary and ternary complexes, disappearance of bosentan monohydrate melting peaks were observed indicating the entrapment of bosentan monohydrate inside the β -cyclodextrin cavity confirming formation of inclusion complexes [25].

FTIR: Fig. 6 displayed IR patterns of all systems. IR spectra of bosentan monohydrate was characterized by IR bands at 3618.42 cm^{-1} (O-H, stretching, OH), 3416.53 cm^{-1} (N-H, stretching), 2953.77 cm^{-1} (C-H stretching aliphatic), 1582.63 cm^{-1} (C-C stretch, aromatic), 1383.58 cm^{-1} and 1170.09 cm^{-1} (S-O stretch, sulphonamide), 1019.55 cm^{-1} (C-O-C, bending). The

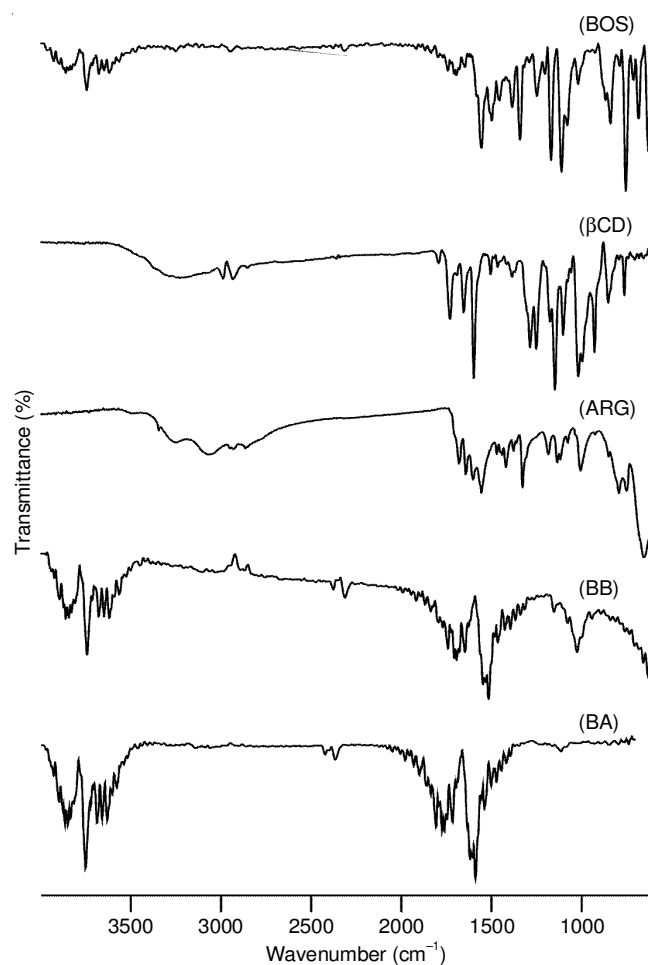


Fig. 6. FTIR spectra of BOS, β CD, ARG, **BB**: β CD + BOS and **BA**: β CD + BOS + ARG; where BOS: bosentan; β CD: β -cyclodextrin; ARG: L-arginine

absorption spectra of β -cyclodextrin had shown prominent peaks at 3225.05 (O-H, stretching, OH), 2933.80 (C-H stretching aliphatic), 1653.83 (H-O-H, bending), 1017.77 (C-O-C, stretching). L-arginine indicating peaks at 3376.72 cm^{-1} and 3338.41 cm^{-1} (broad OH carboxylic or NH guanidine due to intra-molecular hydrogen bonding), 2854.09 cm^{-1} (C-H, stretch), 1680.41 cm^{-1} (C-O, amide), 1336.86 cm^{-1} (C-N stretch) and 1680.41 cm^{-1} (C=O carbonyl COOH).

In case of binary and ternary complexes, the principle absorption peaks of pure drug at 3416.53 cm^{-1} , 2953.77 cm^{-1} , 1383.58 cm^{-1} , 1170.09 cm^{-1} were completely disappeared and some peaks at 3618.42 cm^{-1} , 1582.63 cm^{-1} , 1019.55 cm^{-1} were shifted to 3743.51 cm^{-1} , 1546.68 cm^{-1} , 1025.10 cm^{-1} and 3873.03 cm^{-1} , 1546.87 cm^{-1} , 1100.01 cm^{-1} , respectively. In both the cases the peaks of β -cyclodextrin at 1693.83 cm^{-1} , 928.18 cm^{-1} were shifted to 1693.32 cm^{-1} , 706.42 cm^{-1} and 1693.58 cm^{-1} , 709.9 cm^{-1} , respectively. Further, in complexes, all peaks were smoothened and did not show any new peak indicating non bonded interaction in inclusion complexes [7].

XRPD: The physical state of the drug and complexes could be assigned by examining their respective diffractograms. Fig. 7 presented XRPD patterns of all the samples. The diffractogram of pure bosentan monohydrate, β -cyclodextrin and L-arginine

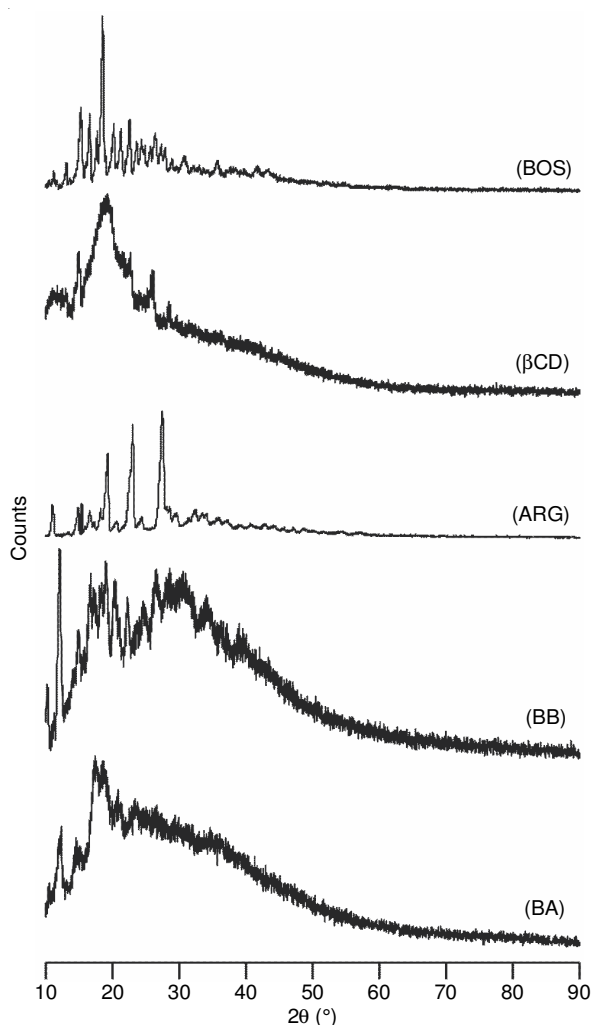


Fig. 7. XRPD patterns of BOS, β CD, ARG, BB: β CD + BOS; BA: β CD + BOS + ARG; where BOS: bosentan; β CD: β -cyclodextrin; ARG: L-arginine

shown peaks at 25.0, 25.1, 25.2, 25.3, 25.4, 25.5 (2 θ) with peak intensities of 604, 624, 599, 601, 665, 772 and 735, 763, 769, 820, 743, 836 and 499, 473, 451, 433, 461, 444 respectively revealing their crystalline nature. The peak intensities of binary and ternary systems were found to be 470, 494, 301, 423, 444, 440 and 605, 641, 580, 606, 573, 629, respectively indicating diffused peak intensities and traces of some crystallinity of β -cyclodextrin and L-arginine. It could be suggested that the inclusion complexes were still exhibiting crystalline nature with a significant reduction in the crystallinity.

SEM: The morphological characteristics of pure drug and its corresponding complexes are shown in Fig. 8. Pure bosentan monohydrate appeared as uneven, oval and rough particles whereas inclusion complexes could be seen with altered shaped, smooth, crystalline particles indicating significant changes in the original morphology of pure drug. The complexes exhibited altered size and shape conforming the presence of new solid phase which might have contributed for modification of physico-chemical properties of drug to certain extent.

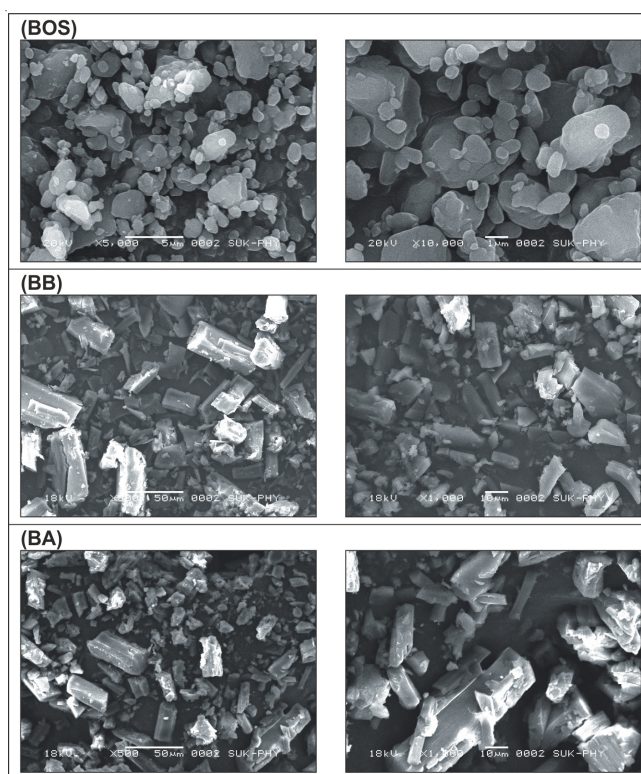


Fig. 8. SEM of BOS and complexes; BB: β CD + BOS; BA: β CD + BOS + ARG; where BOS: bosentan; β CD: β -cyclodextrin; ARG: L-arginine

Percent drug content and saturation solubility studies:

The percentage drug content of the complexes was found to be 18 ± 1.5 (w/w) and 53 ± 2.1 (w/w) respectively. The saturation solubility of bosentan monohydrate and binary and ternary complexes was determined as 17.33 ± 1.5 $\mu\text{g/mL}$, 361.8 ± 5 $\mu\text{g/mL}$, 59272 ± 3.2 $\mu\text{g/mL}$ respectively. The binary and ternary complexes gave 20.9 ± 1.2 and 3486 ± 20 fold increase in solubility respectively, as compared to pure drug alone. Significant improvement in complexation efficiency of ternary system was observed as compared to that of binary system due to incorporation L-arginine. In addition to that solubilizing capacity of

β -cyclodextrin and decreasing the crystallinity of pure drug by freeze drying, resulted in enhancement of aqueous solubility of pure bosentan monohydrate in the complexes.

in vitro Drug release: Fig. 9 displayed drug release profile of pure bosentan monohydrate and the complexes. The release rate profiles were plotted as the percentage of drug dissolved vs. time. The values of % drug release in 10 min (DR₁₀) and dissolution efficiency at 10 min (DE₁₀) were determined.

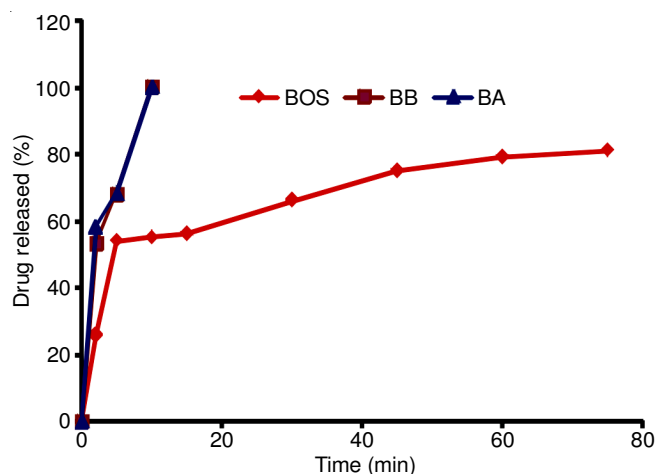


Fig. 9. Dissolution profile of BOS, binary and ternary inclusion complexes at 37 ± 0.5 °C. BB: β CD + BOS; BA: β CD + BOS + ARG; where β CD: β -cyclodextrin; BOS: Bosentan; ARG: L-arginine

The percentage drug release at 10 min for pure drug, binary and ternary systems were observed to be 25.50 ± 2.97 , 99.8 ± 2.57 , 99.84 ± 2.0 , respectively and the dissolution efficiencies of the same at 10 min (DE₁₀) were calculated as 1.59 ± 2.5 , 41.95 ± 2.59 and 41.99 ± 2 , respectively. It was observed that the dissolution rate of pure drug was rapid from inclusion complexes than that of pure drug. A significant difference between the dissolution efficiencies of bosentan monohydrate and complexes ($p < 0.001$) was noted which might be attributed to complexation, greater effectiveness of L-arginine and altered physical state of drug in the complexes.

Protein binding assay: Pharmacological response of any drug mainly depends on the number of free drug molecules rather than the total concentration [20]. The % drug unbound of pure bosentan monohydrate, binary and ternary complexes was observed to be 7.6 ± 1.3 , 98 ± 4 and 98 ± 3.4 , respectively over the period of 5 h. Protein binding of pure drug and inclusion complexes were found to be 92, 2 and 2 %. Observed relevant data clearly indicated that inclusion complexation is useful for increasing availability of pure drug in plasma resulting in the minimization of the therapeutic dose.

log P value: log P value of pure drug, binary and ternary complexes were found to be 3.17 ± 0.9 , 2.66 ± 0.8 and 0.18 ± 0.1 respectively ($p < 0.001$). The decreased log P value of both the complexes indicated enhancement in aqueous solubility [19]. Incorporation of ternary auxiliary substance, L-arginine was responsible for improvement in water solubility of ternary system than that of binary system [14].

Conclusion

In present research work, optimization of various ternary auxiliary substances for improvement of complexation effi-

ciency of β -cyclodextrin towards bosentan monohydrate was performed. *in silico* Data and phase solubility data indicated L-arginine was best ternary substance than that of others. Basic natured L-arginine having the ability to interact with β -cyclodextrins (*via* hydrogen bonding) with weakly acidic drugs (*via* salt formation and electrostatic interactions) perhaps might have contributed for the results. Thermodynamic studies suggested that inclusion phenomenon was entropy driven and spontaneous. Prepared lyophilized complexes gave clear, transparent water solution indicating the complexes possessing lower sized particles of drug, achieved by inclusion phenomenon. The protein binding study resulted inclusion phenomenon was responsible for maintaining the unbound drug in plasma, which resulted in minimization of dose and dose frequency.

ACKNOWLEDGEMENTS

The authors are greatly thankful to the Sun Pharmaceutical Industries Ltd, Vadodara, India for providing gift sample of Bosentan. The authors are thankful to Shivaji University, Kolhapur, India for providing analytical facilities to perform characterization studies. The research given in this paper is financially supported by BARTI (Dr. Babasaheb Ambedkar Research and Training Institute, Pune, India).

REFERENCES

- C. Weber, R. Schmitt, H. Birnboeck, G. Hopfgartner, H. Eggers, J. Meyer, S. van Marle, H.W. Viischer and J.H.G. Jonkman, *J. Clin. Pharmacol.*, **39**, 703 (1999); <https://doi.org/10.1177/00912709922008344>.
- C. Weber, R. Schmitt, H. Birnboeck, G. Hopfgartner, S.P. van Marle, P.A.M. Peeters, J.H.G. Jonkman and C.-R. Jones, *Clin. Pharmacol. Ther.*, **60**, 124 (1996); [https://doi.org/10.1016/S0009-9236\(96\)90127-7](https://doi.org/10.1016/S0009-9236(96)90127-7).
- T.K. Panda, D. Das and L. Panigrahi, *J. Appl. Pharm. Sci.*, **6**, 27 (2016); <https://doi.org/10.7324/JAPS.2016.60904>.
- M.R. Shah, S.P. Sancheti, V.M. Vyas, P.S. Karekar and Y.V. Pore, *Drug Discov. Ther.*, **4**, 70 (2010).
- T. Loftsson, D. Hreinsdottir and M. Masson, *Int. J. Pharm.*, **302**, 18 (2005); <https://doi.org/10.1016/j.ijpharm.2005.05.042>.
- O. Aleem, B. Kuchekar, Y. Pore and S. Late, *J. Pharm. Biomed. Anal.*, **47**, 535 (2008); <https://doi.org/10.1016/j.jpba.2008.02.006>.
- P. Gajare, C. Patil, N. Kalyanea and Y. Pore, *Dig. J. Nanomater. Biostruct.*, **4**, 891 (2009).
- P. Mura, M.T. Faucci and G.P. Bettinetti, *Eur. J. Pharm. Sci.*, **13**, 187 (2001); [https://doi.org/10.1016/S0928-0987\(01\)00093-8](https://doi.org/10.1016/S0928-0987(01)00093-8).
- V. Pokharkar, A. Khanna, V. Venkatapurwar, S. Dhar and L. Mandpe, *Acta Pharm.*, **59**, 121 (2009); <https://doi.org/10.2478/v10007-009-0001-3>.
- M. Shah, P. Karekar, P. Sancheti, V. Vyas and Y. Pore, *Drug Dev. Ind. Pharm.*, **1**, 1 (2008).
- D. Bramhane, N. Saindane and P. Vavia, *J. Incl. Phenom. Macrocycl. Chem.*, **69**, 453 (2011); <https://doi.org/10.1007/s10847-010-9783-7>.
- A. Patil, Y. Pore and B. Kuchekar, *Dig. J. Nanomater. Biostruct.*, **3**, 89 (2008).
- L.S.S. Ribeiro, D.C. Ferreira and F.J.B. Veiga, *Eur. J. Pharm. Sci.*, **20**, 253 (2003); [https://doi.org/10.1016/S0928-0987\(03\)00199-4](https://doi.org/10.1016/S0928-0987(03)00199-4).
- P. Jadhav and Y. Pore, *Bull. Fac. Pharm. Cairo Univ.*, **55**, 147 (2017); <https://doi.org/10.1016/j.bfopcu.2016.12.004>.
- P. Jadhav, B. Petkar, Y. Pore, A. Kulkarni and K. Burade, *Carbohydr. Polym.*, **98**, 1317 (2013); <https://doi.org/10.1016/j.carbpol.2013.07.070>.
- T. Higuchi and K.A. Connors, *Adv. Anal. Chem. Instr.*, **4**, 117 (1965).
- M. Brewster and T. Loftsson, *Adv. Drug Deliv. Rev.*, **59**, 645 (2007); <https://doi.org/10.1016/j.addr.2007.05.012>.

18. C. dos Santos, M.P. Buera and M.F. Mazzobre, *Procedia Food Sci.*, **1**, 355 (2011); <https://doi.org/10.1016/j.profoo.2011.09.055>.
19. S. Talegaonkar, Y. Khan, R.K. Khar, F.J. Ahmad and Z. Khan, *Iran. J. Pharm. Res.*, **6**, 95 (2007).
20. A. Rasheed and C.A. Kumar, *Int. J. Cur. Pharm. Res.*, **1**, 47, (2009).
21. S. Sapte and Y. Pore, *J. Pharm. Anal.*, **6**, 300 (2016); <https://doi.org/10.1016/j.jpha.2016.03.004>.
22. L. Liu and Q. Guo, *J. Phys. Chem. B*, **103**, 3461 (1999); <https://doi.org/10.1021/jp984545f>.
23. M. Shah, Y. Pore, S. Dhawale, K. Burade and B. Kuchekar, *J. Incl. Phenom. Macrocycl. Chem.*, **76**, 391 (2013); <https://doi.org/10.1007/s10847-012-0211-z>.
24. D. Doiphode, S. Gaikwad, Y. Pore, B. Kuchekar and S. Late, *J. Incl. Phenom. Macrocycl. Chem.*, **62**, 43 (2008); <https://doi.org/10.1007/s10847-008-9435-3>.
25. V. Shinde, M. Shelake, S. Shetty, A. Chavan-Patil, Y. Pore and S. Late, *J. Pharm. Pharmacol.*, **60**, 1121 (2008); <https://doi.org/10.1211/jpp.60.9.0002>.