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Effect of β-cyclodextrin and Hydroxypropyl β-cyclodextrin on Aqueous Stability, Solubility and Dissolution of Novel Anti-cancer Drug Rigosertib

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To study Effect of β-cyclodextrin and hydroxypropyl β-cyclodextrin on aqueous stability, solubility and dissolution of novel anti-cancer drug Rigosertib.
Methods: β-cyclodextrin and hydroxypropyl β-cyclodextrin were used to form complex with Rigosertib. The effect of cyclodextrins on drug solubility was studied using phase solubility method. Physico-chemical characterization of drug cyclodextrin complex was performed using differential scanning calorimeter, X-Ray diffraction, Infrared spectroscopy and Scanning electron microscopy. Dissolution profiles of drug-cyclodextrin complexes showed better drug release compared to untreated drug in pH 1, 2, 4 and 5.5 dissolution medium.
Results: Rigosertib exhibits poor solubility and chemical instability in acidic solutions. Poor aqueous stability along with limited solubility in acidic conditions can result in limited oral bioavailability of the drug. Both analogues showed positive effect on drug solubility. Hydroxypropyl β-cyclodextrin performed better compared to β-cyclodextrin to improve drug solubility. In addition,

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presence of both cyclodextrin analogues improved drug stability in acidic solutions. Physico-chemical characterization of drug cyclodextrin complex was performed using differential scanning calorimeter, X-Ray diffraction, Infrared spectroscopy and Scanning electron microscopy. Dissolution profiles of drug-cyclodextrin complexes showed better drug release compared to untreated drug in pH 1, 2, 4 and 5.5 dissolution medium.

Conclusion: Improved drug solubility and chemical stability in acidic conditions can be attributed to complex formation between drug and cyclodextrin.

Keywords: Cyclodextrin; anti-cancer; chemical stability; complex formation; rigosertib; physico-chemical characterization; solubility; dissolution.

1. INTRODUCTION

Rigosertib, also known as ON 01910 Na, is a novel anticancer compound in clinical development by Onconova Therapeutics, Inc. It is a cell cycle modifying agent that belongs to a class of molecules called the benzyl styryl sulfones [1]. Rigosertib has a good safety profile with low toxicity. It exhibits great anti-tumor activity when used alone or in combination with other chemotherapeutic agents. The U.S. FDA has designated Rigosertib as an orphan drug for treatment of Myelodysplastic Syndromes (MDS) and is being investigated for effects against hematological and solid tumors [2]. In Phase I/II studies Rigosertib has shown promising therapeutic results and drug tolerance in patients with advanced solid tumors. Onconova Therapeutics, Inc. is evaluating an oral formulation of Rigosertib in Phase II clinical trials involving transfusion-dependent low risk MDS patients. Rigosertib exhibits poor solubility and chemical instability in acidic solutions. Poor aqueous stability along with limited solubility in acidic conditions can result in limited oral bioavailability of the drug. Authors have discussed drug degradation kinetics in acidic conditions and drug release in previous publication [3].

Cyclodextrins (CDs) are naturally occurring cyclic linked oligosaccharides of α-D-glucopyranose [4]. There are four most commonly occurring CDs in nature: α-cyclodextrin, β-cyclodextrin (BCD), γ-cyclodextrin and δ-cyclodextrin which contain 6, 7, 8 and 9 glucopyranose units respectively [5]. They are cone or toroidal shape cavities containing hydrophobic core and hydrophilic outer surface due to presence of hydroxyl group [6]. Number of glucopyranose units decides the size of cavity [7]. Due to the cavity structure, they can form inclusion complexes by holding drug molecules through non-covalent bonds. These complexes can offer partial or full coverage of drug molecules from surrounding environment. Contributions from Vander Waals bonds, hydrogen bonds and hydrophobic effects help in stable complexation of drugs in apolar cyclodextrin cavity. This unique property (hydrophobic core and hydrophilic outer side) provides dual forces of cohesiveness and friction (friccohesivity), which can be beneficial in formation of monodispersion of drug in cyclodextrin cavity. Formation of such complexes has been shown to improve physicochemical properties of many drugs [8]. They can be used to improve the bioavailability of lipophilic drugs by improvement in solubility and dissolution profile of complexed materials [9,10]. They can also be used to protect drugs or other substances from effects of light and atmosphere by enclosing them [11]. In addition, cyclodextrins can be used to reduce gastrointestinal drug irritation, convert liquid drugs into microcrystalline or amorphous powder, and prevent drug–drug and drug– excipients interactions.

BCD is most common cyclodextrin analogue which occurs naturally. BCD itself or numerous chemically modified analogues are used to improve various drug properties such as solubility, dissolution rate, stability and bioavailability [12-15]. It is an ideal host for the most of the pharmaceutical drugs owing to appropriate cavity size. It possesses limited nephrotoxicity upon oral administration [16,17]. Only drawback related to BCD in formulation development is solubility issues. Limited solubility of BCD can be attributed to strong binding of the molecules in crystal lattice [18]. hydroxypropyl-β-cyclodextrin (HPBCD) is partially substituted poly (hydroxypropyl) ether of BCD. It is one of the BCD analogue approved by FDA for pharmaceutical applications. Studies have shown

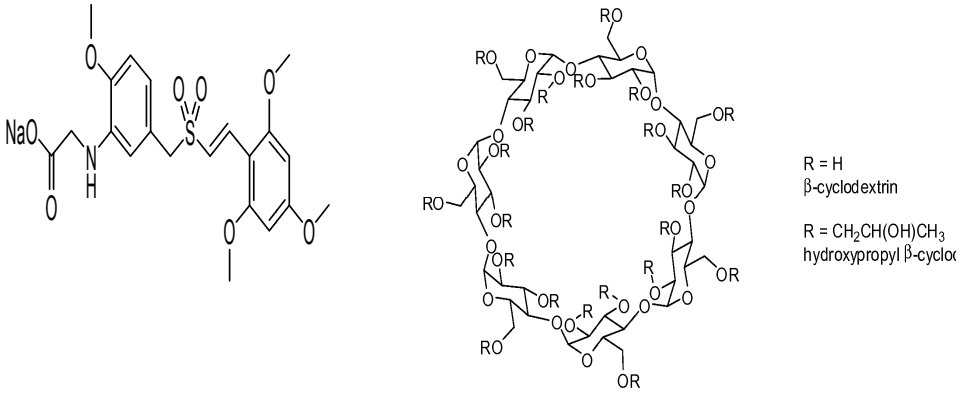


Fig. 1. Chemical structure of Rigosertib, β-cyclodextrin and hydroxypropyl β-cyclodextrin

HPBCD being safe for oral use and toxicologically more benign compared to BCD [19]. It is used to improve solubility, stability and bioavailability of various drugs [20]. HPBCD is found in several marketed drug formulations with oral dose of HPBCD up to 8 g per day [21]. Here, the authors have attempted to demonstrate Effect of β-cyclodextrin and hydroxypropyl β-cyclodextrin on aqueous chemical stability, solubility and dissolution of Rigosertib.

2. MATERIALS AND METHODS

2.1 Materials

Rigosertib (99.5% purity) and degradation product standards were provided by Onconova Therapeutics, Inc. (Newtown, PA). β-cyclodextrin and hydroxypropyl β–cyclodextrin were donated by Roquette, IA, USA. All other materials used were of analytical grades.

2.2 Methods

2.2.1 Analytical method

HPLC method was used for identification and quantification of Rigosertib and degradation products. HPLC system (Shimadzu scientific Inc., OR, USA) including auto sampler and isocratic pump was used for analysis. C-8 column (GL Sciences Inc., CA, USA) with 250 mm X 4.6 mm dimensions and 5 µ particle size was used. Column temperature was maintained at 40 °C using column heater. Isocratic mobile phase with composition of 0.16 % ammonium acetate in water: acetonitrile (60:40) at flow rate of 1 ml/ min was used. 0.2 ml sample was diluted with 0.3 ml Phosphate buffer (pH = 8) and 0.5 ml mobile phase to make it neutral pH before analyzing at 215 nm wavelength using UV detector. Run time for each sample was kept 45 min.

2.2.2 Phase solubility studies

Effects of BCD and HPBCD on Rigosertib solubility were studied by phase solubility method in USP buffer pH 4. Based on its molecular weight of BCD, appropriate amounts of BCD were added to solution. 0 to 7 mM concentration solutions of BCD in pH 4 were prepared and maintained at required temperature (25, 30, 35°C). Drug powder was added to above prepared solution in excess amount in test tubes. Test tubes were sealed using paraffin and stored in incubator shaker. Drug concentrations in the solutions were measured using HPLC at 4 hr (optimized by preliminary experiments) time interval. Similar procedure was performed using HPBCD for phase solubility studies.

2.2.3 Drug degradation and effect of CDs on degradation rate

Rigosertib powder was dissolved in appropriate amount of water depending upon concentration and desired temperature was maintained. 1 N HCl was maintained at the same temperature as Rigosertib solution. Required amount of HCl was added to the drug solution in the beginning of the study. Samples withdrawn from these solutions at predetermined time intervals were neutralized to stop further degradation and analyzed using HPLC. Drug degradation rates in presence of BCD and HPBCD in solution were determined. Appropriate amount of BCD was added along with drug in water to achieve BCD concentration

of 1, 5 and 10 mg/ ml. Similar experiments were performed to study effect of HPBCD on drug degradation. Studies were performed in 0.1 N (pH 1), 0.05 N (pH 1.3), 0.025 N (pH 1.6) HCl concentrations at three different temperatures 25, 30 and 35°C.

2.2.4 Drug-CD complex formation

Solvent evaporation method was used to form drug cyclodextrin complex. Required amount of BCD or HPBCD was dissolved in 40 ml water. 1 g of Rigosertib was added in the solution of CD and stirred to form clear solution. Water was evaporated using RotaVap to get dry powder.

2.2.5 Content uniformity

Content uniformity of drug in prepared complexes was investigated by drug recovery studies where known quantities of Rigosertib, Rigosertib-BCD complex and Rigosertib-HPBCD complex were dissolved in 10 ml mobile phase to get clear solution. Solution was further diluted with mobile phase and buffer before analyzing using HPLC.

2.2.6 Thermal analysis

Calorimetric studies were conducted using Modulated Differential Scanning Calorimetry instrument (MDSC). Accurately weighed samples were hermetically sealed in aluminum pans. Empty sealed aluminum pans were used as a reference. Both pans were heated at rate of 10 °C/min with +/- 1.59 modulations every 60 mins from 10 °C to 250 °C under nitrogen gas flow of 20 ml/min. Thermal analysis of pure drug, excipients, formulations and physical mixtures was performed. Data analysis was performed using Universal Analysis software to measure melting point enthalpy of melting.

2.2.7 X-ray diffraction

X-ray diffraction (XRD) patterns were studied to verify whether the treatment done in section 4.4 (Drug-CD Complexation) caused any structural changes in the compound. A scanning X-ray diffractometer was used in this study. X-ray diffraction patterns were obtained for Rigosertib, BCD, HPBCD, Rigosertib-BCD complex, Rigosertib-HPBCD complex, Rigosertib-BCD physical mixture and Rigosertib-HPBCD physical mixture. The radiations used were generated by a copper Kα filter, with wavelength 1.54 A ° at 35 kV and 30 mA. Glass slide was covered with the sample to be analyzed and scanned over a range from 5° to 40° 2θ degrees, using a scan rate of 1 degree per min and a step scan of 0.02.

2.2.8 Infrared spectroscopy

MAGNA-IR 760 Spectrophotometer (Thermo Scientific, USA) was used to obtain Infrared (IR) spectra for all sample powders. Powdered potassium bromide (KBr) of IR grade stored in desiccators was used as background material. Minute quantity of each sample was triturated with pure KBr using mortar and pestle to form uniform mixture. It was compressed to form semi-transparent film. Each film was scanned (64 scans) in the region of 400 to 4000 cm^{-1} in transmittance mode. EssentialFTIR software was used to detect any shift or disappearance of absorption peak in spectra due to formation of any bond between drug and CD.

2.2.9 Scanning electron microscopy

Scanning electron microscopy (SEM) was conducted to observe surface morphology and texture of pure materials and binary blends. The SEM photographs were taken using JEOL Scanning electron microscope, model 5900 LV. The samples were mounted on double sided carbon tape 31 for SEM imaging. Low Vacuum (LV) mode was used to prevent the samples from charging. The analyses were conducted using 1000X magnification.

2.2.10 Dissolution studies

Rigosertib powder (280 mg) and various drug-cyclodextrin mixtures (equivalent to 280 mg of Rigosertib) were analyzed using USP apparatus-II for in-vitro dissolution studies. The dissolution studies were carried out at 37.2 °C with rotation speed of 75 RPM in 250 ml volumes for pH 1, 2 and 4 and 900 ml for pH 5.5 buffers to mimic gastrointestinal fluid conditions. From dissolution medium, 5 mL aliquots were withdrawn and equal amount of fresh media was replaced at 5, 10, 15, 20, 25, 30, 45, 60, 90, 120, and 180 min of time intervals. Withdrawn samples were filtered using 0.45 µm pore size filters and further diluted with buffer and mobile phase to prevent drug degradation during HPLC analysis.

3. RESULTS AND DISCUSSION

3.1 Phase Solubility Studies

Effect of CD analogues on drug solubility can be determined using phase solubility profile. Various

BCD and HPBCD concentrations were prepared by dissolving them in buffer solutions. Excess amount of drug was added to these solutions to determine saturation solubility. Phase solubility diagram can be used to determine stability constant [22]. Apart from stability constant value stoichiometry of equilibrium process can be determined by phase solubility method. Phase solubility diagram can fall into two categories A and B type. Drug solubility can increase (Type A) or decrease (Type B) as a function of CD concentration. Increment in solubility can be linear (A_L) or non-linear with positive (A_P) or Negative (A_N) deviation. Equilibrium constant K can be calculated from Phase solubility plot using the following equation.

$$K = \frac{m}{S_0(1 - m)} \quad (1)$$

Where: K = Stability constant, S_0 = Solubility of the drug in the absence of cyclodextrin , m = Slope of the drug molar concentration versus CD molar concentration graph.

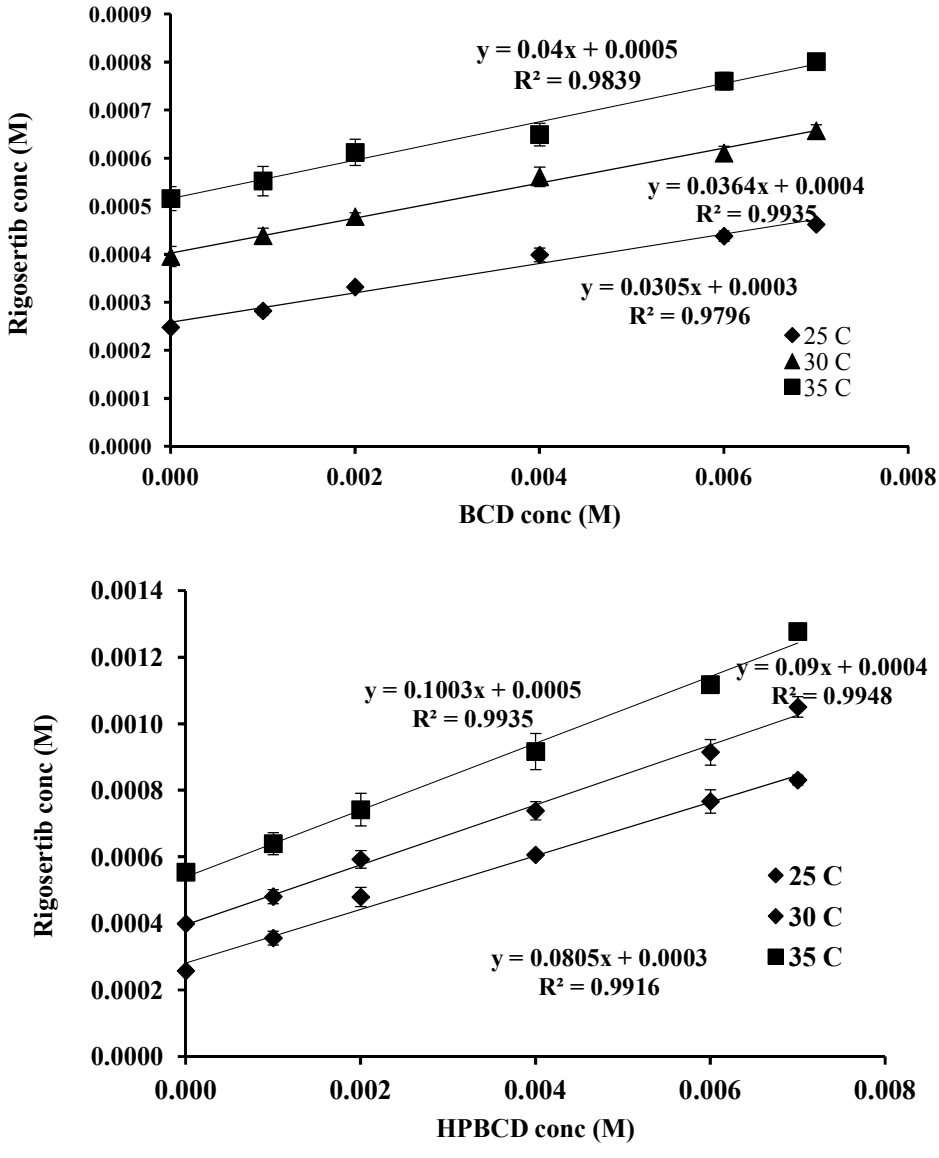


Fig. 2. Effect of BCD and HPBCD on rigosertib solubility

Phase solubility results (Fig. 2) indicate that BCD and HPBCD have positive effect on drug solubility [23]. Positive linear relationship (A_L) was observed for CD concentration on aqueous solubility. Slope of graph was less than 1 for both BCD and HPBCD which suggest 1:1 stoichiometry from drug and CD complex on molar basis. Stability constant or equilibrium constant is reflection of effect of BCD or HPBCD on Rigosertib solubility. Comparison of stability values (Table 1) at various temperature indicate that HPBCD has higher effect on drug solubility compared to BCD. HPBCD is more hydrophilic compared to BCD due to presence of hydroxypropyl group. Therefore, it further improves drug solubility compared to BCD resulting in higher stability constant.

Table 1. Comparison of stability constant values of Rigosertib- BCD and Rigosertib-HPBCD

Temperature		Stability Constant (/M)	
°C	K	BCD	HPBCD
25	298	127	340
30	303	95	248
35	308	81	201

3.1.1 Thermodynamic parameters for complex formation

Van't Hoff equation was used to determine thermodynamic parameters for complexation process. This equation provides information about the temperature dependence of the equilibrium constant. Van't Hoff equation is derived from Gibbs – Helmholtz equation for Gibbs free energy dependence on temperature.

Gibbs equations:

$$\Delta G = -RTLnK \tag{2}$$

$$\Delta G = \Delta H - T\Delta S \tag{3}$$

Where, K= Stability constant, ΔH = Enthalpy of complexation process, ΔS = Entropy of reaction, R = Gas constant, T = Temperature (K).

Combining Equation 2 and 3 yields:

$$-RTLnK = \Delta H - T\Delta S \tag{4}$$

Rearranging Equation 4 yields:

$$LnK = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} \tag{5}$$

Graph of ln K vs. 1/T (Fig. 3) yields straight line with positive slope. Values for enthalpy and entropy of complexation process were calculated using slope (-ΔH/R) and intercept (ΔS/R) of the straight line. Gibbs energy for the reaction was determined using the Equation 2. Gibbs free energies (ΔG) for inclusion complex for Rigosertib–BCD combination were determined to be -12.00 KJ/Mole, -11.47 KJ/Mole and -11.25 KJ/Mole at 25, 30 and 35°C respectively. ΔG values for Rigosertib–HPBCD were calculated as -14.44 KJ/Mole, -13.89 KJ/Mole and -13.58 KJ/Mole at 25, 30 and 35°C respectively. Negative ΔG values for Rigosertib–BCD and Rigosertib–HPBCD combinations under all temperatures examined indicate that the inclusion complexation was a spontaneous process.

Enthalpy values (ΔH) for Rigosertib–BCD and Rigosertib–HPBCD inclusion complexes were found to be -34.37 KJ/Mole and -40.15 KJ/Mole respectively. For Rigosertib–BCD and Rigosertib–HPBCD entropy values were calculated to be -75.23 J/K and -86.40 J/K respectively. Negative enthalpy and entropy values are indicative of exothermic and enthalpy controlled inclusion process [24-27]. Negative enthalpy value can be attributed to Van der Waals interaction. These large negative values associated with less negative entropy values is termed at compensation effect. In this process, water may act as a driving force for complex formation. System energy is lowered when less polar drug molecules replace enthalpy rich water molecules from CD cavity. Stability constant values and thermodynamic parameters suggest ability of BCD and HPBCD to form complex with drug spontaneously. HPBCD forms more stable complex as compared to BCD.

3.2 Drug Degradation in Presence of CD Analogs

Rigosertib rapidly undergoes chemical degradation in acidic conditions. It forms multiple degradation products via various chemical mechanisms. Graph of Rigosertib concentration versus Time indicated first order degradation reaction. This was confirmed by observation of a straight line on a logarithmic plot. Rate constants were calculated for different concentrations of HCl (acidic media) and temperatures using

slopes of logarithmic graph of concentration versus time. A summary of rate constants for drug-degradation reactions and representative charts for drug concentration Vs time (at 0.025N HCl and 25°C) are provided in Table 2 and Fig. 4 respectively. For subject study, BCD was dissolved in respected mediums prior to addition of drug to prepare the inclusion solutions. Effect of BCD on Rigosertib degradation was studied by comparing drug degradation rate in its presence and absence. BCD helps to improve drug aqueous stability in all three HCl concentrations and at different temperature conditions. Increase in BCD concentration significantly improves drug stability. HPBCD presence in solution slows down drug degradation rate at all the experimental conditions (HCl concentration, temperature). Increasing HPBCD concentration from 0 to 10 mg/ml further improves drug stability significantly (data not shown). Effect of increasing BCD and HPBCD concentration on drug degradation rates is almost identical.

Table 2. Summary of Rigosertib degradation rate constants in presence of BCD

HCl Conc (N)	Temperature		BCD Conc (mg/ml) / Rate constants(Min^{-1})			
	°C	K	0	1	5	10
0.1	25	298	0.046	0.031	0.021	0.016
	30	303	0.072	0.059	0.042	0.028
	35	308	0.130	0.091	0.071	0.061
0.05	25	298	0.027	0.018	0.013	0.009
	30	303	0.046	0.027	0.023	0.016
	35	308	0.091	0.066	0.050	0.036
0.025	25	298	0.020	0.015	0.010	0.005
	30	303	0.040	0.027	0.019	0.018
	35	308	0.076	0.059	0.039	0.027

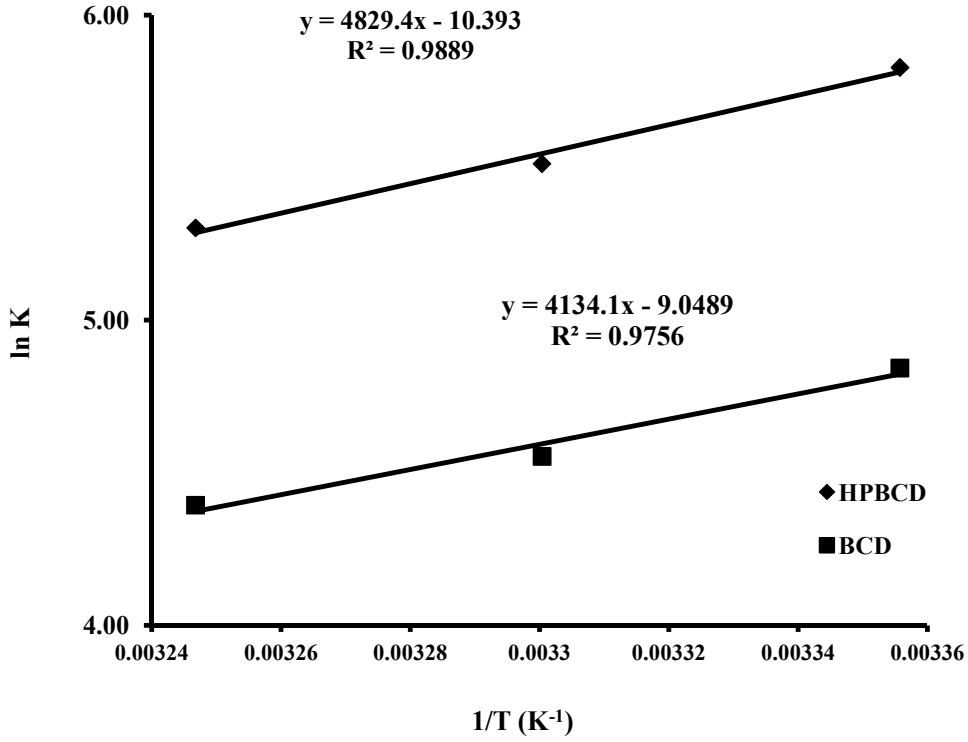


Fig. 3. Van't Hoff plot for Rigosertib - CD complex

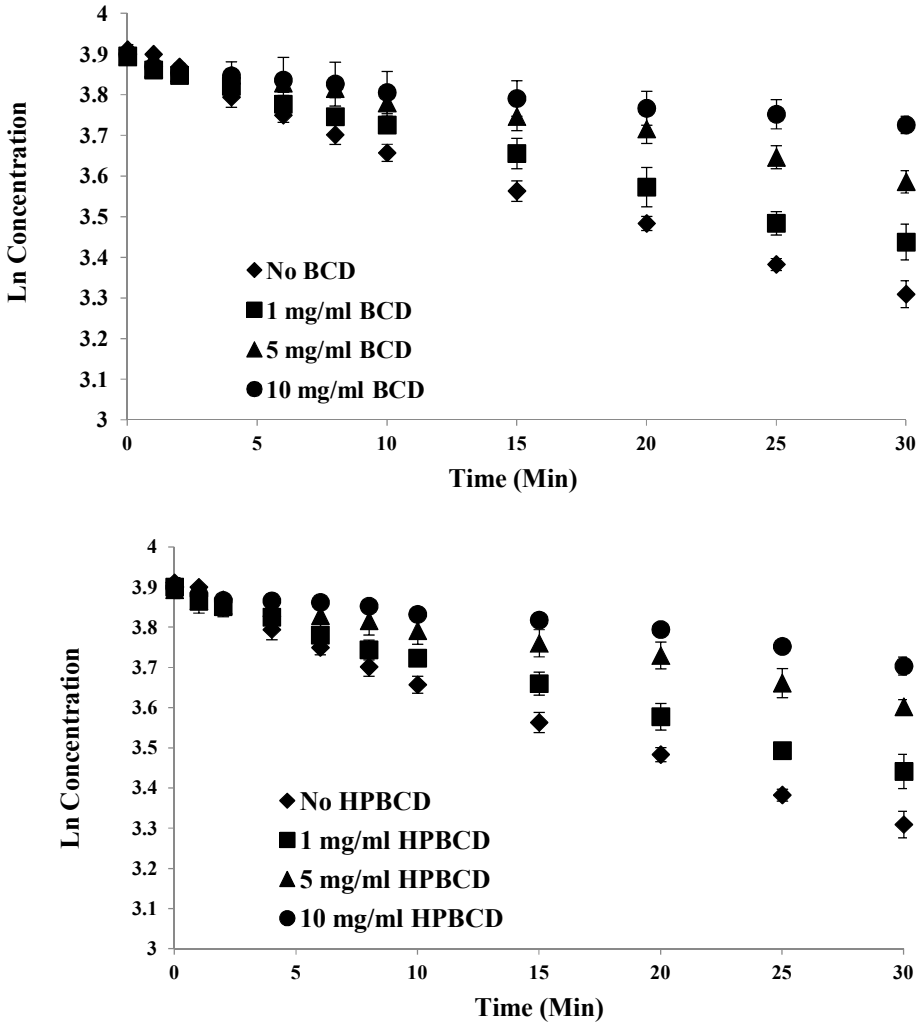


Fig. 4. Comparison of Rigosertib degradation rate in presence of BCD and HPBCD in 0.025 N HCl at 25°C

3.3 Inclusion Complex Formation

The formation of inclusion complexation between Rigosertib and CD analogues various physicochemical tests were confirmed by evaluating MDSC, XRD, IR and SEM analysis [28].

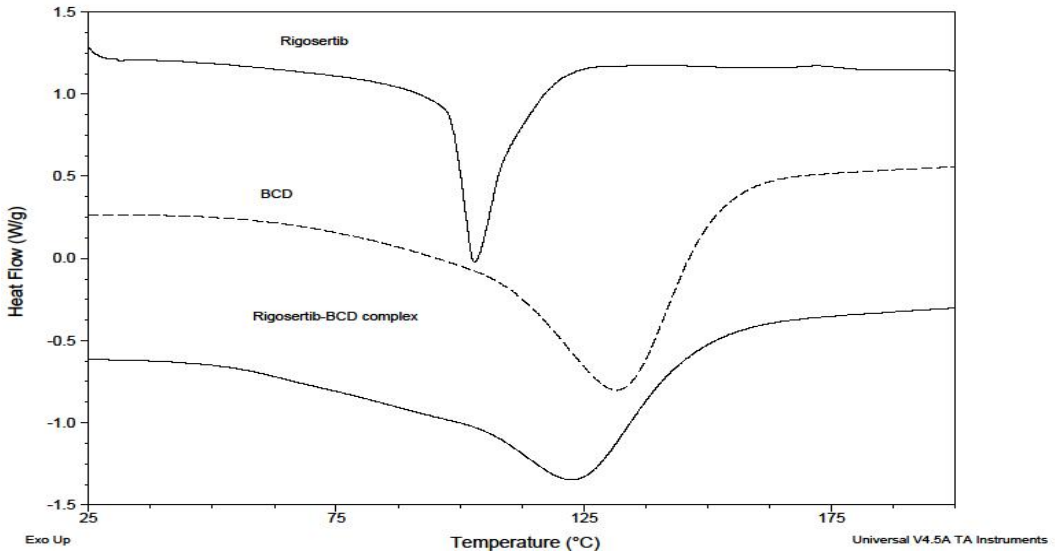
3.3.1 Thermal analysis

Thermal analysis (Fig. 5) of the drug, BCD, HPBCD, complexes and physical mixtures was done using MDSC. Rigosertib melts at 106°C with sharp endothermic peak due to its crystalline nature. BCD has no melting peak in range of 0 to 250 °C temperature range. Thermogram of BCD shows relaxation peak around 120°C which is referred to releasing of water [29,30]. HPBCD thermograms show similar pattern to BCD with only relaxation peak around 100°C due to loss of water by evaporation [31]. Thermogram for physical mixture (not shown) of Rigosertib and BCD showed two peaks corresponding to Rigosertib and water loss, which indicated crystallinity of drug in the physical mixture. Peak occurrence for drug was similar in the mixture but smaller compared to pure drug, which could be due to smaller mass ratio in presence of BCD. Melting peak of Rigosertib does not appear in Rigosertib- BCD complex (Fig. 5) due to formation of inclusion complex [32]. Similar thermal events were observed for Rigosertib-

HPBCD physical mixture and complex formulation. CD analogues form complex with drug by encapsulating (covering) it completely, therefore, DSC thermogram was not able to detect the change in heat flow due to drug melting [33,34].

3.3.2 X-Ray diffraction

X-Ray diffraction studies on the powder samples were performed to study their physical structures and effect of complex formation on crystalline structure of Rigosertib [35]. XRD provides confirmatory information to DSC analysis because it serves as an accurate method to evaluate molecular state of compound or complex [36]. X-ray diffraction has been widely used for crystallographic characterization of inclusion compounds because the formation of an inclusion complex would disrupt the diffraction pattern of the guests (drug). This would make the diffraction pattern of the complex clearly different from the superposition of the diffraction patterns of individual components [37]. The differences in



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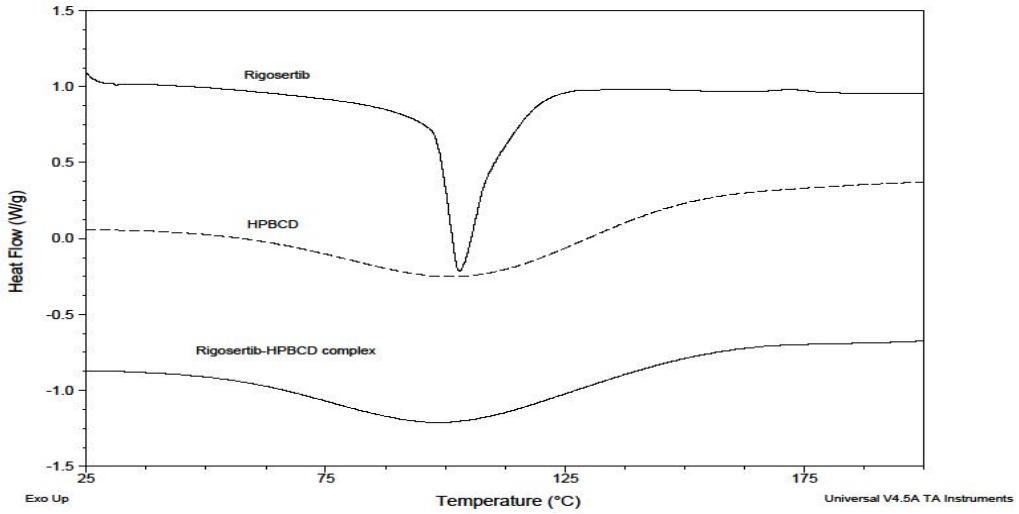
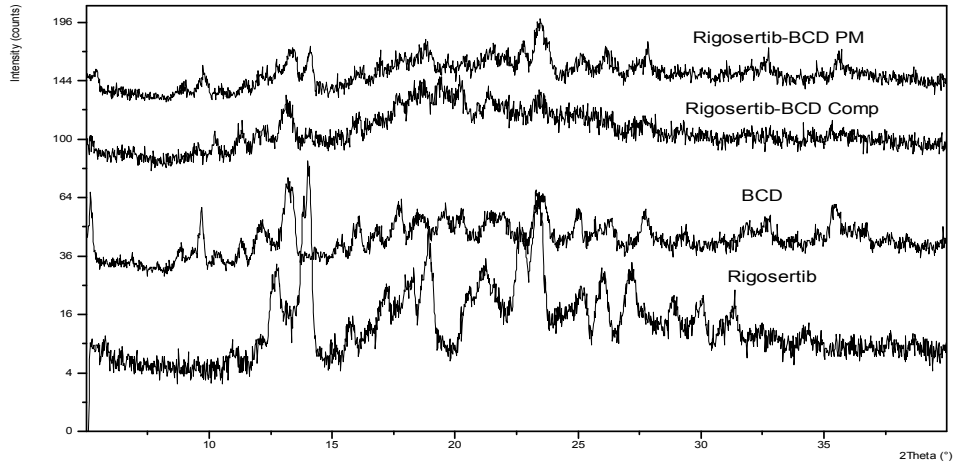


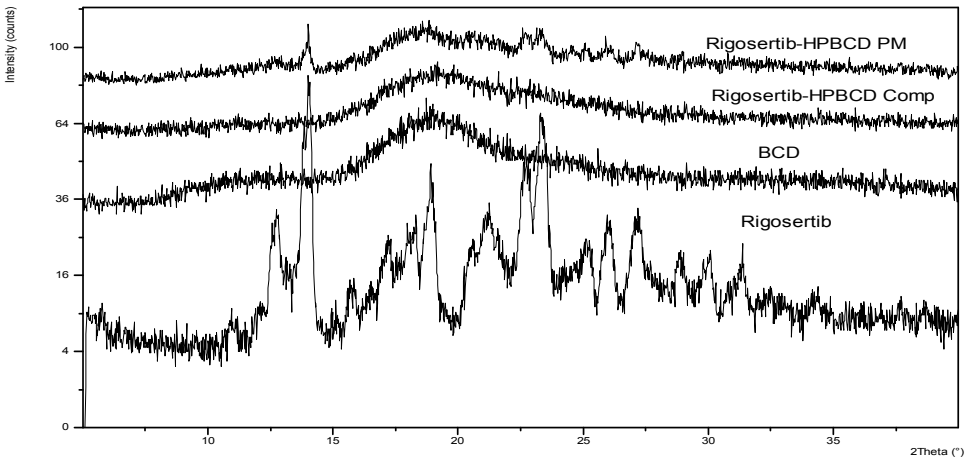
(B)

Fig. 5. DSC thermograms of (A) Rigosertib, BCD and Rigosertib-BCD complex and (B) Rigosertib, HPBCD and Rigosertib-HPBCD complex

the powder crystalline states of the samples were studied by comparing their diffraction patterns (Fig. 6). X-ray diffraction pattern for Rigosertib has distinct diffraction peaks due to its crystalline nature. It exhibits characteristic diffraction peaks with high intensity at 13.88°, 14.07°, 18.95° and 23.33° angles. The pattern also shows peak at 12.78°, 17.27°, 21.28°, 26.08° and 27.18° angles. X-Ray diffraction pattern of BCD exhibits many crystalline peaks between 10 ° and 40 ° angles [38]. Pattern shows intense crystalline peaks at 9.69°, 13.24°, 17.73°, 23.49° and 35.37°. The pattern also shows peak at 11.33°, 12.17°, 16.09°, 18.57°, 19.60°, 20.28°, 24.97° and 32.78° angles with moderate intensity. HPBCD showed flat pattern as typical diffraction profile for amorphous compound due to absence of crystalline nature. In the case of the Rigosertib-BCD complex the diffraction pattern was like BCD pure with reduced intensity. Diffraction peaks corresponding to Rigosertib were absent due to formation of inclusion complex. In the contrast XRD of their physical mixture was simply the super-position of the two patterns of the crystalline Rigosertib and BCD. Reduction in intensities of the peaks was observed due to physical dilutions of powders with each other. XRD pattern of physical mixture of Rigosertib and HPBCD contained peaks like pure Rigosertib powder as expected. Rigosertib–HPBCD complex exhibited halo XRD pattern due to absence of any crystalline structure. It indicated conversion of crystalline drug into amorphous form.



(A)

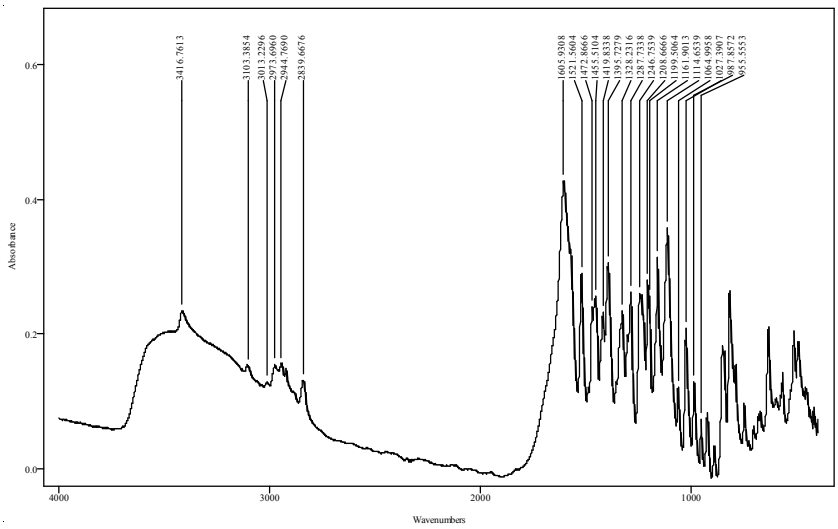


(B)

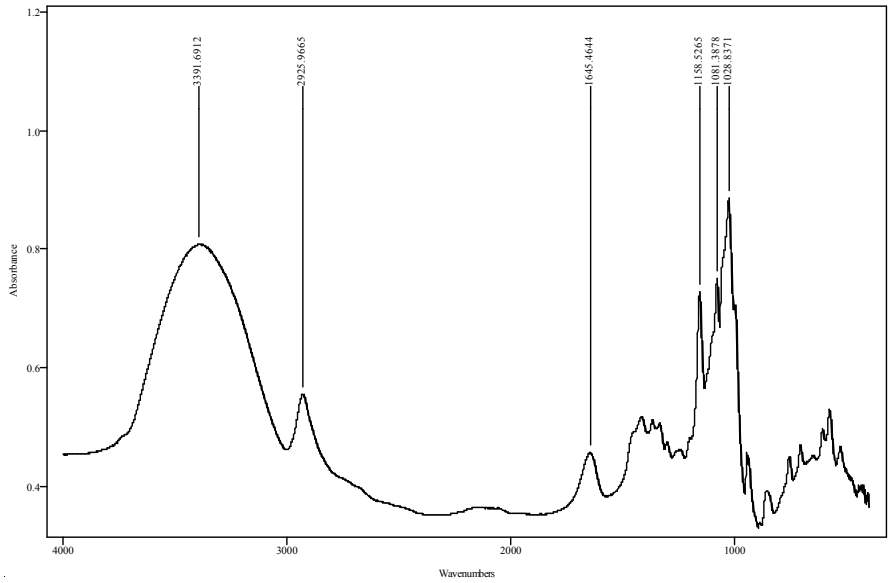
Fig. 6. Overlay of XRD patterns of (A) Rigosertib, BCD, Rigosertib-BCD complex and Rigosertib-BCD physical mixture and (B) Rigosertib, HPBCD, Rigosertib-HPBCD complex and Rigosertib-HPBCD physical mixture

3.3.3 IR Spectroscopy

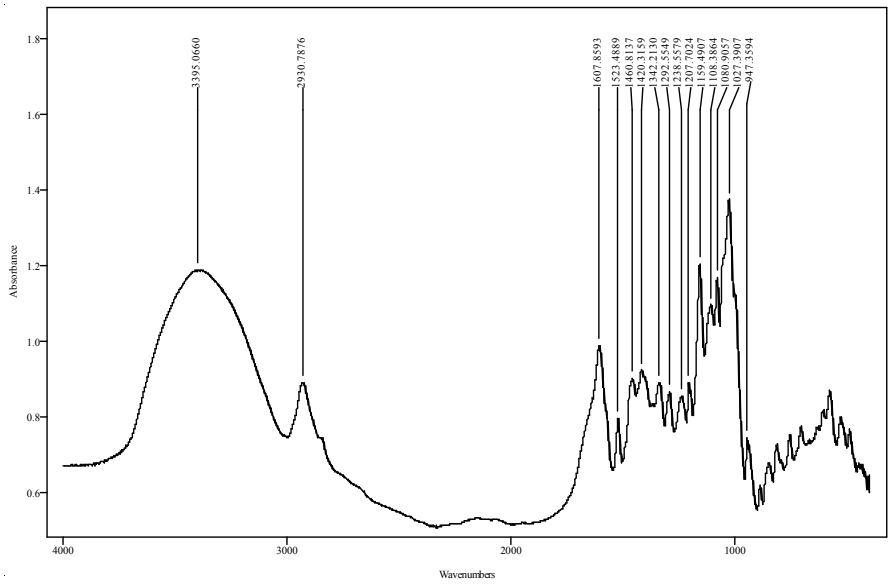
IR spectroscopy is widely used to understand drug excipient interactions. Infrared spectroscopy (Fig. 7) for pure drug and excipients powders was performed to identify their characteristic absorption peaks. IR spectra of physical complexes and physical mixtures were recorded to study any possible chemical interaction or change in structure. Rigosertib has reactive groups like sulfone, secondary amine and carbonyl in chemical structure. An IR spectrum shows peaks at 1329 and 1115 cm^{-1} corresponding to sulfone group. Characteristic peaks for secondary amine groups were recorded at 3414 and 1287 cm^{-1}. IR spectrum of BCD has broad band with a transmittance peak at 3391 cm^{-1} represented the symmetric and asymmetric hydroxyl (-OH) stretching vibration due to the many intermolecular hydrogen bonds and another bond at 2925 cm^{-1} due to C-H stretching vibration. The absorption band at 1647 cm^{-1} can be attributed to H-O-H bending. The peaks at 1028 and 1158 cm^{-1} were respectively asymmetric C-O-C stretching vibration and symmetric C-O-C stretching vibration [39,40]. In the spectrum of HPBCD (data not shown) has band with a transmittance peak at 3395 cm^{-1}, the most characteristic peak due to O-H stretching. Peak at 2929 cm^{-1} represents C-H stretching vibration and 1649 cm^{-1} due to H-O-H bending. The peaks at 1033 and 1158 cm^{-1} were respectively asymmetric C-O-C stretching vibration and symmetric C-O-C stretching vibration [41]. FTIR spectrum of Rigosertib- BCD physical mixture showed superimposition of Rigosertib and BCD peaks (data not shown). It contains peaks at 1115, 1329 (sulfone), 1287 and 3414 cm^{-1}(amine) for Rigosertib and 1028, 1081, 1158, 1645, 1649, 2929 and 3391 cm^{-1} due to BCD. FTIR spectrum of Rigosertib – BCD complex was different compared to physical mixture indicating structural changes or bond formation. Peak was absent at 3414 cm^{-1} for amine group in FTIR spectrum of complex suggesting covering of group by inclusion complex. There was a shift in peak position for amine from 1287 cm^{-1} to 1292 cm^{-1} due to possible chemical interaction. Sulfone group peaks at 1329 and 1115 also shifted to 1342 and 1108 cm^{-1} respectively due to possible hydrogen bonding with BCD hydroxyl group. The spectrum of physical mixture was equivalent to the simple addition of Rigosertib and HPBCD. Spectrum contains characteristic absorption peaks of Rigosertib like at 1115, 1329 (sulfone), 1287 and 3414 cm^{-1}(amine). It also exhibits peaks at 1033, 1158, 1649, 2929 and 3395 cm^{-1} that are characteristic peaks of HPBCD. An FTIR spectrum of physical mixture suggests that Rigosertib without any interaction with HPBCD. For the Rigosertib - HPBCD inclusion complex, the spectrum was very like Rigosertib – BCD complex. Band peak located at 3414 cm^{-1}(amine) present in physical mixture disappeared entirely. The physical mixture bands located at 1288 (amine), 1329 (sulfone) and 3393 (-OH of HPBCD) cm^{-1} had been shifted to 1292, 1340 and 3402 respectively in inclusion complex bands.IR spectroscopy studies suggest that Rigosertib interacts chemically with BCD and HPBCD to form inclusion complex.



(A)



(B)



(C)

Fig. 7. IR Spectra of (A) Rigosertib, (B) BCD and (C) Rigosertib- BCD complex

3.3.4 SEM analysis

Scanning electron microscope helps to study surface morphology of compounds. SEM is a qualitative method used to visualize the surface structure of raw materials or the prepared products. Morphology of pure Rigosertib, BCD, HPBCD powders was investigated by SEM imaging (Figs. 8 and 9). SEM analysis with 1000X magnification on physical mixtures and complexes of Rigosertib with BCD and HPBCD was performed to determine any change in their physical forms. Crystalline nature of Rigosertib was confirmed in SEM images. It exists as a rod-shaped crystal clusters whereas BCD appears to be thick crystals with non-smooth surface. HPBCD was observed as spherical particles with cavity structures [41]. In the physical mixture, the

characteristic BCD crystals mixed with Rigosertib crystals or adhered to their surface were observed. However, the Rigosertib – BCD complex is observed in the form of irregular particles in which the original morphology of both components disappeared, which confirmed the formation of the inclusion complex. In case of Rigosertib and HPBCD no change in morphology was observed for physical mixture whereas complex showed complete change in morphology.

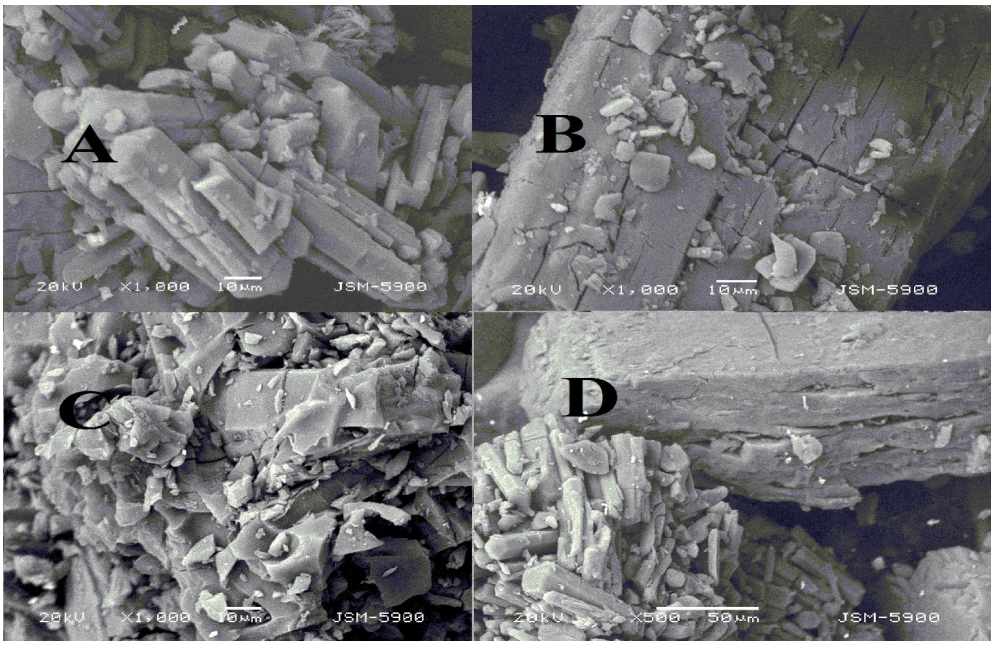


Fig. 8. SEM images of (A) Rigosertib, (B) BCD, (C) Rigosertib - BCD complex and (D) Rigosertib - BCD physical mixture

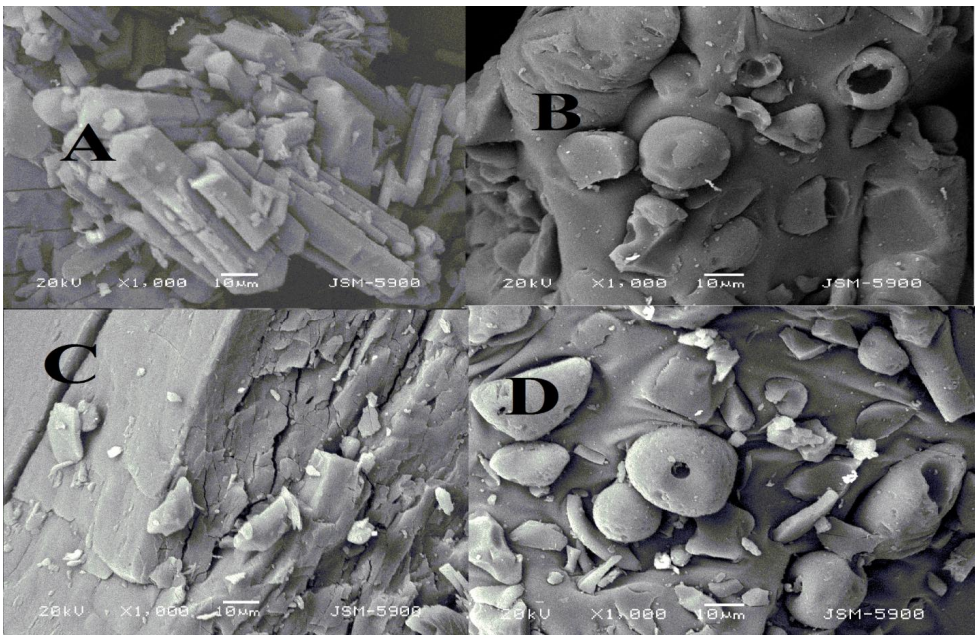


Fig. 9. SEM images of (A) Rigosertib, (B) HPBCD, (C) Rigosertib - HPBCD complex and (D) Rigosertib - HPBCD physical mixture

3.4 Content Uniformity

Content uniformity (Table 3) of drug in prepared complexes was investigated by drug recovery studies in dilution medium. HPLC mobile phase was used as a media for dissolving drug because Rigosertib is very freely soluble in it. Enough drug recovery was observed for both Rigosertib – BCD (102.1%) and Rigosertib–HPBCD complexes (97.1%).

3.5 Cyclodextrin Complex Dissolution Profiles

Dissolution studies were performed on prepared Rigosertib–BCD, Rigosertib– HPBCD complexes and their physical mixtures in various pH buffers. Dissolution profiles of inclusion complexes and physical mixtures were compared with Rigosertib pure powder at pH 1, 2, 4 and 5.5 conditions.

Table 3. % Rigosertib recovered from Rigosertib-BCD and Rigosertib-HPBCD complex

Powder	Weight (mg)	Drug (Theoretical)	AUC	Drug (Actual)	% Recovered	Avg.
Rigosertib	6.4	6.4	961691	6.4	99.3	99.2
	6.4	6.4	949095	6.3	98.0	
	5.4	5.4	819023	5.4	100.2	
Rigosertib-BCD Complex	18.2	5.4	822911	5.4	101.5	102.1
	17.9	5.3	800128	5.3	100.3	
	17.6	5.2	819323	5.4	104.5	
Rigosertib-HPBCD Complex	19.6	4.8	697480	4.6	96.0	97.1
	19.1	4.8	695736	4.6	95.2	
	20.3	5.1	778459	5.1	100.2	

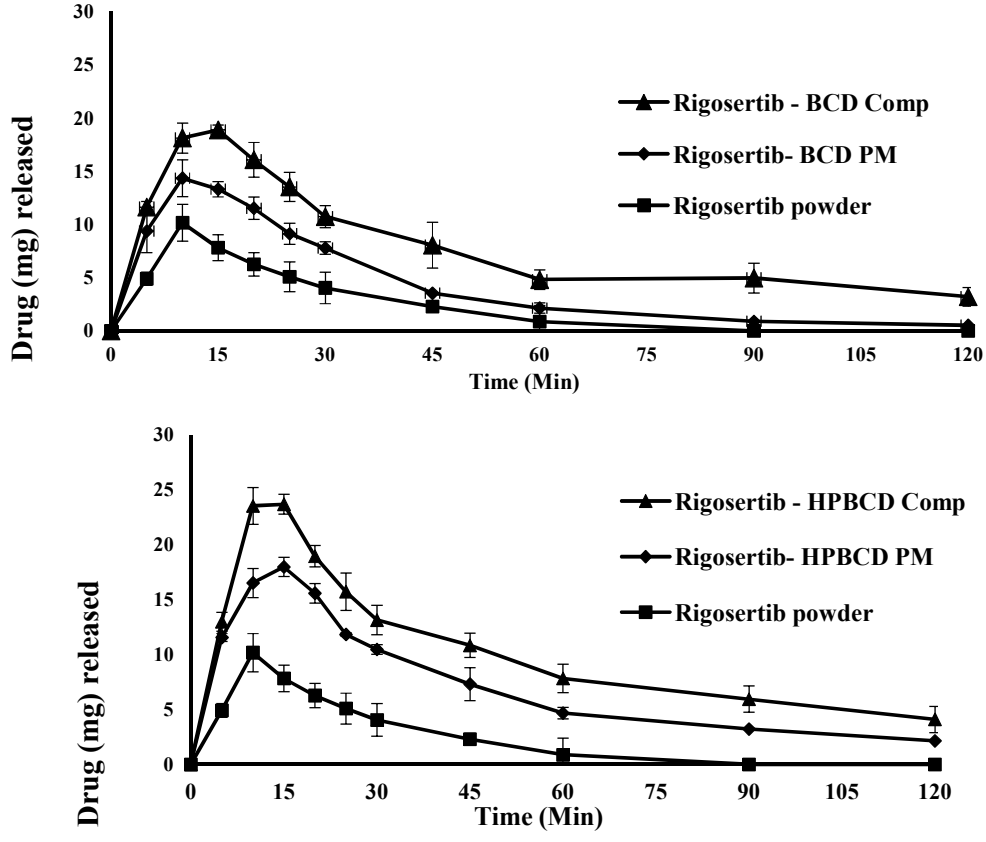


Fig. 10. Overlay of dissolution profiles of Rigosertib, Rigosertib- BCD and Rigosertib-HPBCD in pH 1

3.5.1 pH 1 profile

Dissolution of Rigosertib powder in pH 1 medium yields a very low amount of drug due to extensive chemical degradation in presence of acid and very low solubility at acidic pH (Fig. 10). Dissolution profile for physical mixture of Rigosertib and BCD was slightly better than pure Rigosertib powder. Rigosertib–BCD inclusion complex exhibited further improvement in dissolution profile. Drug released from Rigosertib–HPBCD inclusion complex was almost double than Rigosertib pure. Physical mixture of Rigosertib and HPBCD also shows higher dissolution as compared to Rigosertib pure powder. Improvement in dissolution for CD complex can be attributed to their ability to improve drug stability and solubility in acidic solutions [42]. The possible explanation for higher drug dissolution for Rigosertib-HPBCD complex compared to Rigosertib – BCD complex is that HPBCD has prominent effect on solubility improvement of Rigosertib compared to BCD.

3.5.2 pH 2 profile

Dissolution profiles for Rigosertib powder was like that in pH 1 dissolution medium (Fig. 11). Rigosertib – BCD complex shows higher dissolution compared to pure Rigosertib. Rigosertib–BCD physical mixture shows improved dissolution compared to pure drug but lower than the inclusion complex. Rigosertib–HPBCD complex gives higher drug released compared to its physical mixture and Rigosertib–BCD complex.

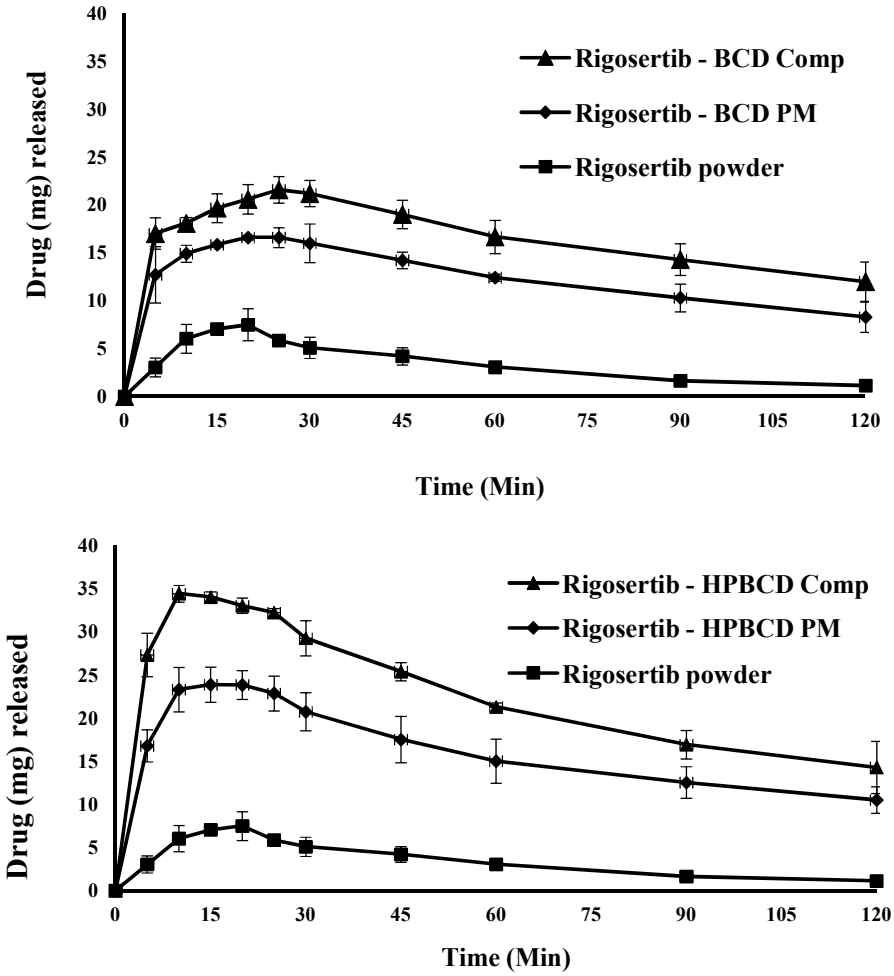


Fig. 11. Overlay of dissolution profiles of Rigosertib, Rigosertib- BCD and Rigosertib-HPBCD in pH 2

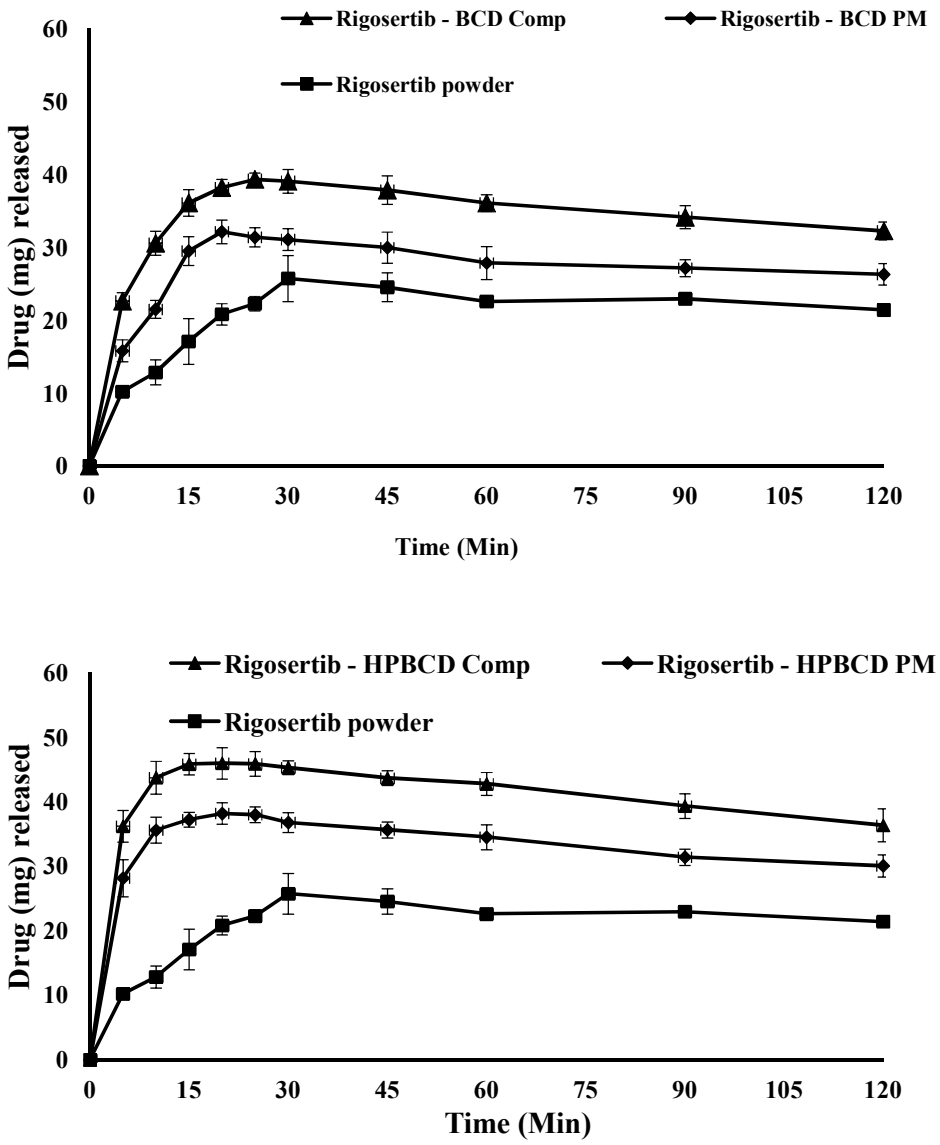


Fig. 12. Overlay of dissolution profiles of Rigosertib, Rigosertib- BCD and Rigosertib-HPBCD in pH 4

3.5.3 pH 4 profile

Dissolution profiles for all the combinations follow patterns like pH 1 and pH 2 mediums. Rigosertib–BCD shows better drug dissolution compared to pure drug, whereas Rigosertib–HPBCD combination gives highest drug released (Fig. 12). Physical mixtures of drug with CD analogues improve drug dissolution profiles but still lower than their respective inclusion complexes.

3.5.4 pH 5.5 profile

Rigosertib is soluble in pH 5.5 buffer giving better dissolution profile for drug powder. Drug released was further increased by Rigosertib–BCD and Rigosertib–HPBCD complex (Fig. 13). Drug release from physical mixtures of drug and CD analogues were comparable to their inclusion complexes.

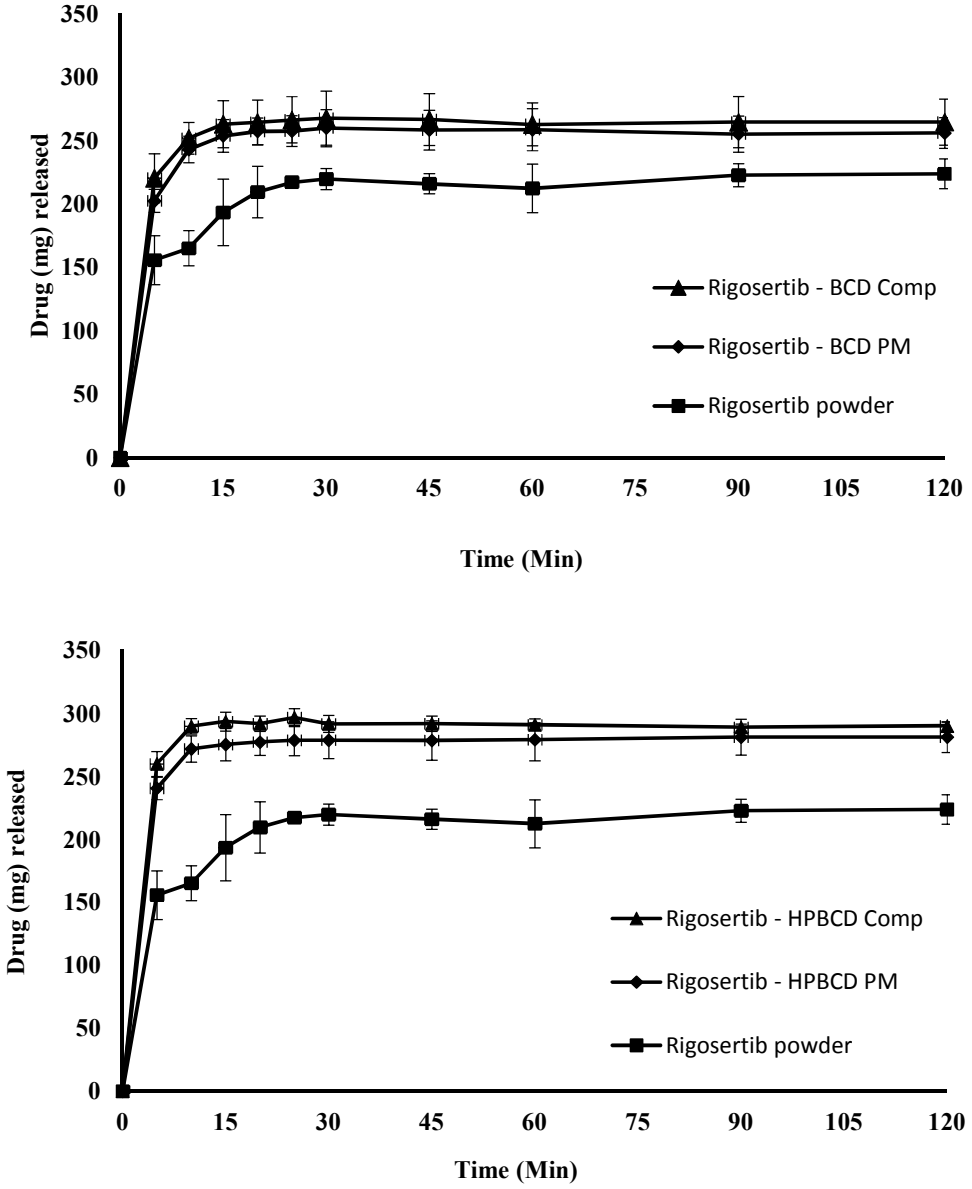


Fig. 13. Overlay of dissolution profiles of Rigosertib, Rigosertib- BCD and Rigosertib-HPBCD in pH 5.5

4. CONCLUSION

The subject research was intended to demonstrate the effect of drug solubility and stability of a novel drug (Rigosertib) using cyclodextrin complexation process. The solubility and drug degradation studies conducted at various pH and temperature conditions clearly indicated poor drug solubility and chemical instability, especially under acid condition. Phase solubility studies results suggested improved drug solubility in presence of BCD and HPBCD. HPBCD shows higher effect on solubility compared to BCD because it is more hydrophilic. Negative Gibbs energy (ΔG) suggests spontaneous reaction between drug and CD to form inclusion complex. Enthalpy (ΔH) values calculated for both BCD and HPBCD were negative, which indicates that reaction is endothermic in nature. From the results, it was

clear that both BCD and HPBCD improve drug stability at all experimental conditions. Increasing BCD and HPBCD further improves drug stability. In DSC thermograms of complexes, crystalline peak for drug disappears completely hinting formation of inclusion complex. XRD data confirmed absence of Rigosertib characteristic pattern in complex. FTIR spectra suggested possible chemical interaction between drug and CD. SEM images of inclusion complexes showed loss of original structures of drug and CD analogs. Comparisons of dissolution profiles of inclusion complexes with Rigosertib pure powder showed higher drug released from inclusion complexes. Higher drug dissolution from inclusion complexes due to ability of CD analogs to improve drug stability and solubility. HPBCD was more effective CD analog as compared to BCD to form inclusion complex due to its ability to improve drug dissolution. The approach to formulate inclusion complexes of Rigosertib with cyclodextrin and its analogues was successful to demonstrate significant improvement in drug solubility and chemical stability.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Chun AW, Cosenza SC, Taft DR, Maniar M. Preclinical pharmacokinetics and in vitro activity of ON 01910.Na, a novel anti-cancer agent. Cancer Chemother Pharmacol. 2009;65(1):177-86.
2. Seetharam M, Fan AC, Tran M, Xu L, Renschler JP, Felsher DW, et al. Treatment of higher risk myelodysplastic syndrome patients unresponsive to hypomethylating agents with ON 01910.Na. Leukemia Research. 2012; 36(1):98-103.
3. Patel H, Maniar M, Chen R, Dave R. Determination of Degradation Kinetics and Effect of Anion Exchange Resin on Dissolution of Novel Anticancer Drug Rigosertib in Acidic Conditions. AAPS Pharm Sci Tech; 2017.
4. Zhang J, Ma PX. Cyclodextrin-based supramolecular systems for drug delivery: Recent progress and future perspective. Advanced Drug Delivery Reviews. 2013; 65(9):1215-33.
5. Ribeiro A, Figueiras A, Santos D, Veiga F. Preparation and solid-state characterization of inclusion complexes formed between miconazole and methyl-beta-cyclodextrin. AAPS Pharm Sci Tech. 2008;9(4):1102-9.
Epub 2008/11/01.
6. Sauceau M, Rodier E, Fages J. Preparation of inclusion complex of piroxicam with cyclodextrin by using supercritical carbon dioxide. The Journal of Supercritical Fluids. 2008;47(2):326-32.
7. Gould S, Scott RC. 2-Hydroxypropyl-beta-cyclodextrin (HP-beta-CD): A toxicology review. Food Chem Toxicol. 2005;43(10): 1451-9.
8. Uekama K, Hirayama F, Irie T. Cyclodextrin Drug Carrier Systems. Chemical Reviews. 1998;98(5):2045-76.
9. Loftssona T, Järvinen T. Cyclodextrins in ophthalmic drug delivery. Advanced Drug Delivery Reviews. 1999;36(1):59-79.
10. Stella V, Rajewski R. Cyclodextrins: Their Future in Drug Formulation and Delivery. Pharm Res. 1997;14(5):556-67.
11. Saenger W. Cyclodextrin Inclusion Compounds in Research and Industry. Angewandte Chemie International Edition in English. 1980;19(5):344-62.
12. Hirayama F, Uekama K. Cyclodextrin-based controlled drug release system. Advanced Drug Delivery Reviews. 1999; 36(1):125-41.
13. Montassier P, Duchêne D, Poelman M-C. Inclusion complexes of tretinoin with cyclodextrins. International Journal of Pharmaceutics. 1997;153(2):199-209.
14. Ficarra R, Ficarra P, Di Bella MR, Raneri D, Tommasini S, Calabro ML, et al. Study of the inclusion complex of atenolol with beta-cyclodextrins. J Pharm Biomed Anal. 2000;23(1):231-6.

15. Wong JW, Yuen KH. Improved oral bioavailability of artemisinin through inclusion complexation with β- and γ-cyclodextrins. International Journal of Pharmaceutics. 2001;227(1–2):177-85.
16. Olivier P, Verwaerde F, Hedges AR. Subchronic toxicity of orally administered beta-cyclodextrin in rats. Journal of the American College of Toxicology. 1991; 10(4):407-19.
17. Bellringer ME, Smith TG, Read R, Gopinath C, Olivier P. β-Cyclodextrin: 52-week toxicity studies in the rat and dog. Food and Chemical Toxicology. 1995; 33(5):367-76.
18. Loftsson T, Jarho P, Masson M, Jarvinen T. Cyclodextrins in drug delivery. Expert Opin Drug Deliv. 2005;2(2):335-51.
19. Gould S, Scott RC. 2-Hydroxypropyl-β-cyclodextrin (HP-β-CD): A toxicology review. Food and Chemical Toxicology. 2005;43(10):1451-9.
20. Govindarajan R, Nagarsenker MS. Formulation studies and in vivo evaluation of a flurbiprofen-hydroxypropyl beta-cyclodextrin system. Pharm Dev Technol. 2005;10(1):105-14.
21. Brewster ME, Loftsson T. Cyclodextrins as pharmaceutical solubilizers. Advanced Drug Delivery Reviews. 2007;59(7):645-66.
22. Higuchi T, Connors K. Advances in Analytical Chemistry and Instrumentation, Chapter 4. Phase Solubility Studies. 1965, 117-212.
23. Tao Y, Han Y, Dong S, Fan X, Wang T, Yan X. Preparation, Solubilization and *In vitro* Anti-tumour Effect of Water-soluble Betulinic Acid/Oligo(polylvinylamino) Bridged bis(β-cyclodextrin)s Complexes, Chemical Science International Journal, 2018;21(2):1-15.
24. Ezz A. Abu-Gharib, Rafat M. EL-Khatib, Lobna A. E. Nassr, Ahmed M. Abu-Dief, Kinetics of base hydrolysis of some chromen-2-one indicator dyes in different solvents at different temperatures, Journal of the Korean Chemical Society. 2011; 55(3):346-353
25. Ezz A. Abu-Gharib, Rafat M. EL-Khatib, Lobna A. E. Nassr, Ahmed M. Abu-Dief, Reactivity trends in the base hydrolysis of 6-nitro-2H-chromen-2-one and 6-nitro-2H-chromen-2-one-3-carboxylic acid in binary mixtures of water with methanol and acetone at different temperatures, Kinetics and Catalysis. 2012;53(2):182–187.
26. Laila H. Abdel-Rahman, Rafat M. El-Khatib, Lobna A. E. Nassr, Ahmed M. Abu-Dief, Reactivity Trends and Kinetic Inspection of Hydroxide Ion Attack and DNA Interaction on Some Pharmacologically Active Agents of Fe(II) Amino Acid Schiff Base Complexes at Different Temperatures, International Journal of Chemical Kinetics. 2014;46(9): 543-553.
27. Ezz A. Abu-Gharib, Rafat M. EL-Khatib, Lobna A. E. Nassr, Ahmed M. Abu-Dief, Kinetics, reactivity, initial-transition state analysis and thermodynamic parameters of base-catalyzed hydrolysis of coumalic acid in solvents with different polarities, Arabian journal of Chemistry. 2017;10:S988–S995
28. Wang X, Luo Z, Xiao Z. Preparation, characterization, and thermal stability of β-cyclodextrin/soybean lecithin inclusion complex. Carbohydrate Polymers. 2014; 101(0):1027-32.
29. Guo P, Su Y, Cheng Q, Pan Q, Li H. Crystal structure determination of the β-cyclodextrin–p-aminobenzoic acid inclusion complex from powder X-ray diffraction data. Carbohydrate Research. 2011;346(7):986-90.
30. Lin SY, Hsu CH, Sheu MT. Curve-fitting FTIR studies of loratadine/hydroxypropyl-β-cyclodextrin inclusion complex induced by co-grinding process. Journal of Pharmaceutical and Biomedical Analysis. 2010;53(3):799-803.
31. Yao Q, You B, Zhou S, Chen M, Wang Y, Li W. Inclusion complexes of cypermethrin and permethrin with monochlorotriazinyl-beta-cyclodextrin: A combined spectroscopy, TG/DSC and DFT study. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2014;117(0): 576-86.
32. Montenegro L, Trapani A, Fini P, Mandracchia D, Latrofa A, Cioffi N, Chiarantini L, Picceri GG, Brundu S and Puglisi G. Chitosan Nanoparticles for Topical Co-administration of the Antioxidants Glutathione and Idebenone: Characterization and *in vitro* Release. British Journal of Pharmaceutical Research. 2014;4(20):2387-2406.
33. Dave RH, Patel H, Donahue E, Patel A. To evaluate the change in release from solid dispersion using sodium lauryl sulfate and model drug sulfathiazole. Drug

Development and Industrial Pharmacy. 2013;39(10):1562-1572.
34. Smith VJ, Rougier NM, de Rossi RH, Caira MR, Buján EI, Fernández MA, et al. Investigation of the inclusion of the herbicide metobromuron in native cyclodextrins by powder X-ray diffraction and isothermal titration calorimetry. Carbohydrate Research. 2009;344(17): 2388-93.
35. Yang LJ, Ma SX, Zhou SY, Chen W, Yuan MW, Yin YQ, et al. Preparation and characterization of inclusion complexes of naringenin with β-cyclodextrin or its derivative. Carbohydrate Polymers. 2013; 98(1):861-9.
36. Dave RH, Patel A, Donahue E, Patel H. To evaluate the effect of addition of an anionic surfactant on solid dispersion using model drug indomethacin. Drug Development and Industrial Pharmacy. 2012;38(8):930-939.
37. Lin SZ, Kohyama N, Tsuruta H. Characterization of steroid/cyclodextrin inclusion compounds by x-ray powder diffractometry and thermal analysis. Ind Health. 1996;34(2):143-8.
38. Sambasevam KP, Mohamad S, Sarih NM, Ismail NA. Synthesis and Characterization of the Inclusion Complex of beta-cyclodextrin and Azomethine. Int J Mol Sci. 2013;14(2):3671-82.
39. Serafini MR, Menezes PP, Costa LP, Lima CM, Quintans LJ Jr., Cardoso JC, et al. Interaction of p-cymene with β-cyclodextrin. J Therm Anal Calorim. 2012; 109(2):951-5.
40. Zhang Y, Ren K, He Z, Li H, Chen T, Lei Y, et al. Development of inclusion complex of brinzolamide with hydroxypropyl-β-cyclodextrin. Carbohydrate Polymers. 2013;98(1):638-43.
41. Qiu N, Cheng X, Wang G, Wang W, Wen J, Zhang Y, et al. Inclusion complex of barbigerone with hydroxypropyl-β-cyclodextrin: Preparation and in vitro evaluation. Carbohydrate Polymers. 2014;101(0):623-30.
42. Kogawa AC, Salgado HRN. Characteristics, complexation and analytical methods of darunavir. British Journal of Pharmaceutical Research. 2014;4(11):1276-1286.

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