Department of Pharmaceutical Chemistry[1], Government College of Pharmacy, Karad, Maharashtra, India; College of Pharmacy and Health Sciences[2], Mercer University, Atlanta, GA, USA

Solid-state characterization and dissolution properties of bicalutamide-β-cyclodextrin inclusion complex

A. L. Patil[1], Y. V. Pore[1], B. S. Kuchekar[1], S. G. Late[2]

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Y. V. Pore, Department of Pharmaceutical Chemistry, Government College of Pharmacy, Karad, Maharashtra, 415124, India
yogeshvpore@rediffmail.com

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The solid-state properties and dissolution profile of bicalutamide β-cyclodextrin (βCD) inclusion complex were investigated. The phase solubility profile of bicalutamide with β-cyclodextrin was classified as A_L-type. Stability constant with 1 : 1 molar ratio was calculated from the phase solubility diagram and the aqueous solubility of bicalutamide was found to be enhanced by 86% for β-cyclodextrin. Binary systems of bicalutamide with βCD were prepared by the kneading method. The solid-state properties of the complex were characterized by differential scanning calorimetry, Fourier transformation-infrared spectroscopy and X-ray powder diffractometry. It could be concluded that bicalutamide could form an inclusion complex with β-cyclodextrin. The dissolution profile of the inclusion complex was determined and compared with those of bicalutamide alone and its physical mixture. The dissolution rate of bicalutamide was significantly increased by complexation with βCD, as compared with pure drug and physical mixture.

1. Introduction

Bicalutamide, chemically, (2*RS*)-4'-cyano-3-(4-fluorophenylsulphonyl)-2-hydroxy-2-methyl-3'-(trifluoromethyl)-propionanilide is an orally active, nonsteroidal antiandrogen (Fradet 2004). It is mainly used in the treatment of prostate cancer (Cockshot et al. 1997). It competitively blocks the growth-stimulating effects of androgens on prostate tumors (Cockshot 2004). The antiandrogenic activity resides almost exclusively in (*R*)-bicalutamide with little activity in (*S*)-bicalutamide (Tucker and Chesterton 1988; Furr et al. 1996; Mukherjee et al. 1996). Though bicalutamide has gained widespread acceptance in the treatment of prostate cancer, it is highly lipophilic drug (log P 2.92) having very low aqueous solubility (5 mg/L) (Cockshot 2004). The drugs with poor aqueous solubility generally show dissolution rate limited absorption (Proudfoot 1991). The low aqueous solubility of bicalutamide may be due to polymorphism and hence the drug has been classified as BCS class II drug according to the biopharmaceutical classification system (Vega et al. 2006). The very low solubility of bicalutamide limits its absorption from the gastrointestinal tract and reduces its oral bioavailability due to poor dissolution. Attempts have been made in order to improve the dissolution rate of bicalutamide in solid dispersion systems (Fuzheng et al. 2006). Improved dissolution rate can be expected to increase oral bioavailability of the drug, which results in reduction of dosing frequency and improves patient compliance. The present work was undertaken to enhance the solubility and dissolution profile of bicalutamide with a beta cyclodextrin (βCD) carrier.

The inclusion complex of bicalutamide with βCD was prepared by the kneading method. The solubility type and the stability constant of the complex were established according to phase solubility studies. The dissolution properties of inclusion complex were studied and compared with bicalutamide alone and physical mixture (PM). Differential scanning calorimetry (DSC), X-ray powder diffractometry (XRD) and Fourier transformation-infrared spectroscopy (FTIR) were used to characterize the solid-state properties of bicalutamide, physical mixture and inclusion complex. The solubility and dissolution behaviour of bicalutamide and its binary systems were further evaluated.

2. Investigations, results and discussion

The % yield of the kneaded product was found to be 94.66%.

The aqueous solubility of the drug increases linearly as a function of βCD. The phase solubility profile of bicalutamide with βCD can be classified as A_L-type. The linear host-guest correlation coefficient $r = 0.9976$ ($r^2 = 0.9951$) with a slope of 0.004037 suggested the formation of a 1 : 1 complex with respect to βCD concentrations. The line equation from the linear regression analysis was found to be as follows:

$$y = 0.004037x + 0.00001271 \quad (1)$$

The apparent stability constant, K_S obtained from the slope of the linear phase solubility diagram was 318.87 M^{-1} (Eq. (1)).

The method used to identify the inclusion complex of drug with CD was differential scanning calorimetry. As

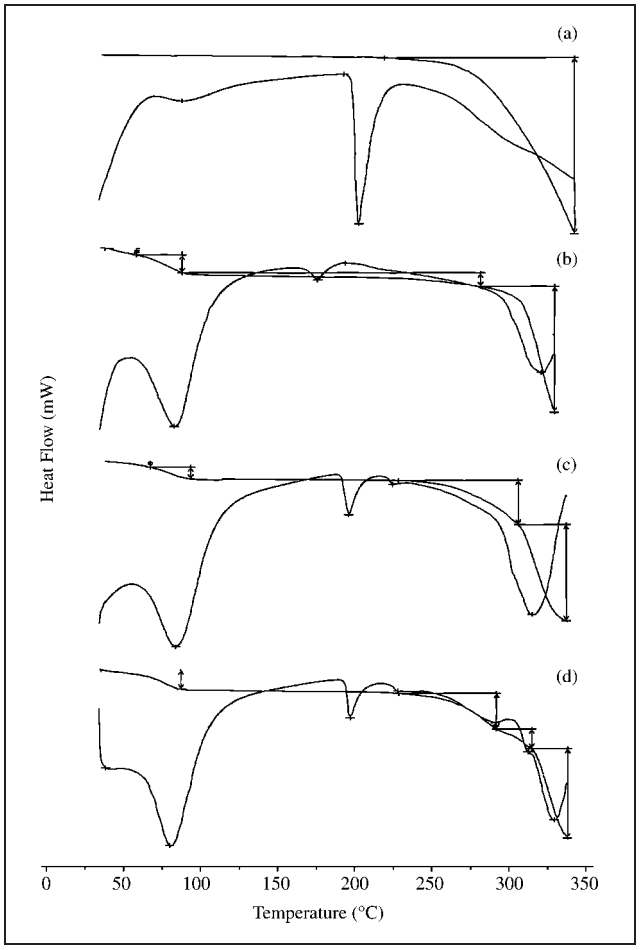

Fig. 1: DSC diagram of bicalutamide-βCD systems: (a) bicalutamide; (b) βCD; (c) physical mixture; (d) inclusion complex

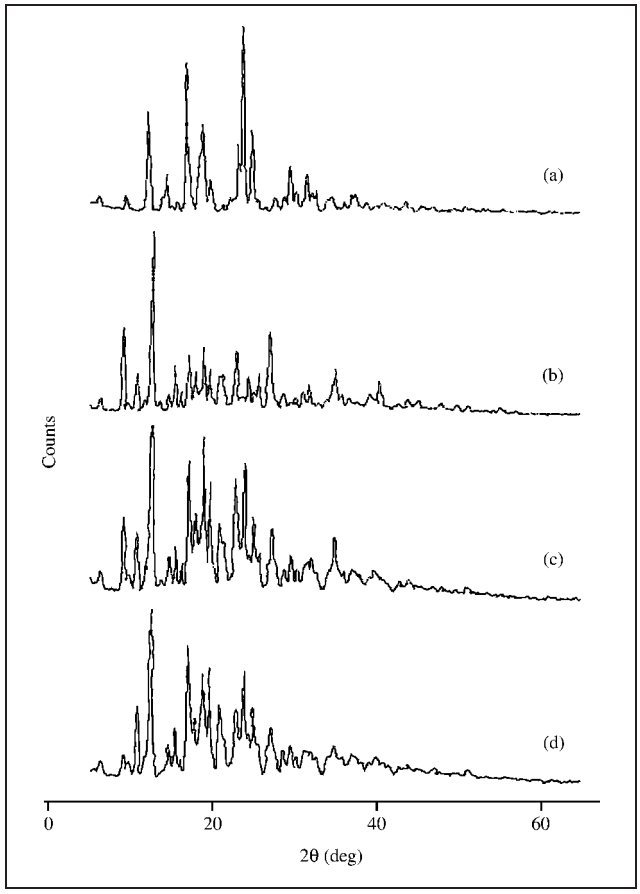

Fig. 2: XRD patterns of bicalutamide-βCD systems: (a) bicalutamide; (b) βCD; (c) physical mixture; (d) inclusion complex

value observed from Fig. 1, DSC thermograms of bicalutamide alone (a) showed an endothermic T_{max} of 198.01 °C, corresponding to the melting point of crystalline form of the drug bicalutamide. In physical mixture (c) it is shifted to lower values, with a decrease in the peak intensity. In inclusion complex (d) the peak of bicalutamide is shifted towards lower temperature 196.87 °C with further decrease in peak intensity. The lower temperature of the inclusion complex was because of melting point depression by the complex (Xianhong et al. 2004). The DSC thermogram of βCD (b) showed a broad endothermic peak at 91.78 °C indicating dehydration process. The peak at 184.37 °C indicated irreversible solid-solid phase transition and the final degradation process has been shown by the broad peak at 328.66 °C. In physical mixture, the peaks of βCD shifted to 85.53 °C and 225.84 °C indicating dehydration and solid-solid phase transition respectively while in inclusion complex, the peaks of βCD shifted to 80.42 °C and 227.55 °C. The DSC thermograms for the complex showed the persistence of the endothermic peak of bicalutamide for the physical mixture and the kneaded product. The kneading process did not substantially affect solid-state properties of PM and the complex, as the thermal behavior of kneaded bicalutamide and PM is similar to the untreated samples.

The XRD pattern (Fig. 2) of bicalutamide (a) showed intense and sharp peaks, indicating its crystalline nature whereas fig. b shows XRD pattern of pure βCD. Crystallinity was determined by comparing some representative peak heights in the diffraction patterns of the binary systems with those of a reference (pure bicalutamide) (Ryan 1986). Bicalutamide (a) showed sharp peaks at 16.97° and 23.85° (2θ) with peak intensities of 538 and 734 respectively. The peak height at 23.85° (2θ) was used for calculating the relative decrease in crystallinity (RDC) of kneaded and physical mixture binary system. The RDC values of corresponding binary systems were 0.3488 and 0.4073 respectively. The diffraction pattern of physical mixture (c) showed peaks of bicalutamide and βCD with little decrease in the peak intensity of bicalutamide indicating reduction in crystallinity. However in kneaded system (d) the crystallinity of bicalutamide was reduced to a greater extent as compared to physical mixture. Further, the peak at 16.97° of bicalutamide in the kneaded system was completely disappeared indicating formation of inclusion complex.

Figure 3 illustrates the FTIR spectra of bicalutamide, βCD, physical mixture and inclusion complex. IR spectrum of bicalutamide (a) is characterized by principal absorption peaks at 3057 cm^{-1} (C−H aromatic), 2939 cm^{-1} (C−H aliphatic asymmetric), 2893 cm^{-1} (C−H aliphatic symmetric), 2231 cm^{-1} (C≡N), 1689 cm^{-1} (C=O), 3577 cm^{-1} (O−H), 3336 cm^{-1} (N−H), 1323 cm^{-1} (S=O), 1595 cm^{-1} (C=C aromatic), 1028 cm^{-1} (C−O), 705 cm^{-1} (p substituted benzene) and 844 cm^{-1} (m and p substituted benzene), 1238 cm^{-1} (C−F monofluorinated benzene). The IR spectrum of βCD (b) shows prominent peaks at 3352 cm^{-1} (O−H), 2922 cm^{-1} (C−H), 1641 cm^{-1} (H−O−H bending), 1151 cm^{-1} (C−O), 1035 cm^{-1} (C−O−C). The intense peaks appeared in the spectrum of bicalutamide and βCD are due to symmetric or asymmetric stretching vibrations of the functional groups. Bicalutamide shows strong absorption peaks at 2231 cm^{-1} and 1689 cm^{-1} indicating presence of cyanide and amide carbonyl group respectively

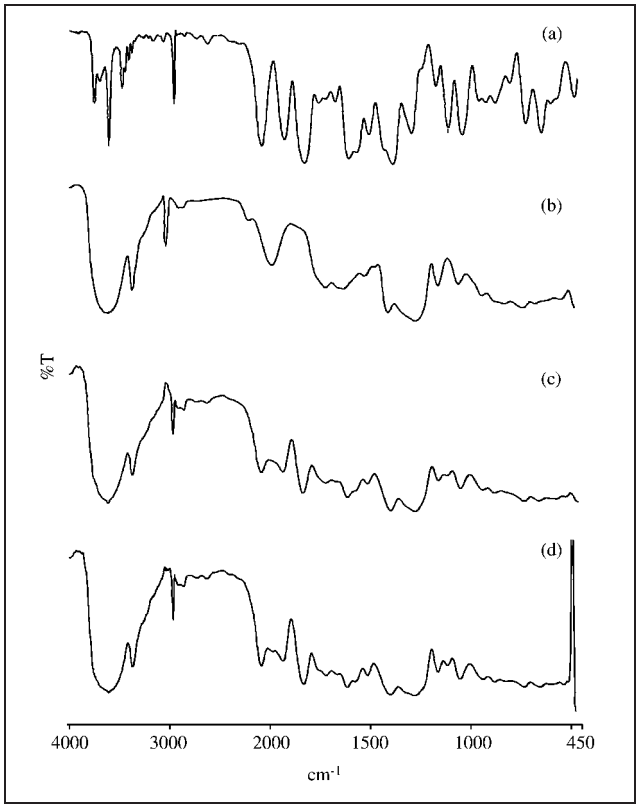

Fig. 3: FTIR spectra of bicalutamide-βCD systems: (a) bicalutamide; (b) βCD; (c) physical mixture; (d) inclusion complex

while, peaks at 705 and 844 cm^{-1} may be assigned to aromatic stretching of the phenyl group in the molecule which is substituted. In IR spectra of PM (c), the peaks at 3057 cm^{-1}, 2939 cm^{-1}, 2893 cm^{-1}, 3577 cm^{-1} and 705 cm^{-1} of bicalutamide are not visible. However, the peak at 2231 cm^{-1} appeared with decreased intensity and the peak at 1689 cm^{-1} was shifted to 1687 cm^{-1}. All other peaks of bicalutamide were smoothened indicating strong physical interaction of bicalutamide with βCD. In IR spectra of inclusion complex (d) the peaks of bicalutamide at 3057 cm^{-1}, 2939 cm^{-1}, 2893 cm^{-1}, 3577 cm^{-1} and 705 cm^{-1} were completely disappeared. Further, the peak at 1238 cm^{-1} of C–F (monofluorinated benzene) was shifted to 1240 cm^{-1} with decrease in peak intensity indicating that aromatic ring of guest has been entrapped in the hydrophobic cavity of host molecule. The peak of OH group of βCD at 3352 cm^{-1} was shifted towards lower frequency 3298 cm^{-1} due to intermolecular hydrogen bonding with bicalutamide (d). The peak at 1641 cm^{-1} in IR spectra of βCD due to water of crystallization, was also disappeared in both PM and inclusion complex (Mukne and Nagarsenker 2004). These changes occurred in IR spectra of samples indicated formation of inclusion complex in solid state.

The binary systems of bicalutamide showed enhancement in the solubility as compared to pure drug alone (Table 1). The 1 : 1 inclusion complex of bicalutamide with βCD showed higher solubility than their physical mixture and pure drug alone. The enhancement in the solubility of complex is mainly attributed to the formation of a stable inclusion complex of bicalutamide with βCD. The stability constant, 318.87 M^{-1} suggests that bicalutamide and βCD are having sufficient affinity towards each other to form stable inclusion complex, as the solubility of complex was found to be increased by 86%. The physical mixture has also shown higher solubility than the pure drug. The enhancement in aqueous solubility of bicalutamide can be explained in terms of wetting property and hydrophilicity of βCD with simultaneous reduction in the crystallinity of the drug caused by the kneading process and inclusion into the hydrophobic cavity of the βCD (Longxiao and Suyan 2006).

Table 1: Solubility data of bicalutamide, physical mixture (PM) and inclusion complex

System	Solubility in water at 25 °C mg/L
Bicalutamide	8.85
Physical mixture (PM)	12.63
Bicalutamide-βCD complex	16.45

The dissolution curves of bicalutamide, physical mixture and inclusion complex in 1% SLS in water at 37 ± 0.5 °C are shown in Fig. 4. The release rate profiles were expressed as the percentage of drug released (vs.) time. The dissolution time of bicalutamide from inclusion complex and physical mixture was determined and $t_{90\%}$ values are reported in Table 2 compared to bicalutamide alone. According to these results, the time required to release 90% drug for physical mixture was 60 min while inclusion complex released 90% drug within 19 min. However, the release of bicalutamide from pure drug was incomplete even in 60 min. Binary systems, PM and complex showed higher dissolution rate than the pure drug. This is because of the hydrophilicity and wetting property of βCD. The kneaded product has shown highest dissolution rate as compared to the physical mixture and pure drug, indicating complete release of bicalutamide from the complex. The enhancement in dissolution rate has been attributed to the formation of an inclusion complex in the solid state with reduction in the crystallinity of bicalutamide, as confirmed by XRD studies. The dissolution rate increase for the physical mixture and inclusion complex is due to greater hydrophilicity, higher wetting effect and ability to form stable inclusion complex of the βCD.

In conclusion, the present investigation shows that bicalutamide can form an inclusion complex with βCD in solid state. The stoichiometry of complex formation is in 1 : 1 molar ratio with better stability constant. From these results, it can be assumed that the formation of the inclusion complex of bicalutamide with βCD can increase the aqueous solubility of bicalutamide. The improved dissolution

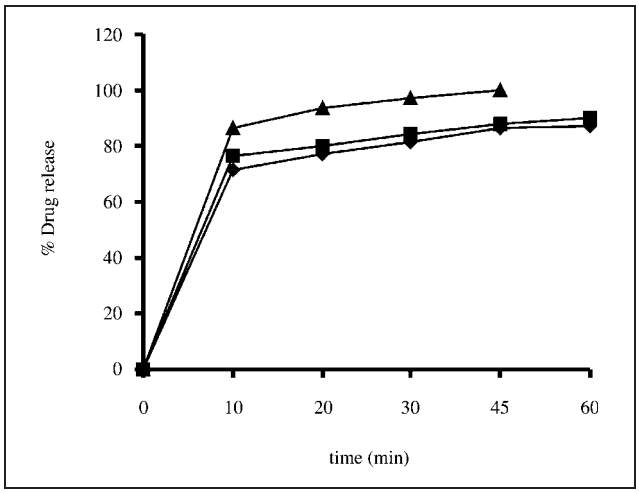

Fig. 4: The dissolution diagram of bicalutamide-βCD systems at 37 °C ± 0.5 °C: (◆) bicalutamide; (■) physical mixture; (▲) inclusion complex

Table 2: The dissolution time of bicalutamide in 1% SLS in water at 37 ± 0.5 °C

Sample source	Dissolution time (min)
Bicalutamide	>60
Physical mixture	60
Inclusion complex	19

rate may be due to increase in solubility, brought about by complexation. From these evidences it can be concluded that the aqueous solubility and dissolution rate of bicalutamide can be significantly increased by forming an inclusion complex with βCD.

3. Experimental

3.1. Materials

Bicalutamide was supplied by Lupin Ltd., Mumbai, India as a gift sample. βCD was provided by Panacea Biotech, Chandigarh, India. All the reagents were of analytical grade. Double distilled water was used throughout the experiment.

3.2. Phase solubility studies

Phase solubility studies were carried out in water according to the method described by Higuchi and Connors (1965). Excess amount of bicalutamide (50 mg) was added to 20 ml of aqueous solution containing various concentrations of βCD (0–0.01 M). Then, the suspensions were shaken on a rotary shaker at 25 ± 2 °C for 7 days. After equilibrium was achieved, the samples were filtered through a 0.45 μm membrane filter and appropriately diluted. The concentration of bicalutamide was determined spectrophotometrically (Shimadzu 1700, Japan) at 269 nm. The apparent stability constant K_s was calculated from phase solubility diagrams with the assumption of 1 : 1 stoichiometry according to the following equation:

$$K_S = \frac{\text{slope}}{S_0(1 - \text{slope})} \quad (2)$$

S_0 is the solubility of bicalutamide in absence of CDs.

3.3. Preparation of solid binary systems

The following binary systems of bicalutamide and βCD were prepared at 1 : 1 molar ratio.

3.3.1. Physical mixture of bicalutamide and β-cyclodextrin

The physical mixture (PM) of bicalutamide and βCD in 1 : 1 molar ratio was prepared by mixing individual components that had previously been sieved through sieve no. 60.

3.3.2. Inclusion complex by the kneading method

Bicalutamide and βCD with 1 : 1 molar ratio were accurately weighed and transferred to mortar. The mixture was then triturated in a mortar with a small volume of water-ethanol (1 : 1 v/v) solution until a homogenous paste was formed. The paste that formed was kneaded for 45 min and then dried at 45 °C in an oven. The dried mass was pulverized and sieved through sieve no. 60.

3.4. Differential scanning calorimetry (DSC)

DSC measurements were performed on a TA SDT 2960 DSC (USA) differential scanning calorimeter. The accurately weighed sample was placed in an aluminum pan. An empty aluminum pan was used as reference. The experiment was carried out in nitrogen atmosphere (flow rate 100 ml/min) at scanning rate of 10 °C/min in the range of 0–350 °C.

3.5. X-Ray powder diffractometry (XRD)

The XRD patterns of bicalutamide, βCD, inclusion complex, and physical mixture were recorded by a Philips Analytic X-Ray – PW 3710 (Holland) diffractometer with tube anode Cu over the interval 5–70°/2θ. The operation data were as follows: Generator tension (voltage) 40 kV, Generator current 30 mA, and scanning speed 2°/min.

3.6. Fourier transformation-infrared spectroscopy (FTIR)

Infrared spectra were obtained using a Perkin-Elmer Spectrum- one FTIR spectrometer or Jasco FTIR 4100 (Japan) using KBr disks. The samples were previously ground and mixed thoroughly with KBr. The KBr disks were prepared by compressing the powder. The scanning range was kept from 4000 to 450 cm^{-1}.

3.7. Solubility studies

Solubility studies were performed according to the method reported by Higuchi and Connors (1965). Excess of pure drug, physical mixture and inclusion complex were added to 20 ml of distilled water taken in stoppered conical flasks and shaken for 24 h in a rotary flask shaker at room temperature. After shaking to achieve equilibrium, appropriate aliquots were withdrawn and filtered through Whatman filter paper no. 41. The filtrate so obtained was analysed spectrophotometrically at 269 nm.

3.8. Dissolution studies

The *in vitro* dissolution rate studies of bicalutamide alone, physical mixture and inclusion complex were performed using USP 6-stage dissolution rate apparatus (Model: Veego DA-6-D tablet dissolution test apparatus, Mumbai) with a paddle stirrer. Dissolution studies were carried out using 1000 ml of 1% SLS (Sodium lauryl sulphate) in water at 37 ± 0.5 °C at 50 rpm. 50 mg of bicalutamide or its equivalent amount of bicalutamide-cyclodextrin complex was added to 1000 ml of 1% SLS in water. 5 ml of samples were withdrawn at time intervals of 10, 20, 30, 45, and 60 min (U.S. FDA 2006). The volume of dissolution medium was adjusted to 1000 ml by replacing each 5 ml aliquot withdrawn with 5 ml of fresh 1% SLS in water. The solution was immediately filtered through 0.45 μm membrane filter, suitably diluted and the concentrations of bicalutamide in samples were determined spectrophotometrically at 269 nm.

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