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EFFECT OF L-ARGININE ON BICALUTAMIDE COMPLEXATION WITH HYDROXYPROPYL-β-CYCLODEXTRIN

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Solid-state properties and dissolution behaviour of bicalutamide-hydroxypropyl-β-cyclodextrin binary and bicalutamide-arginine-hydroxypropyl-β-cyclodextrin ternary systems were investigated. The effect of L-arginine (1% w/v) on stability constant of bicalutamide-hydroxypropyl-β-cyclodextrin was also studied. The phase solubility studies of bicalutamide with HPβCD were performed for seven days while in presence of arginine for two days. Stability constants with 1:1 molar ratio were calculated from the phase solubility diagram. The phase solubility studies revealed that the stability constant was significantly improved in presence of arginine (1% w/v) within two days ($p < 0.001$). The aqueous solubility of bicalutamide was increased by 91% in binary complex and 206% in ternary complex with arginine. The binary and ternary systems prepared by kneading method in equimolar quantities were characterized by Fourier transformation-infrared spectroscopy and X-ray powder diffractometry. The dissolution properties of binary and ternary systems were determined and compared with those of bicalutamide alone. The ternary systems have shown several times faster dissolution rate than binary systems of bicalutamide.

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Keywords: Stability constant; bicalutamide; L-arginine; cyclodextrin; dissolution, ternary system.

1. Introduction

The formulation of poorly water-soluble drugs is one of the most challenging tasks to the formulation experts. The aqueous solubility plays an important role in the development of new chemical entities as successful drugs. An enhancement in the solubility and the dissolution rate can improve the oral bioavailability of such drugs, which further improves the therapeutic efficacy and patient compliance. Various formulation methods were used to enhance the solubility of poorly soluble drugs. Cyclodextrin inclusion complex of hydrophobic drug is one of the techniques to enhance the solubility and dissolution rate of poorly soluble drug [1].

Cyclodextrins served as a versatile carrier for the poorly soluble drugs by increasing its solubility and dissolution rate through formation of inclusion complex. However, high molecular weight, high cost, relatively low water solubility, potential toxicity limits the use of cyclodextrin [2-5]. Hydroxyacids or certain low molecular weight acids can strongly increase solubilizing capacity of cyclodextrin towards basic drugs due to simultaneous effect of salt formation and inclusion complexation [6-10]. The effect of PVP K25, arginine and other hydrophilic polymers at different pH on naproxen solubilization with cyclodextrins has been also reported [11-13].

Bicalutamide (Fig.1), chemically, (2RS)-4'-cyano-3-(4-fluorophenylsulphonyl)-2-hydroxy-2-methyl-3'-(trifluoromethyl)-propionanilide is an orally active, nonsteroidal antiandrogen mainly used in the treatment of prostate cancer [14,15]. It competitively blocks the growth-stimulating effects of androgens on prostate tumors [16]. The antiandrogenic activity resides almost exclusively in (*R*)-bicalutamide with little activity in (*S*)-bicalutamide [17-19]. Though bicalutamide has gained widespread acceptance in the treatment of prostate cancer, it suffers from certain disadvantages such as high lipophilicity (log P 2.92) and poor aqueous solubility (5 mg/L) [16]. The drugs with poor aqueous solubility generally show dissolution rate

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limited absorption [20]. The low aqueous solubility of bicalutamide may be due to polymorphism exhibited by it and hence the drug has been classified as BCS class II drug according to the biopharmaceutical classification system [21]. The very low solubility of bicalutamide limits its absorption from gastrointestinal tract and reduces its oral bioavailability due to poor dissolution. Certain efforts have been taken in order to improve the dissolution rate of bicalutamide in solid dispersion system [22]. Improved dissolution rate can be expected to increase oral bioavailability of drug, which results in reduction of dosing frequency and improves patient compliance.

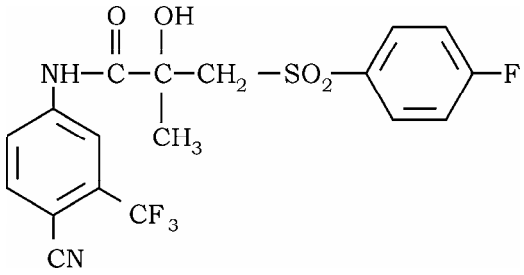

Fig. 1 Chemical structure of bicalutamide.

This work was aimed to investigate the solid-state properties of bicalutamide in its binary and ternary systems with HPβCD and to improve its dissolution profile. The effect of an amino acid, L-arginine 1% (w/v) on phase solubility curve of bicalutamide-HPβCD was also investigated. The solubility type and the stability constants of the complexes were established. The results obtained from phase solubility studies served as a basis for proper choice of L-arginine (1% w/v) as a carrier in ternary systems. The inclusion complexes of bicalutamide with HPβCD were prepared by kneading method. The dissolution properties of inclusion complexes were studied and compared with bicalutamide alone and their corresponding physical mixtures (PM). X-ray powder diffractometry (XRD) and Fourier transformation-infrared spectroscopy (FTIR) were used to characterize the solid-state properties of bicalutamide, physical mixtures and inclusion complexes. The solubility and dissolution behaviour of bicalutamide and its binary and ternary systems were further evaluated.

2. Materials & Methods

2.1 Materials

Bicalutamide was supplied by Lupin Ltd., Mumbai, India as a gift sample. HPβCD was kindly provided by Panacea Biotech, Chandigad, India. L-arginine was purchased from S.D. Fine Chemicals, Mumbai, India. All the reagents were of analytical grade. Double distilled water was used throughout the experiment.

2.2 Methods

2.2.1. Phase solubility studies

Phase solubility studies were carried out in triplicate in water according to the method described by Higuchi and Connors [23]. Excess amount of bicalutamide (50 mg) was added to 20 ml of aqueous solution containing various concentrations of HPβCD (0–0.01 M) with or without fixed concentrations of L-arginine (1% w/v). Then, the suspensions were shaken on rotary shaker at 25 ± 2°C for 7 days for binary system and 2 days for ternary system. After equilibrium was achieved, the samples were filtered through 0.45 μm membrane filter and appropriately diluted. The concentration of bicalutamide was determined spectrophotometrically (Shimadzu 1700, Japan) at 269nm. The apparent 1:1 stability constant were calculated from the phase solubility diagrams, according to the following equation:

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

S_0 is the solubility of bicalutamide in absence of CDs.

The results obtained from phase solubility studies were statistically validated using ANOVA (Tukey-Kramer Multiple Comparisons Test).

2.2.2. Preparation of solid binary and ternary systems

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

Preparation of physical mixtures of binary and ternary systems

The physical mixtures (PM) of binary and ternary systems in 1:1 molar ratio were prepared by mixing individual components that had previously been sieved through sieve no. 60.

Preparation of inclusion complexes by kneading method

Bicalutamide and HPβCD with 1:1 molar ratio were accurately weighed and transferred to mortar. Similarly bicalutamide, HPβCD, L-arginine 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 till 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.

2.2.3. 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}.

2.2.4. X-ray powder diffractometry (XRD)

The XRD patterns of bicalutamide, HPβCD, L-arginine, inclusion complex, and physical mixture for binary and ternary systems were recorded by using 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.

2.2.5. Solubility studies

Solubility studies were performed according to the method reported by Higuchi and Connors [23]. Excess of pure drug, physical mixture and inclusion complex for binary and ternary systems were added to 20 ml of distilled water taken in stoppered conical flasks and shaken for 24 hrs in 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.

2.2.6. Dissolution studies

The dissolution rate studies of bicalutamide alone, physical mixture and inclusion complex of binary and ternary systems were performed in a dissolution apparatus (Lab India) using the paddle method, according to USP Type II apparatus. 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 binary and ternary 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 [24]. 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.

3. Results and discussion

3.1. Determination of % yield of binary and ternary systems

The % yield of the kneaded products for binary system and ternary systems was found to be 93.20% and 94.80% respectively.

3.2. Phase solubility studies of binary and ternary systems

The phase-solubility diagram for the complex formation between bicalutamide and HPβCD in binary and ternary systems with 1% (w/v) arginine is presented in Fig. 2. This plot showed that the aqueous solubility of the drug increases linearly as a function of HPβCD concentration. The phase solubility profile for binary and ternary systems can be classified as A_L-type. For binary system the linear host-guest correlation coefficient r =0.9987 (r^2 = 0.9975) with a slope of 0.002369 suggested the formation of a 1:1 complex with respect to HPβCD concentrations. For ternary system with 1% (w/v) arginine, r = 0.9965 (r^2= 0.9931) with a slope 0.003199 suggested the formation of 1:1 complex. The line equations from the linear regression analysis for these systems were

$$y = 0.002369x + 0.00001472 \text{ (binary system)} \quad (2)$$

$$y = 0.003199x + 0.00001642 \text{ (ternary system with 1\% (w/v) arginine)} \quad (3)$$

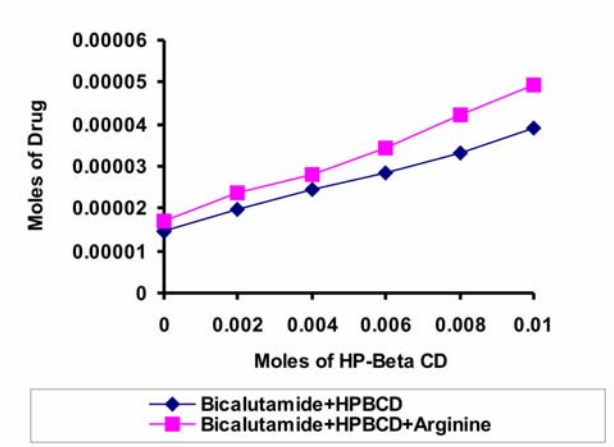

Fig. 2 Phase solubility diagram of bicalutamide–HPβCD system in presence and absence of L-arginine in water.

The apparent stability constants $K_{1:1}$ obtained from the slope of the linear phase solubility diagram for binary and ternary systems are represented in Table 1. The phase solubility curve of L-arginine in 1% (w/v) shows significant increase in the slope of the curve with little increase in the intrinsic solubility of drug. This indicated that L-arginine has improved the stability constant remarkably when used in the concentration of 1% (w/v) ($p < 0.001$) as the equilibrium has achieved within two days. The significant enhancement obtained in stability constant of bicalutamide might be attributed to electrostatic effect of arginine with drug and HPβCD. L-arginine interacts simultaneously with the drug (via electrostatic interaction and salt formation) and HPβCD (via hydrogen bonding) [12]. Form these studies it could be concluded that arginine has not only improved the complexation efficiency of HPβCD towards bicalutamide but also promoted the rate of complex formation.

Table 1. Effect of amino acid, L-arginine (1% w/v) on the intrinsic solubility ($S_{o)}$, slope of phase-solubility diagrams and stability constant (K_c) for binary and ternary systems of bicalutamide with HPβCD.

System	S_0 (μg/mL)	Slope	r^2	K_c (M^{-1})* Mean ± S.D.	K_{TS}/K_{BS}
Drug-HPβCD	6.27	0.002369	0.9975	161.36 ± 2.27	---
Drug-HPβCD-arginine	7.25	0.003199	0.9931	195.91 ± 2.48 †	1.21

K_{TS}/K_{BS} is the ratio of K_c for ternary and binary complexes; * Indicates mean of three readings; S.D.: Standard deviation. †: *p* value compared to bicalutamide-HPβCD ($p < 0.001$) i.e. significant.

3.3. Fourier transformation-infrared spectroscopy (FTIR)

Fig. 3A illustrates the FTIR spectra of bicalutamide, HPβCD, physical mixture and inclusion complex in binary system. 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), 844 cm^{-1} (*p* substituted benzene), 1238 cm^{-1} (monofluorinated benzene) and 1137 cm^{-1} (C-F of CF_3). The IR spectrum of HPβCD (b) shows prominent peaks at 3390 cm^{-1} (O-H), 2928 cm^{-1} (C-H), 1647 cm^{-1} (H-O-H bending). In IR spectra of PM (c), the peaks at 3057 cm^{-1}, 2939 cm^{-1}, 2893 cm^{-1}, 3577 cm^{-1} of bicalutamide are not visible whereas the peak at 3336 cm^{-1} was shifted to 3338 cm^{-1}. However, the peak at 2231 cm^{-1} appeared with decreased intensity and shifted to 2230 cm^{-1} while the peak at 1689 cm^{-1} and 844 cm^{-1} was shifted to 1688 cm^{-1} and 840 cm^{-1} respectively. The peak at 1137 cm^{-1} (C-F of CF_3) was also shifted to 1179 cm^{-1}. All other peaks of bicalutamide were smoothened indicating strong physical interaction of bicalutamide with HPβCD. 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 844 cm^{-1} were completely disappeared. Further, the peak at 1238 cm^{-1} of C-F (monofluorinated benzene) was shifted to 1242 cm^{-1} with decrease in peak intensity. The peak at 1137 cm^{-1} (C-F of CF_3) was also shifted to 1142 cm^{-1}. However, the peak of bicalutamide at 3336 cm^{-1} (N-H) was observed at 3338 cm^{-1} in both PM and complex due to intermolecular hydrogen bonding. These changes occurred in IR spectra of samples indicated that aromatic ring of guest has been entrapped in the hydrophobic cavity of host molecule and formation of inclusion complex in solid state. In both the binary systems (PM and complex) the broad peak of HPβCD (b) at 3390 cm^{-1} was disappeared. However, the intensity of the peak was strongly reduced in complex. This suggested that, bicalutamide could form inclusion complex with HPβCD in solid state.

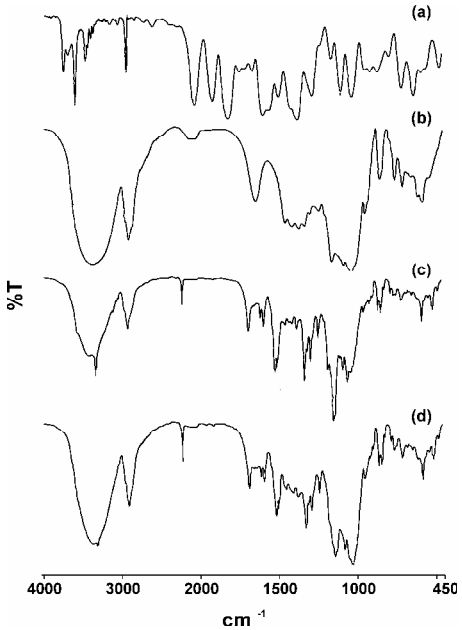

Fig. 3A FTIR spectra of bicalutamide-HPβCD binary systems: (a) bicalutamide; (b) HPβCD; (c) physical mixture; (d) inclusion complex.

Both the ternary systems of bicalutamide (PM and complex) have shown somewhat different pattern of IR spectra as compared to the binary systems (Fig 3B). Pure L-arginine (c) shows typical absorption peaks of amino acid at 3106 cm^{-1} (N-H stretch of NH_3), 1645 cm^{-1} (NH_3 bending), 1605 cm^{-1} (carboxylate ion) and 1472 cm^{-1} (C=N). All these peaks of L-arginine have been disappered in both PM (d) and complex (e). However, the peak of drug at 844 cm^{-1} was shifted to 840 cm^{-1} in both PM and complex. An additional peak was observed in complex at 1613 cm^{-1}, which may be because of salt formation. Both the binary and ternary systems of bicalutamide-HPβCD did not show any new peak, indicating no chemical bond formation between drug and HPβCD in the complexes.

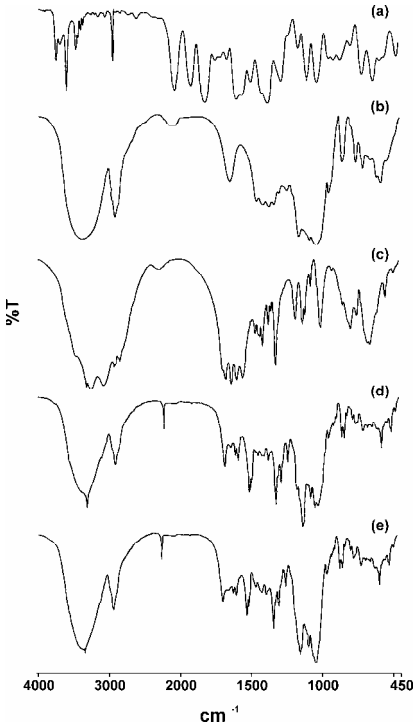

Fig. 3B FTIR spectra of bicalutamide-HPβCD ternary systems: (a) bicalutamide; (b) HPβCD; (c) arginine; (d) physical mixture; (e) inclusion complex.

Bicalutamide serves as an excellent candidate for cyclodextrin inclusion complexation because of presence of two substituted aromatic rings. Therefore, it is very critical to determine as to which aromatic ring has been entrapped in the cyclodextrin cavity. However, the entrapment of aromatic ring depends upon its hydrophobic nature, overcrowding and nature of the subsistent on it. In bicalutamide, monofluoro substituted aromatic ring is more hydrophobic and less overcrowded than trifluromethyl substituted aromatic ring. Further presence of acetamido and cyano functional groups increases its polarity with decreasing its affinity towards cyclodextrin. In monofluoro substituted aromatic ring, fluorine also increases its hydrophobic character increasing its ability to get entrapped in the cavity of host molecule [25].

3.4. X-ray powder diffractometry (XRD)

The XRD pattern of bicalutamide showed (Fig. 4) intense and sharp peaks, indicating its crystalline nature. Crystallinity was determined by comparing some representative peak heights in the diffraction patterns of the binary and ternary systems with those of a reference (pure bicalutamide [26]. The peak intensities of pure bicalutamide and its corresponding binary and ternary systems are presented in Table 2. 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 of binary systems (Fig. 4A). The RDC values of corresponding binary systems were 0.2343 and 0.2861 respectively. The diffraction pattern of physical mixture (c) showed peaks of bicalutamide and HPβCD 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 kneaded system was completely disappeared indicating formation of inclusion complex. The RDC values of ternary complex of bicalutamide-arginine-HPβCD (Fig. 4B, e) can not be calculated as the peak of bicalutamide at the same angle has been completely disappeared in this ternary system. Only PM (d) shows peak at 16.95° with RDC value of 0.3085. The diffraction pattern of all binary and ternary systems of bicalutamide also showed other peaks of drug with decrease in the peak intensity indicating reduction in crystallinity. However in the ternary systems, the crystallinity of bicalutamide was reduced to a greater extent, evidenced by complete disappearance of intense of peaks of bicalutamide.

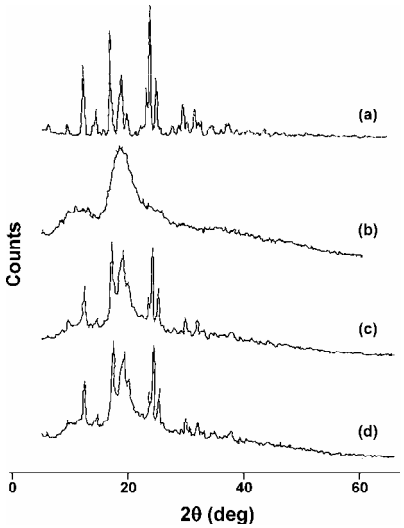

Fig. 4A XRD patterns of bicalutamide-HPβCD binary systems: (a) bicalutamide; (b) HPβCD; (c) physical mixture; (d) inclusion complex.

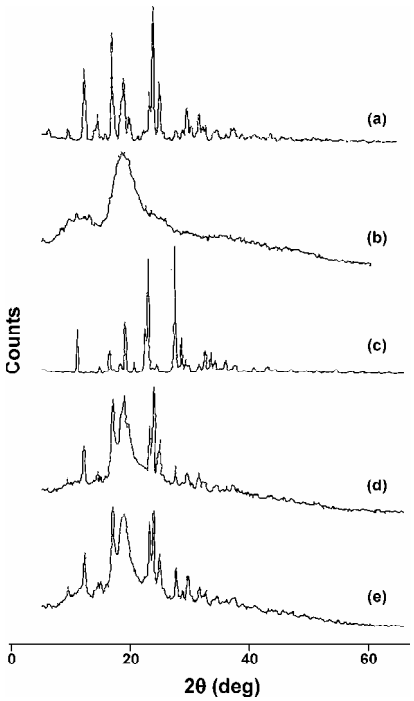

Fig. 4B XRD patterns of bicalutamide-HPβCD ternary systems: (a) bicalutamide; (b) HPβCD; (c) arginine; (d) physical mixture; (e) inclusion complex.

Table 2. Peak intensities of bicalutamide in the XRD patterns of bicalutamide- HPβCD binary and ternary systems.

2θ	Drug	Drug: HPβCD binary system		Drug: Arginine: HPβCD ternary system	
		PM	KN	PM	KN
16.97	538	199	----	166	159
19.05	335	142	77	123	121
23.85	734	210	172	164	----
24.98	303	90	72	94	66

PM: Physical mixture; KN: Kneaded product (complex)

3.5. Solubility studies

The binary and ternary systems of bicalutamide showed enhancement in the solubility as compared to pure drug alone (Table 3). The 1:1 inclusion complex of binary and ternary systems showed higher solubility than their physical mixture and pure drug alone. The enhancement in the solubility of complex is mainly attributed due to the formation of stable inclusion complex of bicalutamide with HPβCD. The stability constant, 161.36 ± 2.27 M^{-1} for binary system within 7 days suggested that HPβCD and bicalutamide are having sufficient affinity towards each other to form stable inclusion complex. The solubility of complex was increased by 91%. 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 hydrophillicity of HPβCD with simultaneous reduction in the crystallinity of the drug caused by the kneading process and inclusion into the hydrophobic cavity of the HPβCD [27]. However in case of ternary system with L-arginine 1% (w/v), the solubility of complex was increased by 206%, compared to drug as there was significant improvement in stability constant (195.91 ± 2.48 M^{-1}) within 2 days.

Table 3. Solubility study of bicalutamide with HPβCD in binary system and ternary system with 1% (w/v) L-arginine in water.

System	Solubility in water at 25°C μg/mL* (Mean ± S.D.)	S.E.M.
Bicalutamide	6.40 ± 0.59	0.34
PM binary	10.82 ± 0.95	0.55
KN binary	12.20 ± 0.71	0.41
PM ternary	16.51 ± 0.52	0.30
KN ternary	19.59 ± 0.83	0.48

* Indicates mean of three readings; S.D.: standard deviation; S.E.M.: Standard error of mean; PM: physical mixture; KN: kneaded product (complex)

3.6. Dissolution rate studies

The dissolution curves of bicalutamide, physical mixtures and inclusion complexes for binary and ternary systems in 1% SLS in 1000ml water at 37 ± 0.5°C are shown in Fig. 5. The release rate profiles were expressed as the percentage of drug released (vs.) time. The dissolution time of bicalutamide from inclusion complexes and physical mixtures were determined and $t_{90\%}$ values are reported in Table 4 compared to bicalutamide alone. According to these results, for the binary systems, the time required to release 90% drug from physical mixture was 45 min while inclusion complex released 90% drug within 15 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 higher hydrophillicity and wetting property of HPβCD.

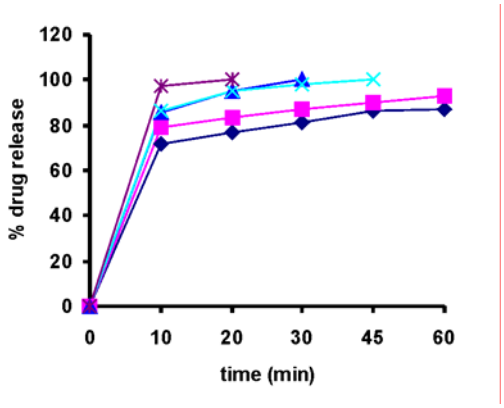

Fig. 5 The dissolution diagram of bicalutamide-HPβCD systems at 37℃ ± 0.5℃: (◆) bicalutamide; (■) physical mixture binary; (▲) inclusion complex binary; (✳) physical mixture ternary; (✱) inclusion complex ternary.

Table 4. The dissolution time of bicalutamide in 1% SLS in water at 37 ±. 0.5℃ from binary and ternary systems.

Sample source	Dissolution time (min) t_{90}
Bicalutamide	> 60
Physical mixture binary	45
Inclusion complex binary	15
Physical mixture ternary	13
Inclusion complex ternary	< 10

For ternary systems, the time required to release 90% drug from physical mixture was 13 min while inclusion complex released 90% drug in < 10 min. The increased dissolution rate in ternary systems of bicalutamide is attributed due to presence of L-arginine, which can form salt with drug giving amphiphilic structure characterized by a strongly hydrophobic portion and a hydrophilic polar head. In the presence HPβCD, the hydrophobic portion of drug molecule can interact with the hydrophobic cyclodextrin cavity to form inclusion complex, where as at the same time, the hydrophilic portion lowers the aqueous surface tension by acting as a surfactant toward the cyclodextrin complexes, and thus increasing its wettability and dissolution [12].

4. Conclusion

The present investigation shows that bicalutamide can form inclusion complex with HPβCD in solid state. The stoichiometry of complex formation is in 1:1 molar ratio in both binary and ternary systems. The stability constant of bicalutamide in presence of arginine was increased significantly within two days of phase solubility studies. The aqueous solubility of bicalutamide was increased by 206% when L-arginine was used as an auxiliary substance in ternary complex system. All ternary systems have shown better dissolution performances than binary systems indicating proper choice of arginine as a carrier. Thus addition of arginine in ternary or co-complexes of HPβCD can be beneficial in terms of improvement in complexation efficiency, rate of complex formation and enhanced dissolution properties of poorly water soluble bicalutamide.

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