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Preparation and evaluation of binary and ternary inclusion complex of Itraconazole

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Abstract

The aim of this work was to assess the advantage of ternary complexes of itraconazole over its binary complex with β-cyclodextrin (β-CD) so as to explore the possibility of further improving the solubility, dissolution rate and complexation efficiency of itraconazole -βCD by addition of auxillary substance plasdone S-630. The phase solubility study performed in the presence of fixed amount of plasdone S-630 showed a linear, A_L type diagram with a seven fold enhancement in the complexation efficiency and the stability constant was calculated as 887.60 M^{-1}. Of the various preparation techniques used ternary complexes prepared by freeze drying exhibited highest $\%DE_{90}$ of 30.12. The freeze dried and microwave irradiated itraconazole -βCD – plasdone S-630 terneray complexes were characterized by SEM, XRD, DSC and ^{1}HNMR.

Keywords: Itraconazole, ternary complexes, β-cyclodextrin, Plasdone S-630, complexation efficiency

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INTRODUCTION

Itraconazole is a potent broad spectrum triazole antifungal drug that has been in clinical use for over a decade. It is a weakly basic compound (pKa=3.7) which can only be ionized at low pH such as in gastric juice with a very poor aqueous solubility. It is also very lipophilic with n-octanol/water log partition coefficient of 5.66 at a pH of 8.1[1]. The drug has a pH dependent dissolution resulting in low and variable oral absorption. Based on the biopharmaceutical classification system, itraconazole is class II compound meaning that its oral bioavailability is determined by dissolution rate in the GI tract. Initially introduced as a capsule formulation, itraconazole was marketed for the treatment of both superficial (onychomycosis) and systemic (blastomycosis, histoplasmosis) fungal infections. To overcome the pharmacokinetic deficiencies of the capsule formulation, two new formulations of itraconazole (oral solution and IV formulation) were developed [2].The improved absorption and consistent plasma levels achieved with these new formulations has opened up the possibility of using itraconazole in more seriously-ill patients with invasive fungal infections. However, predicting an appropriate dosing strategy for itraconazole in the critically-ill patient remains a challenge due to the dose-

dependent pharmacokinetic profile, potential for multiple drug interactions, and substantial inter-patient variability in drug metabolism [3].

Research inputs are continually aiming to meet out the challanges of pH dependent variable oral absorption. Investigators [4-6] have shown that various methods can be employed to enhance the complexation efficiency of CDs namely by drug ionization, salt formation, cosolvents,ion pairing, and by the combination of two or more methods. In all the cases, a synergistic effect on the solubilizing power of cyclodextrins was seen. In the present work, the effect of auxiliary substance on the complexation and dissolution rate of ITZ with β-CD was investigated. This approach has been reported earlier with PEG 6000 and HPMC 2910 E5 [7], Pluronic F68 and Hydroxypropylmethylcellulose [3] and TPGS 1000 and PVPVA 64 [8]. In this study the authors intend to use Plasdone S 630 to increase the solubility of both the complex and the drug itself.

Plasdone S-630 copovidone is a synthetic random copolymer consisting of N-vinyl-2-pyrrolidone and vinyl acetate in 60:40 ratio. A freely flowing material mainly composed of spherical particles has good plasticity, relatively low glass-transition temperature (Tg'105–108 8C), and low hygroscopicity. In addition, the material has been shown to absorb as much as three times less water than povidone at a given relative humidity [9]. Owing to these attributes Plasdone S 630 was selected as an auxiliary substance for improving the complexation efficiency. Thus the study was designed so that the ternary complexes made using Plasdone S630 were compared with the dissolution of binary complexes of itraconazole.

MATERIALS AND METHODS

Materials

Itraconazole (ITR) was kindly donated from Ranbaxy Labs, Gurgaon. Plasdone® S-630 was supplied as a gift by ISP Technologies, Mumbai. All other chemicals and solvents used were of pharmaceutical grade.

Methods

Phase Solubility Studies

The stability constants for inclusion complex formation between itraconazole and β-CDs were determined using the phase solubility method [10]. Phase solubility diagrams were obtained at 37˚C±0.5^{o}C in double distilled water (pH 6.80). An excess amount of itraconazole (50mg) was added to 10ml aqueous solutions containing increasing concentrations of the CDs (0-15mM). The suspensions were shaken for 72h after which the equilibrium was reached. Then they were filtered, appropriately diluted and analyzed by UV spectrophotometer (Shimadzu, pharmaspec 1700, Tokyo, Japan) at 255 nm.

The apparent stability constant (Ks) and complexation efficiency (C.E) of the complexes were calculated from the slope of the phase solubility diagrams according to the following equations [11]

$K = slope / S_0 (1\text{-}slope)$ … ..Eq. 1

Complexation efficiency (CE) = Slope/ (1-Slope) ……Eq. 2

Where was S_0 the intrinsic solubility of itraconazole in absence of cyclodextrins.

Equilibrium Solubility Study of Pure Drug With Different Concentration Range of Auxillary Substance

Solubilities were determined by adding excess amount of drug to aqueous solution containing (0.04-0.8 % w/v) of plasdone S-630 in double distilled water (pH-6.80). Suspensions formed were then subjected to temperature controlled wrist action shakers at 60^0C for 1hr. After cooling to ambient temperature, the samples were allowed to equilibrate on a water bath shaker for three days at 37^0C±0.5^0C. After equilibration, the samples were analyzed spectrophotometrically.

Phase Solubility Studies With Optimized Concentration of Auxillary Substance

Excess amount of drug was added to 10 ml of double distilled water containing increasing concentration of β-CD (0-15mM) with fixed polymer concentration of 0.4%w/v plasdone S-630. The above suspensions were subjected to temperature controlled wrist action shaker at 60^0C for 1hr, allowed to equilibrate for 72 h on a water bath shaker and analyzed spectrophotometrically at 255 nm. Apparent stability constant (Ks) and Complexation efficiency (C.E) were estimated.

Preparation of Solid Binary And Ternary Complexes

Physical Mixture

It was prepared by simple mixing, in a mortar with pestle for 10 min, the powders of both components previously sieved.

Kneading

Kneaded product was obtained by adding small amount of water to β-CD without (binary) and with (ternary) optimized concentration of auxillary substance plasdone S-630. The itraconazole powder was then added slowly. After wetting the physical mixture in a mortar, the resultant systems were kneaded for 45 min. and then dried in a microwave at 320 watts for 4 min. The temperature (60^0C) was maintained throughout the preparation. The dried mass was finally ground and sifted through a 100 mesh sieve.

Co-evaporation

Equimolar amount of β-CD and itraconazole was dissolved in double distilled water, 40% w/v plasdone S-630 was added in ternary system and stirred the solution. 25% (v/v) ammonia solution was added drop wise. The solvent was removed at reduced pressure in rotary evaporator at 45°C until paste was obtained. It was dried at 45°C for 3 hours and passed through 100 mesh sieve.

Microwave Irradiation

Equimolar quantities of itraconazole and β-CD were taken in a flask. Minimum amount of solvent mixture (methanol: water (1:1v/v)) was added. In case of ternary complex plasdone S-630 40% w/v is added. Mixture was kept for 2 min at 60°C in the microwave oven. More solvent was added to remove residual free drug. Filtered and dried in vacuum oven.

Freeze –drying

Freeze dried product was prepared by dissolving an equimolar mixture of itraconazole and cyclodextrin in double distilled water and shaken for 24h, ammonia solution (25%v/v) was added to it drop wise till a clear solution was obtained. The solution was frozen overnight in petridishes at -45^oC and lyophilized in a freeze drier at -45^oC for 48h. Secondary drying was carried out at room temperature.

Evaluation of Prepared Complexes

Dissolution Study

In vitro drug release study of pure drug, binary and ternary mixtures were investigated according to USP paddle type apparatus II method in 0.1 N HCl at an agitation speed of 100 rpm at 37˚C±0.5ºC. Sample equivalent to 50 mg was used in each test. Samples (5 ml) were withdrawn at predetermined time intervals till 2 hours filtered, diluted suitably and analyzed spectrophotometrically at 255 nm. The dissolution runs were made in triplicate and the data was analysed for model independent parameters using PCP Disso2.0v software, Pune, India.

Characterization of Optimized Ternary Mixtures

Differential Scanning Calorimetry

Differential scanning calorimetry (Perkin Elmer DSC6) measurements were conducted on 5 mg samples at a heating rate of 10ºC/min over a temperature range of 5-250ºC. A nitrogen purge (20ml/min) was maintained throughout the runs, using an empty sealed pan as a reference. Temperature and heat flow calibrations were performed using indium as a standard.

X-Ray Diffractometry

Powder x-ray diffraction patterns were obtained with an x-ray diffractometer (PW3040/60 X'Pert PRO, Netherland) using Ni-filtered CuK (α) radiation (λ= 1.5405980Aº) at scan step size of 0.020º under a voltage of 40kV and a current of 30mA for the generator. The investigation was performed in the 2θ range of 5-50ºC.

Fourier Transform Infrared Spectroscopy

A Jasco FT-761 apparatus (Japan) was used for FTIR spectroscopy. Each sample was analysed as a KBr disk in 400-4000cm^{-1} wave number range. The number of scans was adjusted automatically as a function of sample concentration in the disk.

Scanning Electron Microscopy

The micrographs of the systems were obtained by scanning electron microscope. Samples were previously coated with silver and obtained photomicrographs were examined at a magnification ratio of 4000X.

Nuclear Magnetic Resonance

To determine the nature of proton or protonated group in itraconazole and complexation of itraconazole, the NMR spectrum (^{1}HNMR) in dimethyl sulphoxide (DMSO) were recorded on Bruker Avance 400, FT-NMR spectrometer, 300 MHz, using TMS (tetra methyl silane) as internal standard. Chemical shifts were recorded in ppm.

RESULTS AND DISCUSSION

Phase Solubility Studies

The phase solubility diagrams at 37^{o}C were obtained by plotting the apparent equilibrium concentrations of the drug against β-cyclodextrin concentration (Fig.1). It can be observed that

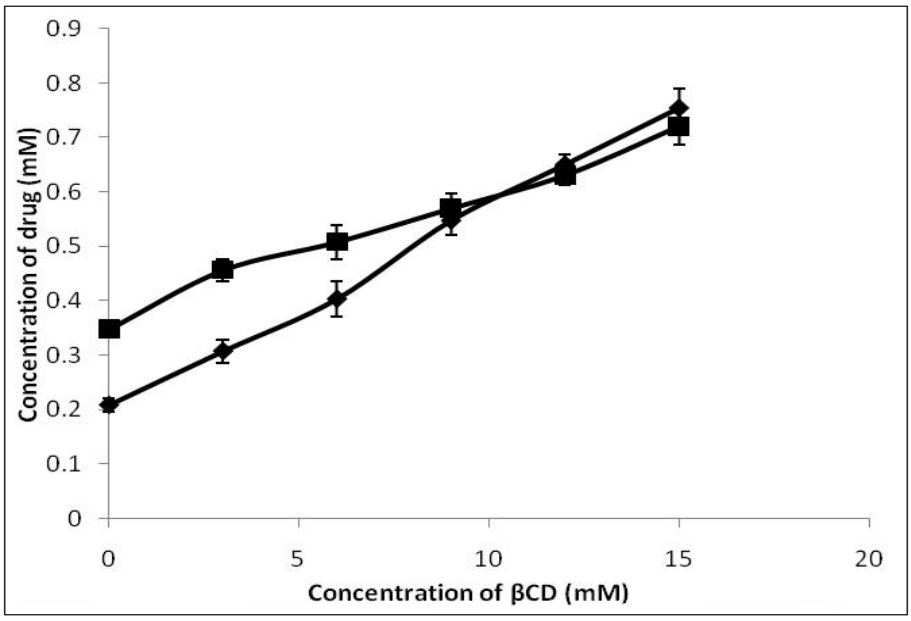


Figure 1. Phase solubility diagram of Itraconazole β-CD system (- -) and in the presence of an optimized concentration of plasdone S-630 (--) in double distilled water at 37ºC

apparent solubility of itraconazole increased linearly as a function of β- cyclodextrin over the entire concentration range studied. Linearity was characteristic of A_L type system [12] and suggested that water soluble complex was formed in solution. Furthermore, a slope value of 0.0363 for β-CD itraconazole (ITR) complexes respectively indicated formation of inclusion complexes in the molar ratio of 1:1. The apparent stability constant (Ks) of the 1:1complexes for β-CD itraconazole complex was calculated and reported in Table 1.The lower stability constant and complexation efficiency values obtained showed that complexes formed were not stable.

Table 1. Correlation coefficient, slope, intercept, stability constant, Complexation efficiency (C.E) and Drug: β-CD ratio from phase solubility diagram

Solubilising component	**Correlation coefficient**	**Slope**	**Intercept**	**Stability constant (Ks)(M^{-1})**	**Complexation efficiency**	**Drug: β-CD ratio**
β-CD	0.9994	0.0363	0.2134	177.0	0.037	1:28
β-CD - Plasdone S 630	0.984	0.236	3.66	887.0	0.308	1:4.25

The phase solubility study in the presence of optimized concentration of auxiliary substance Plasdone S630 determined as 0.4%w/v by equilibrium solubility studies (Figure. 2.) also displayed A_L type curve (Figure 1) with the slope value of 0.236, that was less than 1, indicating 1: 1 complex formation with a stability constant of 887 and a significantly higher complexation efficiency of 0.308 that was 8.34 times more than the binary system. Thus a reduction in the amount of β-CD (6 fold) for complexation is suggested as the calculations

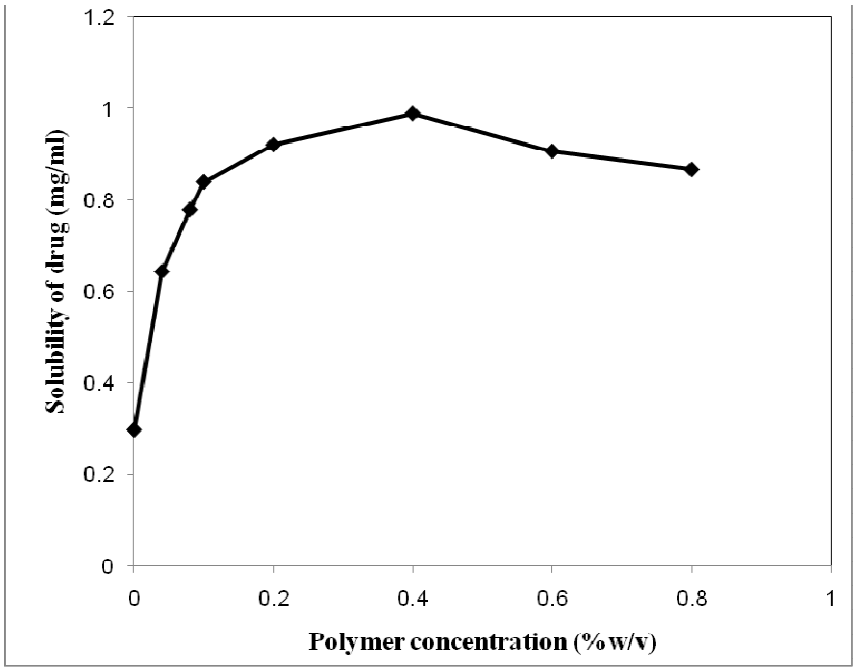


Figure 2. Equilibrium solubility curve of drug in the presence of varying Plasdone S 630 concentrations

indicate that five molecules of β-CD are required for complexation in the presence of auxillary substance that is anticipated to reduce the toxicity issues related to β-CD , economical and promote dissolution. The possible mechanism of promoting dissolution is the solubilizing effect due to weak polymeric drug interactions. Addition of polymer could also contribute to improvement of complexing ability of CD's by establishing interactions such as hydrophobic bonds and vander walls such as dispersion forces or hydrogen bonds and promoting the release of high energy water molecules present in the cavity [13].

Drug content determination

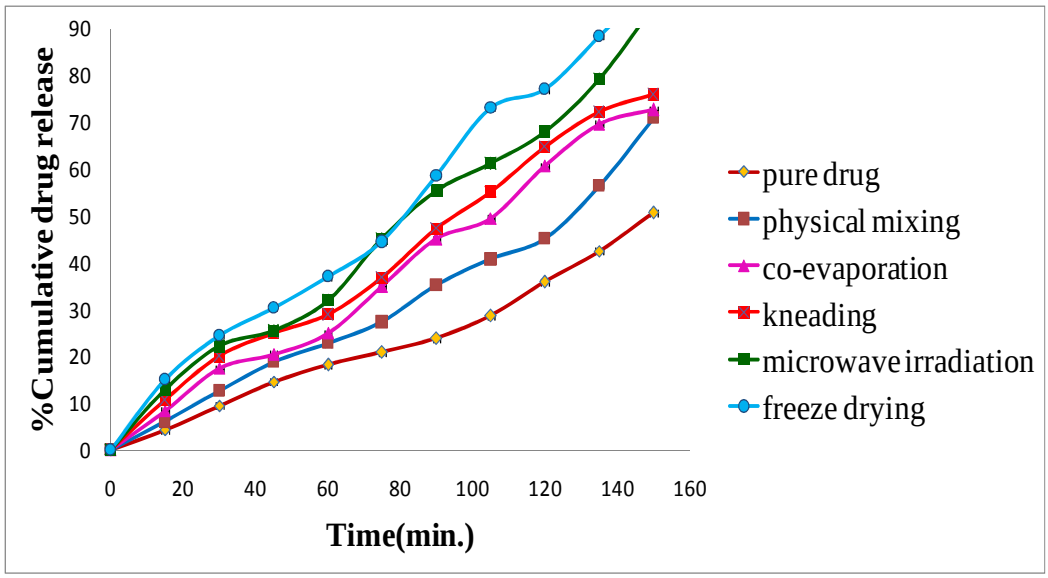
The actual drug content in binary mixture and ternary mixture was determined. The results are in Table 2. Binary and ternary mixtures containing different auxillary substances in their optimized concentration showed good agreement between theoretical and actual drug content.

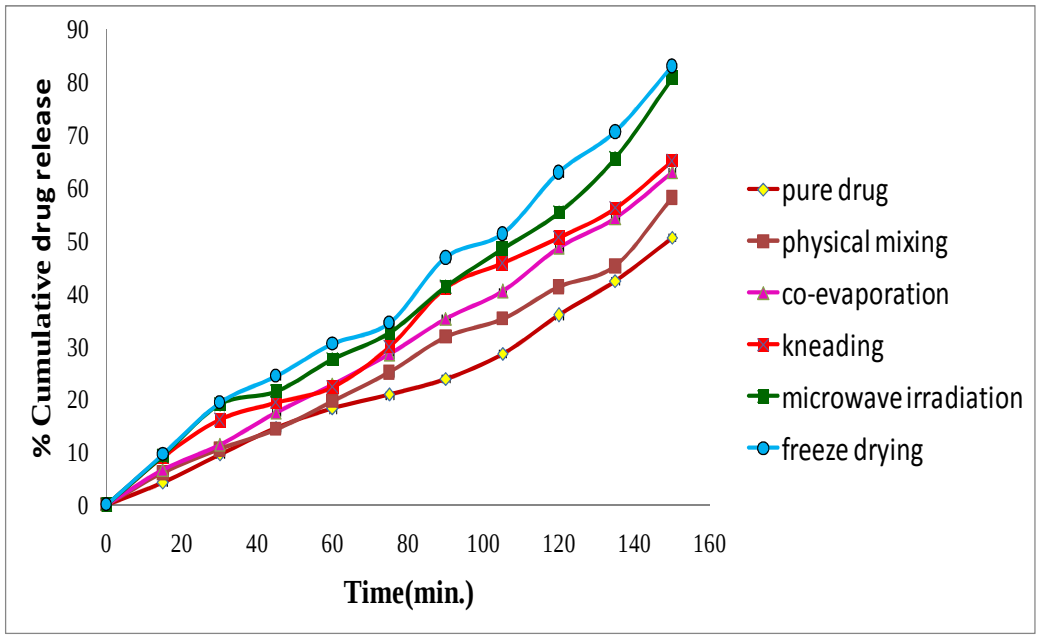
Table 2. Percent drug content of itraconazole in binary and ternary systems

Systems	Binary system		Ternary system	
	Theoretical drug content	Actual drug content	Theoretical drug content	Actual drug content
Physical mixing	100	97.10 ± 1.92	100	97.18 ± 1.17
Co-evaporation	100	98 ± 1.02	100	98.23 ± 1.22
Kneading	100	98.14 ± 2.06	100	99 ± 2.29
Microwave irradiation	100	99.37 ± 1.91	100	99.45 ± 1.63
Freeze drying	100	99.45 ± 2.24	100	99.71 ± 2.89

***In vitro* drug dissolution studies**

In vitro dissolution profiles of ternary complexes(Figure 3a) were compared to those of binary complexes(Figure 3b). A marked increase in dissolution was evident in ternary mixtures than binary mixtures due to very strong affinity of itraconazole for β-CD for plasdone S-630. Comparison of binary ITR/β-CD with the ternary system showed that addition of plasdone S-630 lead to a vast improvement in the complexation efficiency. A very high increase of the drug dissolution observed in case of freeze dried product was probably due to several reasons like the formation of soluble inclusion complexes, amorphisation of drug, better wettability, reduction of particle size and consequently increase in solubility [14].





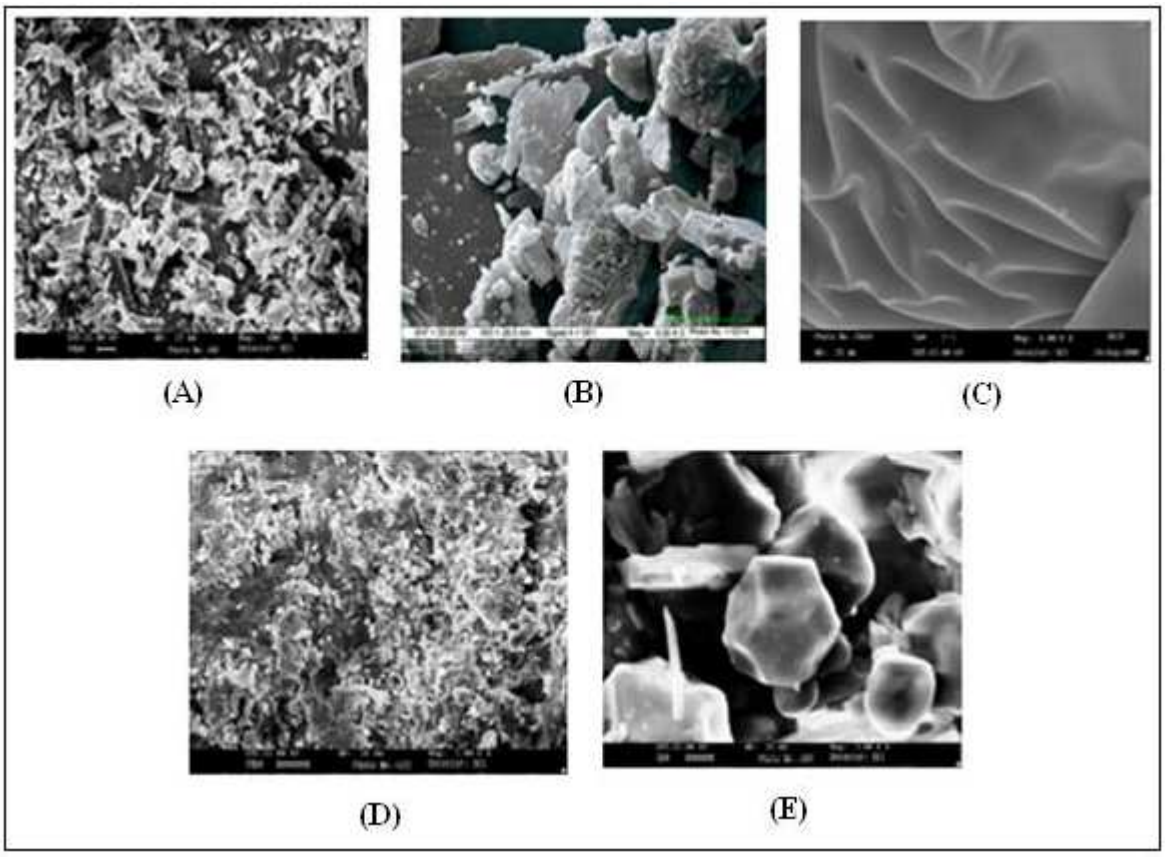
Solid inclusion complexes prepared by freeze drying and microwave irradiation methods exhibited higher rates of dissolution and dissolution efficiencies than the corresponding physical mixing, kneading and co-evaporation. An increase of 1.23 fold (freeze drying) and 1.17 fold (microwave irradiation) in dissolution efficiency was observed respectively with 1:1 M solid

inclusion complexes of ITR-β-CD with and without plasdone S-630. A comparison between the complexes prepared by various methods was made by calculation of dissolution efficiency.
Dissolution efficiency (DE) was calculated from the area under the dissolution curve at time t (measured using the trapezoidal rule) and expressed as percentage of the area of the rectangle described by 100% dissolution in the same time [15]. Considering the DE values (Table 3), the dissolution rate of itraconazole increased in the order: drug < physical mixing < coevaporation < kneading <microwave irradiation < freeze dried product suggesting that dissolution rate was influenced by method used for preparation of the binary and ternary complexes. DE_{60}, DE_{90}, for binary and ternary complexes were consequently highest for ternary freeze dried product.

Table 3 Model independent parameters for binary and ternary complexes

Methods	**Binary complex**		**Ternary complex**	
	% DE_{60}	**% DE_{90}**	**%DE_{60}**	**% DE_{90}**
Pure drug	9.2	13.14	9.2	13.14
Physical mixing	10	15.14	12.18	17.48
Co-evaporation	11.66	17.33	17.46	23.27
Kneading	13.57	19.28	14.58	21.36
Microwave irradiation	15.78	21.64	19.18	27.59
Freeze drying	17.05	22.97	22.14	30.12

Scanning Electron Microscopy (SEM)
The observation of size, shape and surface topography of pure drug, binary and ternary complexes were done by SEM (Figure 4). From SEM analysis it can be seen that pure ITR particles appeared as crystals with smooth surfaces. β-CD particles consisted of three dimensional parallelogram crystals of irregular size whereas Plasdone S 630 melted at the temp used for analysis and appeared as smooth surface with numerous folds. Microscopic examination of ITR-β-CD physical mixtures for ternary systems showed the presence of ITR crystals mixed and adhered on the surface of β-CD particles, revealing no apparent interaction between species in solid state [5].



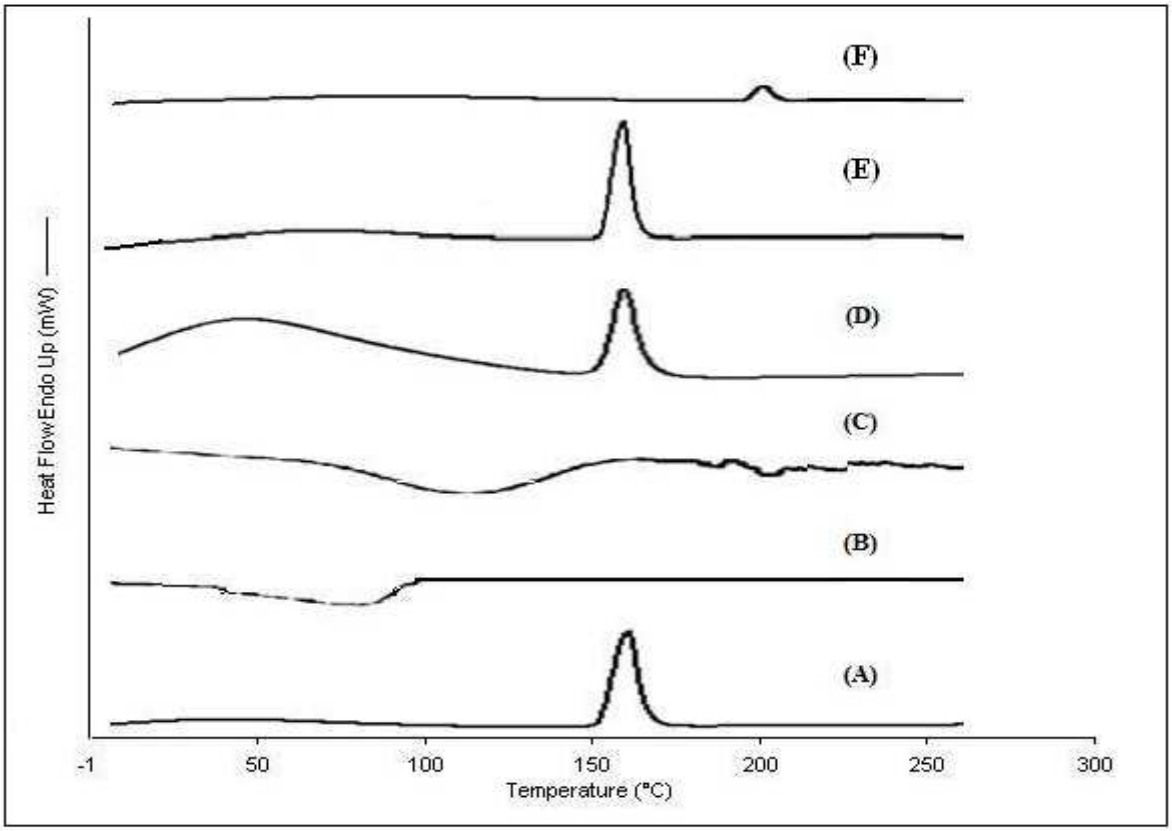


Figure 5. DSC thermograms of (A) itraconazole, (B) β-CD, (C) Palsdone S630, (D) physical mixing ternary, (E) Kneading ternary, (F) Freeze drying ternary

On the contrary, a drastic change in the original morphology and shape of both ITR and β-CD particles were observed in the ternary products. The freeze dried product appeared as a single component as shown in Fig 4 that may be a consequence of a crystalline habitus change in the system or it may support the evidence of existence of single phase.

Differential Scanning Calorimetry

Differential scanning calorimetry was used for solid state characterization of binary and ternary systems. Pure itraconazole had a sharp endothermic peak at 169.2°C that corresponded to the melting point of Itraconazole [16].

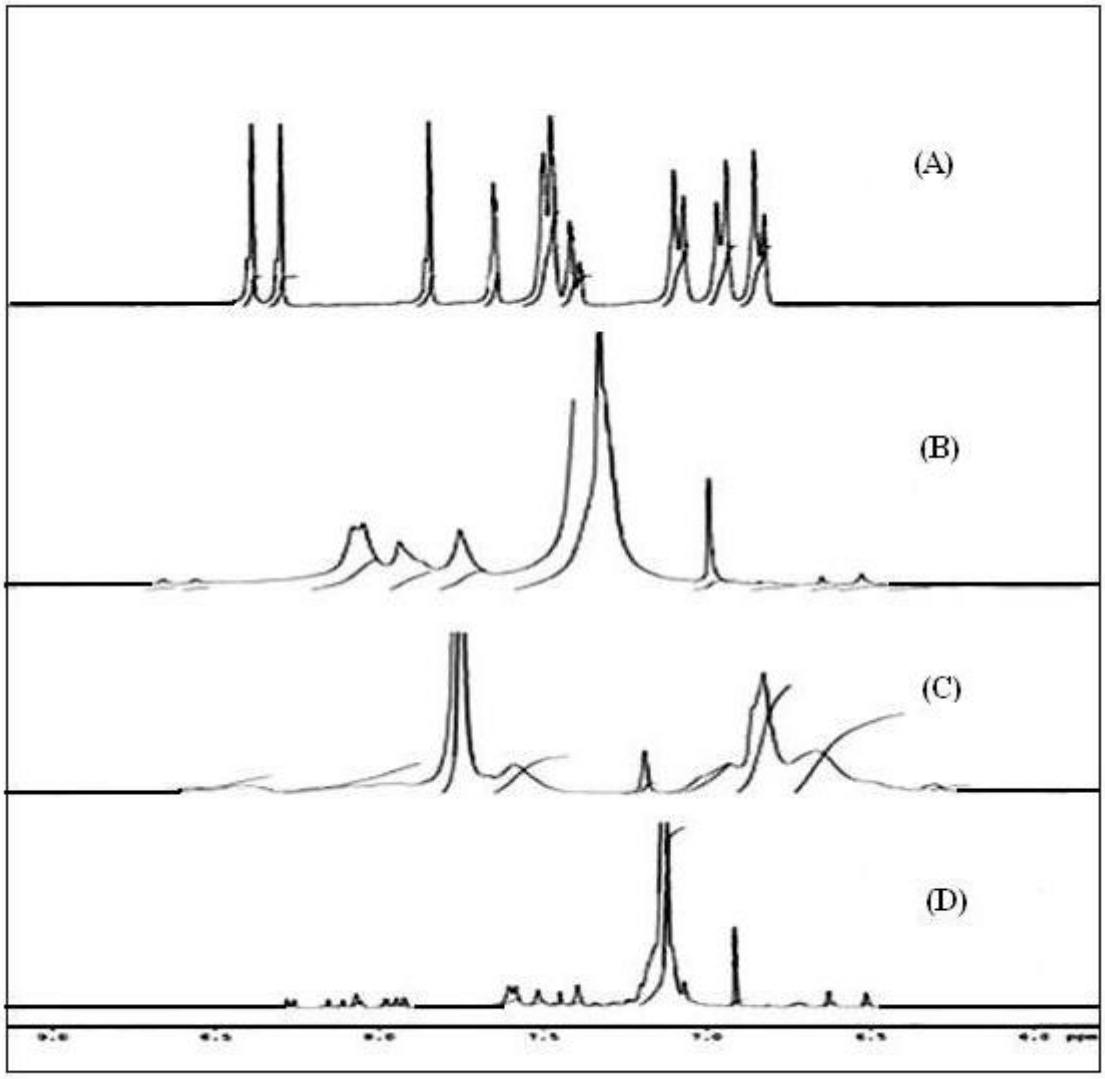


Figure 5. NMR spectra of itraconazole (A), β-cyclodextrin (B) Plasdone S-630 (C) and freeze dried ternary complex (D)

These thermograms revealed the disappearance of the sharp endothermic peak and appearance of the broad endotherm in case of ternary system. This sharp endotherm was clear in case of drug/β-CD binary system and was attributed to the melting of the remaining crystalline drug as shown in

Figure 5. The DSC curve of β-CD showed the liberation of crystal water as an endothermal effect peaked at about 166^0C whereas broader endotherms were associated with water loss from amorphous Plasdone S-630. DSC curves of ITR-β-CD-Plasdone S-630 ternary freeze dried products exhibited the complete disappearance of the endothermal melting peak of itraconazole and this disappearance of endothermic peak may be attributed to an amorphous state and/or to an inclusion complexation, So these results suggest that only ternary freeze dried products can be considered as true inclusion complexes, differing from simple physical mixtures.

Nuclear Magnetic Resonance

The NMR spectra of the ternary complex demonstrated the intrinsic peaks of Itraconazole. Slight peak shifts and overlapping occurred due to molecular interactions between Itraconazole, β-cyclodextrin and plasdone S-630 as shown in Fig 6 [17].

X-Ray Diffraction

Powder x-ray diffractometry (XRD) is a useful method for the detection of cyclodextrin complexation in powder or microcrystalline states. X-ray results confirm that itraconazole is present as an amorphous state in ternary freeze dried product. The diffraction pattern of the complex is supposed to be clearly distinct from that of the superposition of each of the components if a true inclusion complex is formed as shown in Fig. 7.

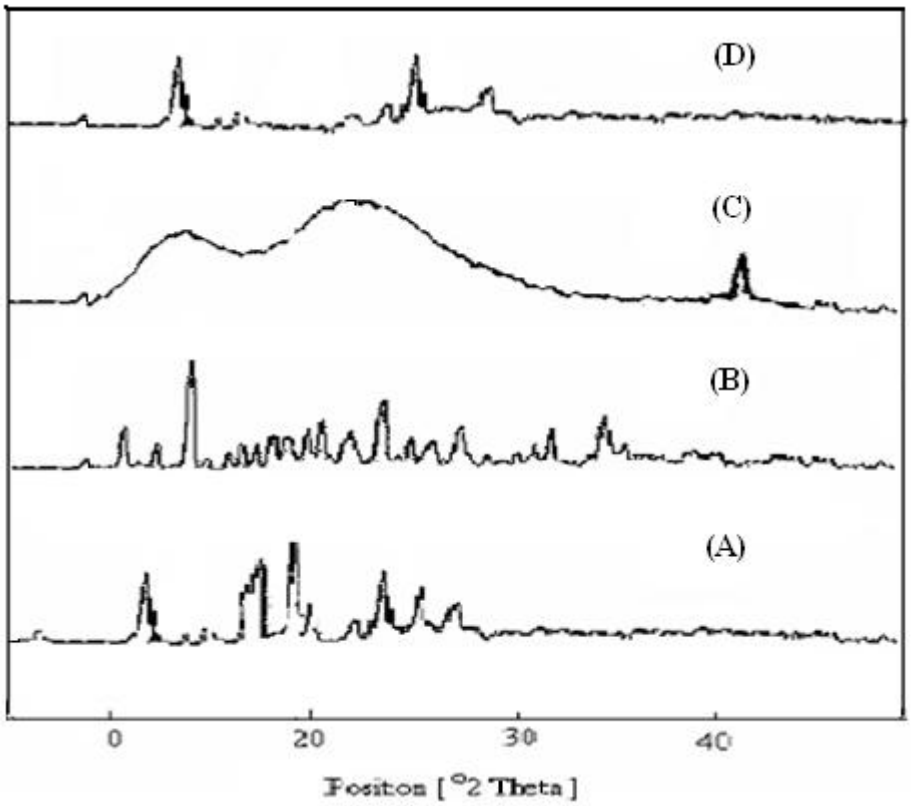


Figure 6. XRD pattern of (A) Itraconazole, (B) β-cyclodextrin, (C) Plasdone S-630, (D) Freeze dried ternary complex

A lower intensity of the diffraction peaks and overlapping of some itraconazole peaks with the peaks of β-CD was also observed, which was attributed to the reduction in particle size during the preparation of the physical mixtures. For ternary ITR-β-CD freeze dried products the obtained patterns were diffused indicating the amorphous state reached by the lyophilization technique [18].

CONCLUSION

Complexation with itraconazole - β-cyclodextrin significantly improved the solubility and the dissolution behaviour of itraconazole. The ternary systems clearly signified superiority over binary systems in terms of in vitro drug release studies. It can be concluded that addition of Plasdone S630 in ternary complexes of β-cyclodextrin can be beneficial in terms of improving C.E., stability constant and dissolution properties of poorly water soluble drugs.

Acknowledgments
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