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Solubility enhancement and physicochemical characterization of inclusion complexes of itraconazole

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ABSTRACT

Itraconazole (ITZ) is an orally active, triazole antifungal drug. It is used for the management of local and systemic fungal infections. However, the effectiveness of ITZ is limited due to its poor aqueous solubility and low dissolution rate. In the present study, inclusion complexes of ITZ and hydroxyl propyl β-cyclodextrin (HPβCD) were prepared to enhance solubility and in vitro dissolution rate of the drug. Inclusion complex in solution was studied by phase solubility technique. The phase solubility study showed an A_P type diagram which is a sign of the development of an inclusion complex in 1:1 molar ratio in solution with the apparent stability constant (K_a) 263 M^{-1}. Solid inclusion complexes of ITZ-HPβCD were prepared in different ratios by numerous methods such as physical mixing, kneading, co-evaporation and spray drying methods. The prepared solid inclusion complexes were characterized by UV, differential scanning calorimetry and Fourier transform infrared (FTIR) spectroscopy. The result of studies confirmed inclusion of ITZ molecule into hollow space of cyclodextrin. From the in vitro studies it can be concluded that inclusion complexes of ITZ with HPβCD can improve the solubility and dissolution rate of ITZ significantly. In compare to all other methods, an inclusion complex obtained by spray drying method showed superior solubility and drug dissolution rate compared to other methods. The in vitro release from all the formulations followed first order kinetic.

Key Words: Itraconazole, hydroxyl propyl β-cyclodextrin, complexation, spray drying method.

INTRODUCTION

Itraconazole (ITZ) is a synthetic triazole antifungal agent having topical and systemic efficacy against familiar fungi such as *Aspergillus* and other filamentous fungi. Itraconazole is weakly

basic (pKa =3.7) and highly hydrophobic (log P= 6.2) [1]. It shows pH dependent solubility and can be solubilized only under extremely acidic condition (4 µg/ml). ITZ is practically insoluble in water. The very poor aqueous solubility of the drug gives rises to difficulties in pharmaceutical formulation and may lead to a large inconsistent dissolution rate and bioavailability. To outweigh this problem, efforts must be made to get better the aqueous solubility of ITZ [2-3].

A lot of technical methods exclusively micronization, co-grinding, formation of solvates, salt formation and solid dispersions have been used to improve the solubility and dissolution characteristics of poorly water soluble drugs. However, conventional methods used to prepare these systems have serious limitations on their applicability in the market, often involving physical instabilities of the solid dispersions on storage, problems of grinding or difficulties in removing the toxic organic solvent and all poorly water soluble drugs are not suitable for improving their solubility by salt formation [4].

Cyclodextrins are cyclic (α-1, 4)-linked oligosaccharides of α-D-glucopyranose, having a relatively hydrophobic inner cavity and hydrophilic outer exterior. As the outside surface of these molecules is hydrophilic and the inside surface hydrophobic, they are capable to include, totally or partially, in their cavity large guest molecules by non-covalent interaction forces (hydrogen bonds, Vander Waals forces). Physical and chemical properties of the integrated guest molecules may thus be satisfactorily modified, and especially the physical stability and the aqueous solubility can be improved. In modern years, cyclodextrins and their derivatives have produced extensive consideration in the pharmaceutical field due to their appreciation by various regulatory agencies. They play a considerable role in formulation and development of various dosage forms by improving solubility, dissolution rate, chemical stability and absorption of drugs. Among cyclodextrin, hydroxyl propyl β-cyclodextrin is the most extensively considered compound for drug complexation due to its proficient aqueous solubility and low toxicity (greater than 500 mg/ml at room temperature compared to 18 mg/ml for β -Cyclodextrin) [5].

The objectives of the present study are to prepare solid inclusion complex of ITZ-HPβCD using different techniques and to study the effect of concentration of HPβCD and method of preparation on *in vitro* dissolution profile of ITZ and complexes. The prepared complexes are also to be evaluated by UV, differential scanning calorimetry and Fourier transform infrared (FTIR) spectroscopy.

MATERIALS AND METHODS

ITZ was a generous gift from IPCA Laboratories, Salvas. HPβCD was purchased from Aatur chemicals, Vadodara. All reagents used were of analytical reagents grade. Double distilled water was used for all the experiments.

Phase Solubility Study

A phase solubility study was performed at room temperature for the determination of stability constant formation between ITZ and HPβCD, according to method described by Higuchi and Connors. Excess amount of ITZ was added to phosphate buffer solutions (pH 6.8) containing

various concentration of HPβCD (0.003-0.015M) in a series of stoppered conical flasks. The suspensions were sonicated for 15 min and then shaken for 48 hours on a rotary flask shaker. After attainment of equilibrium, the contents of the flasks were filtered through Whatman filter paper (0.45 µm). The filtered solutions were appropriately diluted with phosphate buffer and assayed for ITZ using UV spectrophotometer (Shimadzu UV-2101PC, Japan) at 244 nm against blank prepared using same concentration of HPβCD in phosphate buffer solutions (pH 6.8) so as to cancel any absorbance that may be exhibited by the CD molecules. The apparent stability constant (K_a) according the hypothesis of 1:1 stoichiometric ratio of complexes was calculated from the slope of the linear portion of the phase solubility diagrams using the following equation [6]:

$$K_a = \text{Slope} / S_0 (1- \text{Slope}) \quad \ldots\ldots\ldots\ldots (1)$$

Where, S_0 is intrinsic aqueous solubility of ITZ.

Prepration of solid complexes

The solid inclusion complexes of ITZ & HPβCD were prepared by different techniques at three different molar ratios which are described below in detail [Table 1].

A] Physical mixture

The physical mixtures of ITZ and HPβCD were prepared by homogeneous blending of previously pulverized powder of both components (#60) together in a mortar with pestle for 30 min. These powdered physical mixtures were then stored in the room at controlled temperature (25°±2°C) and humidity conditions (Relative humidity 40-50%) for comparison with the corresponding solid complex powders [7].

B] Kneading method

ITZ and HPβCD were triturated in a mortar with a small volume of water-methanol solution. The thick slurry was kneaded for 45 min and then dried in a vacuum oven at 40^0C. The dried mass was pulverized and sieved through British Standard Sieve 60# (180-µm diameter) and stored at temperature of 25°±2°C and relative humidity between 40-50% [7].

C] Co evaporation method

The aqueous solution of HPβCD was added to an alcoholic solution of ITZ. The resulting mixture was stirred for 1 hour and evaporated at a temperature of 45^0C until dry. The dried mass was pulverized and sieved through British Standard Sieve 60# (180-µm diameter) and stored at temperature of 25°±2°C and relative humidity between 40-50% [8].

D] Spray drying method

The drug was dissolved in a mixture of methylene chloride and methanol. HPβCD was dissolved in distilled water with the help of a magnetic stirrer. Both the solutions were mixed slowly and drop wise together on a magnetic stirrer for 30 min. The resulting solution was fed to mini spray dryer (Labultima-222, Mumbai, India) and sprayed in the chamber from a nozzle with diameter 0.7 mm under the atomization pressure of 2.5 kg/cm^2 with a feed rate of 3 ml/min and aspiration of 25 m^3/h. The inlet temperature was kept at 70^0C and out let temperature $50^0\pm5^0$C. The product

thus obtained was collected, packed and doubly wrapped in an aluminum foil and stored in a desiccator till further use [9].

Characterization of solid complexes

Physical Appearance

All the batches of ITZ-HPβCD inclusion complex were evaluated for color and appearance.

Saturation Solubility Studies

The saturation solubility study was carried out to determine increase in the solubility of pure ITZ as compared with the physical mixture (PM) and inclusion complexes. The known excess amount of drug, PM and inclusion complexes were added to the 250 ml conical flasks containing 25 ml of phosphate buffer solution (pH 6.8). Then the sealed flasks were maintained at 25°C for 48 hours. The saturated solution was sonicated for 20 min and then centrifuged. Then, the supernatant were withdrawn through Whatman filter paper. The concentration of ITZ was determined by UV spectrophotometer at 244 nm [9].

Percentage practical yield

Percentage practical yield helps in selection of appropriate method of production and it gives efficiency of any method. So these were determined to know about percent practical yield (PY) from the following equation [10].

$$PY\ (\%) = \frac{\text{Practical Mass (Inclusion complex)}}{\text{Theoretical Mass (Drug + Carrier)}} \times 100$$

Drug content

ITZ- HPβCD complex equivalent to 10 mg of ITZ were weighed accurately and dissolved in 100 ml of methanol. Diluted suitably and drug content was analyzed at 244 nm by UV spectrophotometer. The concentration was calculated using the standard calibration curve of ITZ in methanol. The actual drug content was calculated using the following equation as follows [10]:

$$\%\ \text{Drug content} = \frac{\text{Actual ITZ content in weight quantity of inclusion complex}}{\text{Theoretical amount of ITZ inclusion complex}} \times 100$$

Mean particle size

The solid inclusion complexes were dispersed in liquid paraffin and mounted on clean glass slides and placed on the mechanical stage of the microscope (Aatur Instruments, Vadodara). An ocular micrometer was fitted with the microscope which was calibrated with the use of stage micrometer under 10×45 magnification. A particle size of 150 particles was measured using a calibrated stage micrometer and ocular micrometer. From the data, the average particle size was calculated [11].

Fourier transform infrared spectroscopy (FTIR)

FTIR spectra of pure ITZ, HPβCD and with its complexes were obtained by FTIR-8400S, CE (Shimadzu, Japan) spectrophotometer. The procedure consisted of dispersing samples with KBr

and compressing into disc by applying a pressure for 5 min in a hydraulic press. The pellet was placed in the light path and the scanning range used was 4000 to 400 cm^{-1} to obtain spectra [12].

Spectroscopic Studies

Complex formation between ITZ and HPβCD was studied by UV spectrophotometric method. UV spectra of the inclusion complex and pure ITZ were taken by using UV spectrophotometer. The scans were recorded from 200 to 400 nm [12].

Differential scanning calorimetry

Differential scanning calorimetry (DSC) thermograms of the drug, HPβCD, and prepared solid inclusion complexes were recorded on the Shimadzu TGE-50 DSC instrument (Shimadzu Corporation, Japan). These thermograms represent the rates of heat uptake from sample. About 2-5 mg samples were sealed in aluminum pans and scanned at a heating rate of $10^0C\ min^{-1}$ over a temperature range of 30 to 300^0C under a nitrogen gas stream [12].

***In-vitro* dissolution studies**

Dissolution studies of ITZ pure drug, physical mixture and solid inclusion complex were carried out using a digital USP dissolution type II apparatus (Lab India, Mumbai), at a paddle rotation speed of 75 rpm in 900 ml of phosphate buffer (pH = 6.8) as the dissolution medium at 37±0.5°C. ITZ (100 mg) or its inclusion complex equivalent to 100 mg of ITZ was used in each test. The samples of dissolution medium were withdrawn sintered glass filter at predetermined time intervals and replaced by an equal volume of fresh dissolution medium. The samples were suitably diluted and the ITZ content was analyzed by measuring its absorbance spectrophotometrically at 244 nm using phosphate buffer (pH = 6.8). The dissolution experiments were conducted in triplicate and the mean of three dissolution testes was recorded.

Analysis of *in vitro* dissolution profile

The dissolution profiles of all the solid inclusion complexes were subjected to the kinetic analysis to evaluate the drug release mechanism. The release data were fitted to zero order, first order, Higuchi model and Hixson-Crowell equations and the kinetic modeling of drug release was determined. The dissolution profiles were also evaluated on the basis of dissolution efficiency (DE) parameter at 45 (DE_{45}%) and 120 (DE_{120}%) minutes and the dissolved percentage (DP) at 45 and 120 minutes.

$$DE = \frac{\int_0^t y.dt}{y_{100}{}^{.t}}.100\%$$

Another dissolution parameter, mean dissolution time (MDT), which is a measure of the rate of the dissolution process was calculated using following formula:

$$MDT = \frac{\sum_{i=1}^{i=n} t_{mid} * \Delta M}{\sum_{i=1}^{i=n} \Delta M}$$

Where i is the dissolution sample number; n is the number of observation; t_{mid} is the midpoint time between i and i-1; ΔM = additional amount of drug dissolved between i and i-1 [13].

Statistical analysis

A statistically significant difference between different methods at different molar ratios was calculated using a one-way analysis of variance (ANOVA). P<0.01 was considered to be significant for interpretation of the results using the Instant Graphpad Prism Software [14].

RESULTS AND DISCUSSION

Phase solubility studies were carried out for assessment of the affinity between HPβCD and drug molecule in water before preparing inclusion complex. The phase solubility diagram for the complex formation of ITZ with HPβCD [Figure 1] illustrates linear increase of aqueous solubility of the drug (r^2 = 0.9912) as the concentration of HPβCD increased over the entire concentration range studied and can be classified as A_P-type following the Higuchi and Connors classification. The linear correlation coefficient of ITZ-HPβCD with a slope smaller than 1 indicated the increase in solubility was due to the formation of 1:1 water soluble complex in solution with respect to HPβCD concentrations. The apparent stability constant (Ka) calculated was found to be 263 M^{-1} which was inside range of 200-5000 M^{-1}. This value of stability constant (Ka) indicated that the complex formed is a bit stable and recommended that β-CD is appropriate for the improved dissolution properties and hence better bioavailability of ITZ.

The saturation solubility data for PM and complexes of ITZ-HPβCD are shown in Table 2. The whole inclusion complexes prepared were found to be fine and free flowing powders. The percentage practical yield of the prepared solid inclusion complexes was found to be in the range of 84.23±1.08 to 93.74±0.07 %. Percentage drug content of the complexes was found within the range of 73.45±1.02 to 92.85±0.24 %. The mean particle size for spray dried inclusion complexes showed the least mean particle size as compared to complex prepared by other methods. Hence solubility of these complexes was observed to be high compared to other formulations [Table 2].

UV spectroscopy studies of pure drug, physical mixture and inclusion complexes are shown in figure 2. There was no change in the position of λ_{max} for PM and all inclusion complexes, which indicates there is no chemical interaction between these binary systems. Physical mixture and inclusion complexes show more absorbance as compare to pure drug, which denote that the solubility of drug is being increased with HPβCD in physical mixture and inclusion complexes as well.

The dissolution of ITZ was rapid and higher from all the solid inclusion complexes when compared with ITZ pure drug [Figure 3]. The hydrophilic external surface of HPβCD acts as surfactant and helps to lower interfacial tension between insoluble drug in its hydrophobic interior cavity and surrounded dissolution medium and thus improve wetting of drug. The enhancement of dissolution rate mainly depends on different methods used for complexation and ITZ-HPβCD molar ratio [Table 3]. The highest dissolution rate and dissolution efficiency was obtained by spray drying method as compared to other complexation methods. Because in spray

drying rapid solvent evaporation causes amorphization of the drug, since very less time is available for crystal structure progression along with the inclusion of drug in cavity of the cyclodextrin and thus resulting in uniform globules obtained in the spray drying process.

The results of various kinetic models and mechanism of drug release from ITZ- HPβCD inclusion complex are shown in Table 4. The results showed that all formulations best fitted with zero order kinetics, as they contain highest regression coefficient values (0.9511 to 0.99928).

Statistical treatment of dissolution efficiencies of ITZ-HPβCD complexes formulated with different methods and different molar ratios was done using a one-way analysis of variance (ANOVA). For all the methods, the P values in between 1:1 Vs 1:2 and 1:1 Vs 1:3 molar ratios were found to be less than 0.01 [Table 5]. But the P values for 1:2 Vs 1:3 were found to be greater than 0.05, which shows that there was an irrelevant difference between 1:2 and 1:3 with regard to dissolution efficiencies.

The major peaks of FTIR spectrum of pure ITZ and ITZ-HPβCD complexes were shown in figure 4. The IR spectrum's of pure ITZ shoed characteristic peaks at 3394, 3325 and 3032 cm^{-1} due to absorption of amine group, 2924 cm^{-1} resulted from –CH stretching band and a sharp peak occurred at 1651 cm^{-1} due to –C=O stretching vibration. The peaks observed at 1558 cm^{-1} may be confirmed to the presence of –C=N bond. A peak observed at 1504 and 1442 cm^{-1} can be attributed to –C-H deformation. The spectrum peak points of the pure ITZ and inclusion complexes are near by same. So there is no chemical interaction between the ITZ and HPβCD which confirmed the stability of the drug with its complex.

ITZ and HPβCD showed an endothermic peak at 168.3^0C and 113^0C respectively corresponding to its melting point in DSC thermograms. Except inclusion complex prepared by spray drying method in all other complex ITZ endothermic peak was appear but its shape and size were different than pure drug [Figure 5]. It showed formation of partial complex where as in spray drying inclusion complex ITZ peak was disappear and showed formation of true inclusion complex in solid state.

Table 1. Formulation ingredients and preparation method of ITZ- HPβCD inclusion complex

Batch Code	Composition		Method	Ratio
	ITZ (mg)	HPβCD (mg)		
PM1	1.69	3.31	Physical mixture	1:1
CE1	1.69	3.31	Co-evaporated method	1:1
KN1	1.69	3.31	Kneading method	1:1
SPR1	1.69	3.31	Spray drying method	1:1
PM2	1.02	3.98	Physical mixture	1:2
CE2	1.02	3.98	Co-evaporated method	1:2
KN2	1.02	3.98	Kneading method	1:2
SPR2	1.02	3.98	Spray drying method	1:2
PM3	0.73	4.27	Physical mixture	1:3
CE3	0.73	4.27	Co-evaporated method	1:3
KN3	0.73	4.27	Kneading method	1:3
SPR3	0.73	4.27	Spray drying method	1:3

Table 2. Comparison of particle size, % yield and drug content of different ITZ- HPβCD solid inclusion complex

Batch Code	Saturation Solubility (µg/ml)	Mean particle size (µ)	Powder yield (%)	Drug content (%)
Pure drug	2.35	--	--	--
PM1	3.01	25.14	91.21±0.3	73.45±1.02
CE1	6.69	19.12	85.34±1.22	80.45±0.649
KN1	6.87	17.21	92.12±0.56	86.45±0.75
SPR1	10.17	12.69	85.55±1.59	90.5±0.42
PM2	8.92	23.42	88.35±0.46	76.24±1.36
CE2	11.19	20.17	87.67±1.11	82.41±0.646
KN2	13.32	18.02	93.01±0.02	87.99±0.94
SPR2	15.94	9.89	86.48±1.66	91.23±0.006
PM3	8.30	26.71	90.33±0.46	77.87±1.47
CE3	10.89	20.39	88.21±1.24	83.21±1.021
KN3	12.93	16.50	93.74±0.07	88.11±0.473
SPR3	15.19	10.97	84.23±1.08	92.85±0.241

**Average of three determinations*

Table 3. Comparison of Dissolution Parameters of different ITZ- HPβCD inclusion complex

Batch Code	% Cumulative drug released		DE_{45}%	DE_{120}%	MDT (min)
	Q_{45}	Q_{120}			
Pure drug	11.36	29.98	5.58	16.46	57.23
PM1	14.74	32.33	7.17	19.61	49.12
CE1	20.60	40.22	11.30	24.7	49.24
KN1	25.34	48.50	13.72	29.34	48.54
SPR1	29.34	60.27	17.29	36.94	48.72
PM2	25.42	55.01	14.14	32.76	50.50
CE2	39.11	65.14	19.73	42.66	43.68
KN2	53.26	82.50	27.46	54.63	41.96
SPR2	63.23	96.10	33.07	64.44	40.54
PM3	24.80	54.37	13.67	32.25	49.58
CE3	39.00	64.96	19.35	42.39	42.03
KN3	52.89	81.87	27.18	54.27	40.64
SPR3	63.00	95.90	32.76	64.10	40.08

Table 4. Comparison of different kinetic models applied on the *in vitro* dissolution profile of ITZ- HPβCD inclusion complex

Batch Code	Regression coefficient			
	Zero order	First order	Higuchi	Hixson-Crowell
Pure drug	0.9553	0.9831	0.9303	0.9809
PM1	0.9374	0.9511	0.9368	0.9471
CE1	0.9487	0.9724	0.9699	0.9654
KN1	0.9465	0.9771	0.9722	0.9686
SPR1	0.9541	0.9874	0.9749	0.9795
PM2	0.9569	0.9928	0.9616	0.9762
CE2	0.9108	0.9632	0.9617	0.9487
KN2	0.8904	0.9847	0.9634	0.9625
SPR2	0.8784	0.9919	0.9623	0.9892
PM3	0.9523	0.9776	0.9175	0.9710
CE3	0.9040	0.9730	0.9700	0.9401
KN3	0.8840	0.9764	0.9718	0.9537
SPR3	0.8765	0.9913	0.9719	0.9841

Figure 1. Phase solubility diagram of Itraconazole- HPβCD

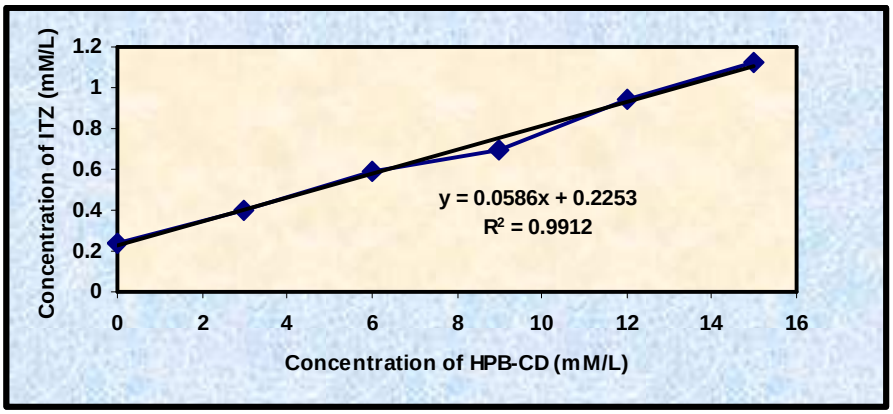


Table 5. Statistical treatment of dissolution efficiencies of ITZ- HPβCD complexes

Method	Trial	DE%			ANOVA Parameters			Dunnett's Parameters		t-Test Parameters for 1:2 and 1:3			
		1:1	1:2	1:3	Calculated value (F)	d.F	Significance	Ratio	Significance	Calculated value	d.F	Table value	Significance
Physical Mixture (PM)	1	19.61	32.76	32.25	864.3	2,6	P<0.05	1:1 vs 1:2	P<0.01	11.25	4	2.623	P>0.05 NS
	2	19.47	32.59	31.00				1:1 vs 1:3	P<0.01				
	3	20.02	32.98	31.97				1:2 vs 1:3	P>0.05				
Kneading (KN)	1	29.00	54.63	54.27	4398	2,6	P<0.05	1:1 vs 1:2	P<0.01	2.265	4	1.561	P>0.05 NS
	2	28.97	54.46	53.91				1:1 vs 1:3	P<0.01				
	3	29.95	54.89	54.44				1:2 vs 1:3	P<0.01				
Co evaporation (CE)	1	24	40.49	42.39	411.7	2,6	P<0.05	1:1 vs 1:2	P<0.01	3.128	4	0.1947	P>0.05 NS
	2	24.56	42.44	41.00				1:1 vs 1:3	P<0.01				
	3	24.81	42.79	41.85				1:2 vs 1:3	P<0.01				
Spray drying (SPR)	1	36.82	64.44	64.1	6983	2,6	P<0.05	1:1 vs 1:2	P<0.01	1.276	4	1.568	P>0.05 NS
	2	36.55	64.13	63.69				1:1 vs 1:3	P<0.01				
	3	37.15	64.85	64.32				1:2 vs 1:3	P<0.01				

Figure 2. UV spectroscopy studies

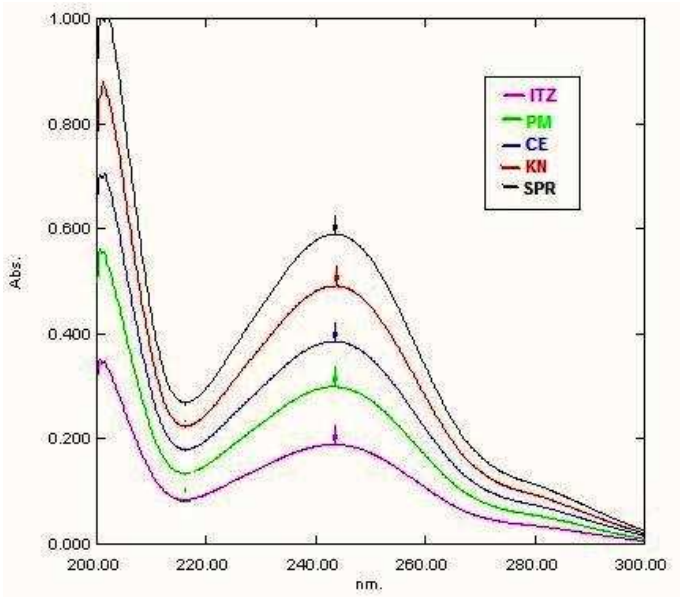


Figure 4. FTIR spectra of pure drug and ITZ- HPβCD complexes

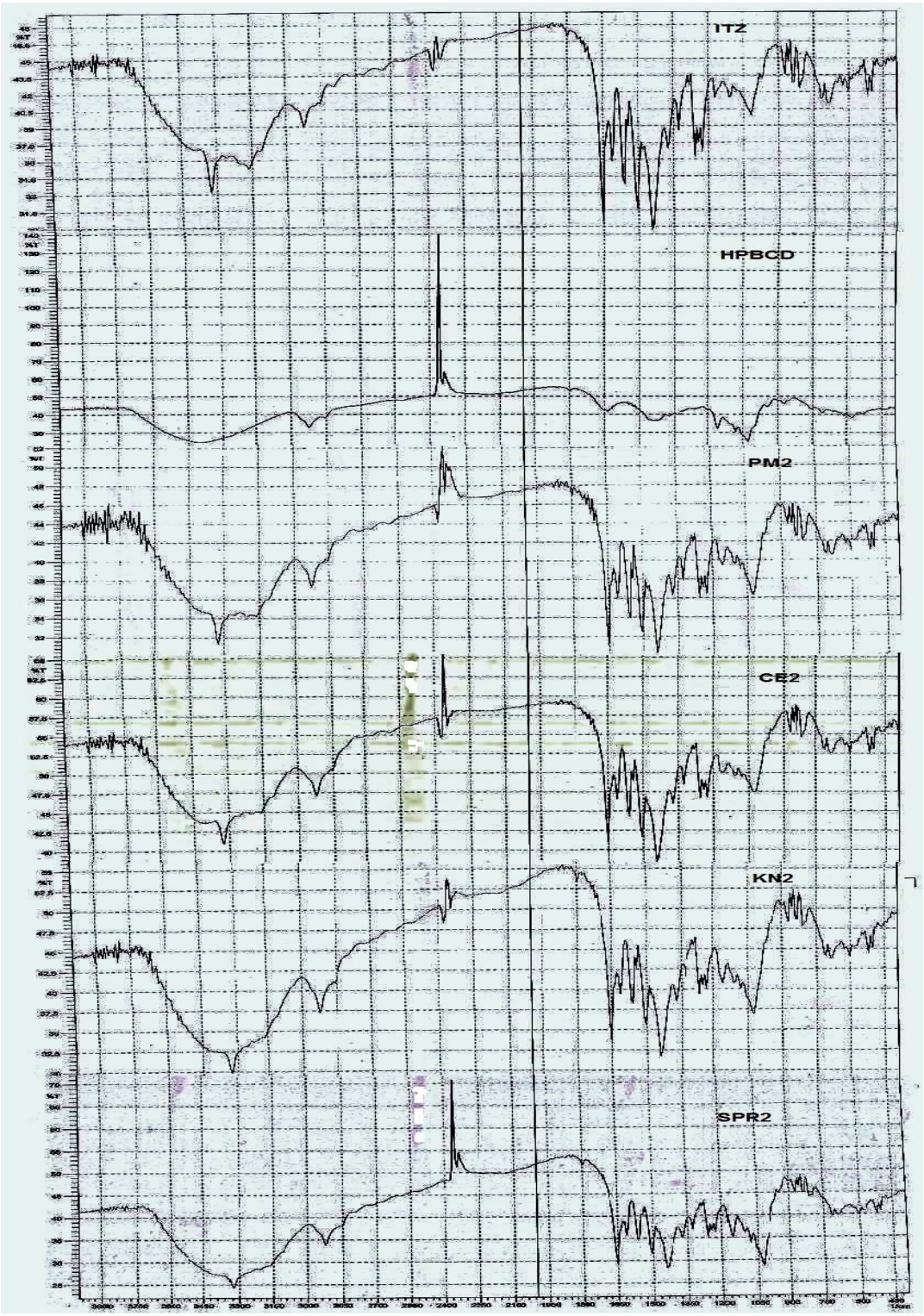


Figure 5. DSC thermograms of pure ITZ and its inclusion complex

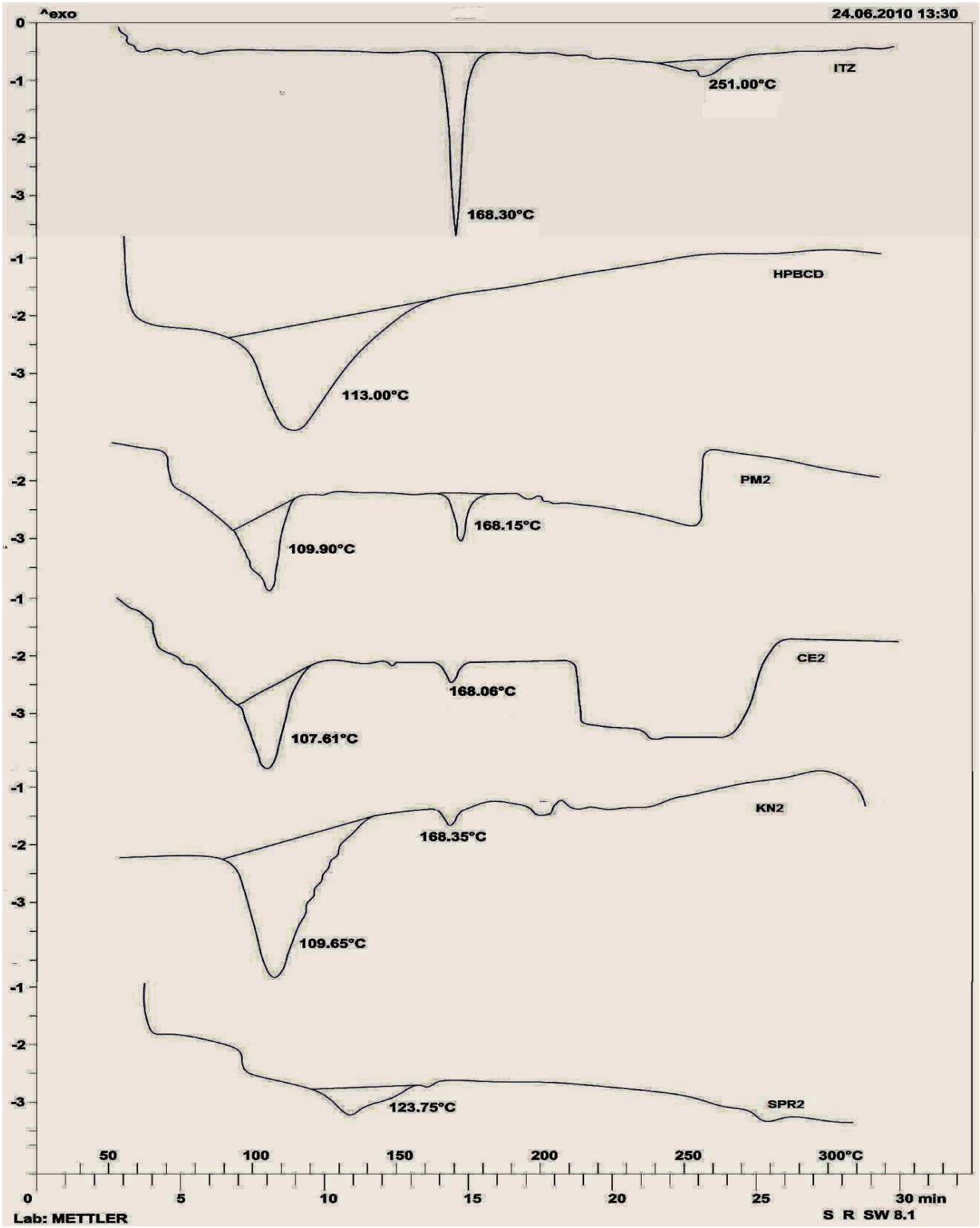
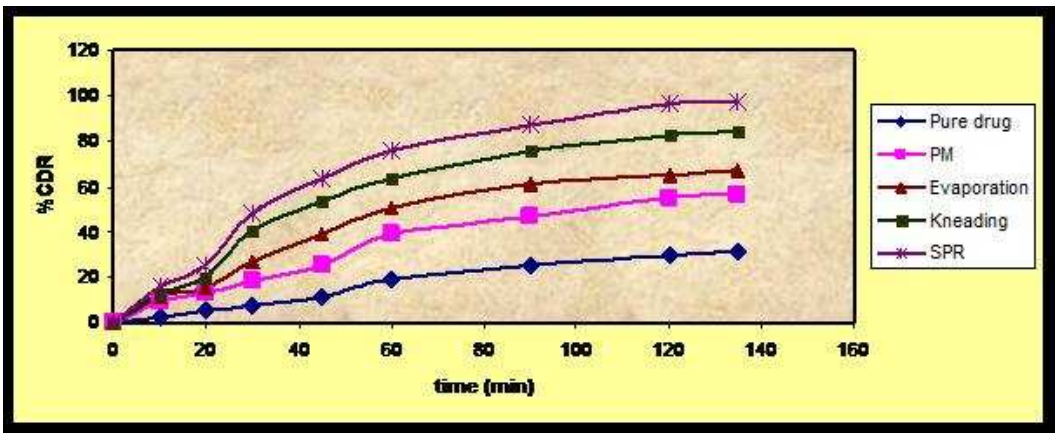


Figure 3. Comparison of drug release profile of various ITZ- HPβCD complexes (1:2)



CONCLUSION

The solubility and dissolution rate of ITZ can be improved by preparing inclusion complex with HPβCD. Highest increase in solubility and dissolution rate was achieved by ITZ-HPβCD complex (1:2) prepared by spray drying method in comparison with pure drug and complexes prepared by other methods. Moreover DSC study revealed that spray drying method was successful for producing solid inclusion complexes with HPβCD.

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