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ANALYSIS

STUDY OF PROCESS INDUCED POLYMORPHIC TRANSFORMATIONS IN FLUCONAZOLE DRUG

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Abstract: The polymorphic form-I of the fluconazole drug commonly crystallized from the solution phase could be obtained by the solid state transformation of form-II employing different process parameters. As received fluconazole-II drug melted at 138.4˚C. The molten drug undercooled almost to ambient temperature of 30˚C and solidified to a glassy mass which, on ageing for 48 h transformed to a white powder which could be identified as fluconazole-I. The same glassy mass on heating at 5˚C/min, without ageing, also underwent polymorphic transformation to fluconazole-I above 81˚C. The application of uniaxial pressure of 200 kg/cm^2 on as received fluconazole-II sample also yielded form-I of the drug. This phase transformation was enhanced by the application of pressure (200 kg/cm^2) on the as received sample aged for 36 months. The phase transformation was concluded from the difference in differential scanning calorimetric (DSC) curves of the original sample (form-II) and the products obtained by adopting the different processing routes. The DSC patterns of fluconozole-I obtained by different methods were found to be identical. The phase transformation in the as received drug (form-II) induced by different process parameters, concluded from the DSC data was corroborated by X- ray diffraction (XRD) studies and scanning electron microscope (SEM) photographs of the two polymorphic forms. The intrinsic dissolution rates of polymorphic form-I and -II and the influence of crystal habit on the drug dissolution process have also been studied.

Keywords: fluconazole, polymorphism, thermal cycling, ageing, high-pressure, DSC, XRD, SEM.

Polymorphism of drugs is the most common phenomenon (1) and has great bearing on their efficacy (2). Different polymorphs of a drug may exhibit significantly different biological activities due to their different solubility and dissolution rate (3). Drugs can undergo polymorphic transformations when subjected to temperature as well as pressure changes (4, 5) and other processing parameters (6). The knowledge of such transformations is extremely important in the formulation of drug, and the potential for process-induced solid phase transformations must, therefore, be evaluated during design and development of the formulations and manufacturing processes.

The polymorphic transformations of the drugs have been studied extensively in recent years employing differential scanning calorimetry (DSC), supported by other complementary techniques like X-ray diffraction, FT-IR and scanning electron microscopy (SEM) (7-9).

In our earlier publication (7), we showed that fluconazole drug existed in two polymorphic forms designated as (I) and (II). As received drug, designated as fluconazole-II, melted at 138.4^{o}C. The melt of this drug undercooled almost up to room temperature and on holding, solidified to a glassy mass, which on heating above 81^{o}C, transformed to a new polymorphic form – fluconazole-I.

In the present work we examined the effect of pressure on the commercial fluconazole-II sample and the sample aged at room temperature (30^{o}C) for 36 months. The results indicated that fluconazole-II, on application of pressure also transformed to fluconazole-I, which is crystallographically distinct from form-II and identical to that obtained by ageing or heating of the glassy supercooled molten fluconazole.

The solubility studies carried out on the two forms of the drug, employing *in-vitro* dissolution profiling, showed that fluconazole-I obtained by any of the above methods dissolved relatively slowly compared to the original drug (form-II) under identical conditions (10).

EXPERIMENTAL

Materials

Fluconazole-(II) used in this investigation was

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procured from Dr. Reddy's Laboratories Ltd., India. Purity of the drug was found to be 99.8% as analyzed by HPLC. The reversed phase HPLC system (Shimadzu, Model: LC 10 AT-vp) consisting of a solvent delivery pump and variable UV detector (set at 261 nm) was used for purity determination. A nonpolar stationary phase (Lichrosphere C18, 10 µm particle size, 4 mm × 250 mm; Merck) column was used. The mobile phase consisted of 70% 0.05 M potassium dihydrogen *ortho* phosphate, 20% acetonitrile and 10% methanol. The flow rate of the mobile phase was maintained at 1.0 mL/min. The injection volume used was 20 µL. The fluconazole sample on drying in air oven at 105°C for 3 h showed a mass loss of 0.3%, which could be attributed to the loss of adsorbed moisture.

The high pressure form of the fluconazole drug was obtained by the application of uniaxial pressure of 200 kg/cm^2 for 10 min on the as received powder and 36 months aged sample. The pressure on the powder sample was applied by containing it in 1 cm diameter hydraulic press die and plunger set.

Instrumental methods

Differential Scanning Calorimetry (DSC)

The DSC curves for the two polymorphic forms of the drug were recorded at the heating and cooling rates of 5°C/min in flowing nitrogen, employing differential scanning calorimeter (Shimadzu, Model: DSC 60). The sample investigated in the present study was heated up to 160°C. About 8 mg sample was used in each set of experiments. The built-in software which facilitated the determination of phase transformation temperatures from the recorded curves has been provided with the instrument. The instrument was calibrated for temperature and enthalpy employing the standard reference materials and the procedure recommended by Hohne et al. (11).

X-ray diffraction

The X-ray diffraction (XRD) patterns for different forms of the drug were recorded using Philips X-ray diffractometer (Model: PW1710) employing Cu Kα radiation. The original X-ray beam was mono-chromatized using graphite monochromator. The scanning speed of 2° 2θ min^{-1} was used in the range of 10 – 40°.

Scanning electron microscopic (SEM) studies

Experiments were carried out by mounting sample on specimen stubs with double-sided adhesive tape. The drug samples investigated in this study were very sensitive to electron beam and developed considerable charge on being bombarded by electrons. The charging of the specimen causes artifacts and also focusing problem in the SEM. To avoid charging, the specimen were coated with a thin layer (250 – 300 Å) of conducting material (gold) using sputter coater and examined in Philips (Model: XL-30) apparatus. Scanning electron microscope at 12 kV accelerating voltage with tilt angle of 45° was used with suitable magnification of 5000×.

Dissolution studies

Solubilities of two polymorphic forms of the drug were determined using *in vitro* dissolution profile. Dissolution test conditions were selected according to British pharmacopoeia (12). Dissolution tests were carried out using USP-30 rotating basket method with dissolution apparatus from Electrolab Instruments, India (Model: TDT-08L), which contains six jars with six auto sample withdrawals with 0.8 µm prefilters and dissolution medium replacement facility, using a rotational speed of 100 rpm at 37 ± 0.1°C. The dissolution experiment was conducted for 45 min. Dissolution

Table 1. Enthalpy of transformation derived from manually resolved composite DSC peaks

	Fluconazole form-I		Fluconazole form-II	
Figures	Melting (Onset)	Heat of fusion mJ/mg	Melting (peak)	Heat of fusion mJ/mg
Fig. 1(a)	—	—	138.4°C (Onset) 141.3°C (Peak)	99.2
Fig. 1(c)	135.6	92.0 * †	141.2	100.4 *
Fig. 1(d)	135.5	107.5 * †	141.2	103.0 *
Fig. 1(e)	135.7	98.5 * †	140.5	103.0 *
Fig. 1(f)	135.8	6.1	141.4	95.0
Fig. 1(g)	135.7	102.0 * †	141.3	96.0 *

* Heat of fusion calculated for the manually resolved overlapping peaks.

† Could also involve contribution from enthalpy of transformation of fluconazole-I to fluconazole-II.

Table 2. Comparison of the XRD data obtained for the form-I and form-II of fluconazole in the present study with that reported in the literature.

Form-I (melting range 135-136°C)				Form-II (melting range 138-140°C) c			
Observations recorded in Analytical profiles for drug substances		Experimental observations		Observations recorded in Analytical profiles for drug substances		Experimental observations	
2Θ	Intensity (%)	2Θ	Intensity (%)	2Θ	Intensity (%)	2Θ	Intensity (%)
12.20	33.0	—	—	12.25	10.0	12.45	17.2
13.70	14.2	—	—	13.50	10.0	—	—
14.90	14.2	15.0	12.7	15.2	56.25	15.4	78.0
15.90	68.5	16.0	43.4	—	—	16.0	24.1
16.20	38.0	16.3	87.8	—	—	—	—
—	—	—	—	16.50	100	16.7	100
17.00	29.4	16.8	65.8	—	—	—	—
18.15	30.8	18.10	12.0	—	—	—	—
—	—	—	—	—	—	18.4	28.3
—	—	—	—	—	—	18.8	39.9
19.75	100	19.9	100	20.00	93.7	19.8	42.1
—	—	—	—	21.30	18.75	21.1	34.9
—	—	—	—	22.25	20.0	22.2	23.0
22.85	35.0	—	—	—	—	—	
23.80	90.0	23.70	25.0	23.80	11.0	23.6	52.6
—	—	—	—	—	—	24.0	46.8
24.70	88.0	24.9	14.7	—	—	—	—
25.45	64.4	25.6	19.0	25.35	23.75	25.5	70.9
—	—	—	—	26.0	22.5	25.9	43.7
—	—	—	—	26.4	13.75	26.2	21.3
26.90	36.4	27.1	12.2	—	—	—	—
—	—	—	—	27.3	12.75	27.5	32.8
27.80	49.0	28.0	12.4	—	—	—	—
29.00	33.6	29.2	17.4	28.8	22.5	28.6	11.1
—	—	—	—	29.6	21.5	29.35	11.1
30.10	29.4	—	—	30.3	27.5	30.2	14.0
—	—	—	—	—	—	—	—
32.15	43.0	32.0	10.0	32.0	11.0	32.0	10.0
32.95	26.6	—	—	—	—	—	—

runs for all powder samples of fluconazole form-II and form-I (obtained by heating of glassy fluconazole above 81°C , ageing the glassy material for 48 h and application of high pressure) were performed six times. Powder samples for each run were accurately weighed (150 mg) on Mettler Toledo analytical balance (AEG 285). The drug in the filtrate was determined at 261 nm using a double beam spectrophotometer (Shimadzu UV-1601). The cumulative percent of drug dissolved was calculated for each run using the absorbance of fluconazole standard with concentration of 150 µg/mL.

RESULTS AND DISCUSSION

DSC observation

The DSC patterns were recorded for commercial fluconazole-II during first heating (Fig. 1a) and

Table 3. Data on the dissolution profiles for fluconazole-II and fluconazole-I obtained by different processing methods.

Time (minutes)	Average % fluconazole-II (as received) dissolved	Average cumulative % fluconazole-I dissolved (form-I obtained by heating glassy mass above 81˚C)	Average cumulative % fluconazole-I dissolved (form-I obtained by ageing of glassy mass)	Average cumulative % fluconazole-I dissolved (form-I obtained by application of high pressure on fluconazole-II)	Average cumulative % fluconazole (I) dissolved (form-I obtained by application of high pressure on 36 months aged fluconazole-II)
5	30.6	16.5	15.1	21.1	20.0
10	71.3	53.3	51.9	56.6	55.1
15	81.7	75.8	73.1	76.3	73.6
20	91.8	83.8	81.5	86.2	84.9
30	99.9	94.1	91.6	93.3	92.0
45	100	99.8	97.0	100	98.9

The values of solubility listed in the Table are the mean of six experiments with the standard deviation not exceeding $\pm$ 1.5%

cooling (Fig. 1b) cycle. During the first heating cycle a sharp endothermic symmetric peak (Fig. 1a) due to melting beginning at 138.4^{o}C was observed. Considerable under-cooling was seen for molten fluconazole drug during cooling up to 30^{o}C (Fig. 1b). The undercooled mass was solid at room temperature (30˚C) and exhibited glassy appearance.

The DSC pattern presented in Fig.1c was obtained for the glassy mass formed from undercooled molten fluconazole. The sample was heated at 5^{o}C/min in flowing nitrogen. Two peaks were observed: an exothermic peak around 81^{o}C (7) followed by a sharp twin endothermic peak around 135^{o}C. The nature of this DSC pattern suggests that possibly the glassy meta-stable fluconazole transformed to a crystalline form irreversibly around 81^{o}C (7), which on further heating either transformed to its original form-II or melted independently or simultaneously along with the original form, obtained from the glassy mass during heating. The DSC pattern (Fig. 1d) recorded for the sample obtained after ageing of the glassy mass at the ambient temperature of 30^{o}C for 48 h did not show any exothermic peak around 81^{o}C , but showed the sharp twin endothermic peak identical to that in Figure 1c. This observation suggests that the glassy form of the drug on ageing for 48 h at 30^{o}C has also undergone a type of polymorphic transformation similar to that observed during heating of the glassy mass (7).

The DSC pattern for the sample obtained by application of uni-axial pressure of 200 kg/cm^{2} on fluconazole-II is presented in Figure 1e. The comparison of this curve with the curves in Figures 1c and 1d, suggests that the application of high pressure on fluconazole-II also results in its polymorphic transformation, yielding the crystallographic form similar to that obtained by heating or ageing of the glassy mass resulting from the undercooled molten fluconazole.

The DSC pattern in Figure 1f recorded for the sample aged for 36 months in ambient air at 30^{o}C was found to be different compared to Figures 1c to 1e and consisted of a small peak beginning at 135.8^{o}C preceding the major endothermic peak due to melting of fluconazole-II (139^{o}C). The minor peak in this pattern could be due to form-I produced due to ageing of the commercial drug. The magnitude of the area of this peak compared to the area of the major peak beginning at 139^{o}C, however, indicates that the amount of fluconazole-I formed after ageing of fluconazole-II for 36 months was not more than 7%. The application of 200 kg/cm^{2} pressure on the 36 months aged sample also yielded the DSC pattern (Fig.1g) identical to that in Figure-1e, which was obtained by application of the same pressure on as received sample. The effect of application of pressure on the aged sample was thus identical to that applied on as received sample.

The curves in Figures-1c to 1g indicate that fluconazole-II sample subjected to various processing parameters, perhaps, transform to different crystallographic form, which begins to undergo physical changes (phase transformation / melting) around 135^{o}C.

The composite peaks in Figures-1c to 1g comprising of two overlapping peaks obtained in the

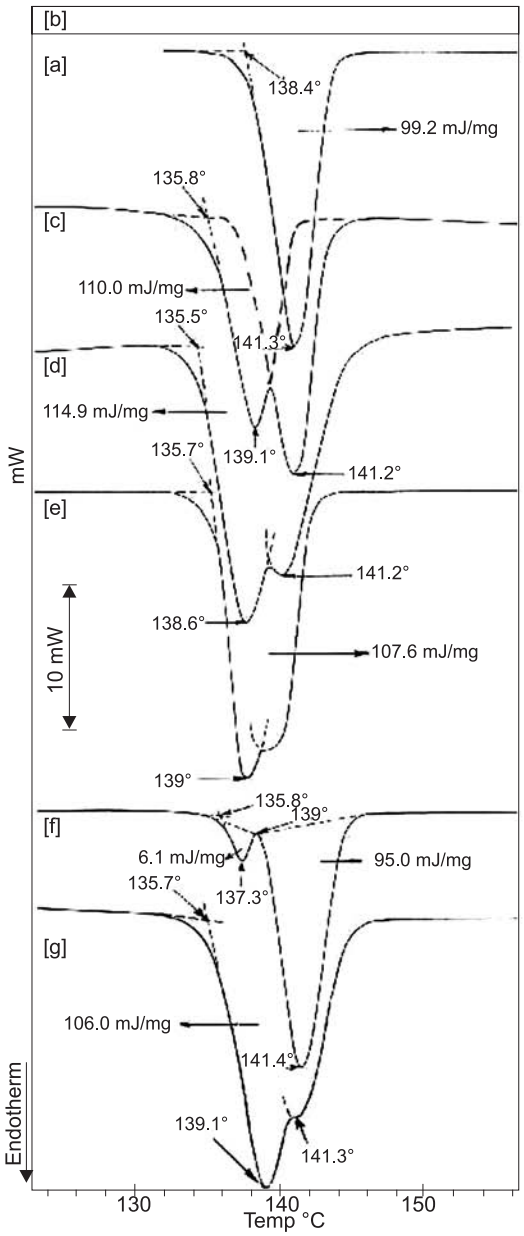

Figure 1. Differential scanning calorimetric scans for: (a) heating cycle for commercial fluconazole-II; (b) cooling cycle for fluconazole-II; (c) fluconazole-I obtained when glassy fluconazole sample was heated above 81°C (obtained by freezing the molten fluconazole mass); (d) fluconazole-I obtained when glassy fluconazole sample was aged for 48 h; (e) fluconazole-I obtained by application of uniaxial pressure of 200 kg/cm^2 on fluconazole-II; (f) fluconazole-I obtained from fluconazole (II) aged for 36 months; (g) fluconazole-I obtained by application of uniaxial pressure of 200 kg/cm^2 on 36 months aged fluconazole-II

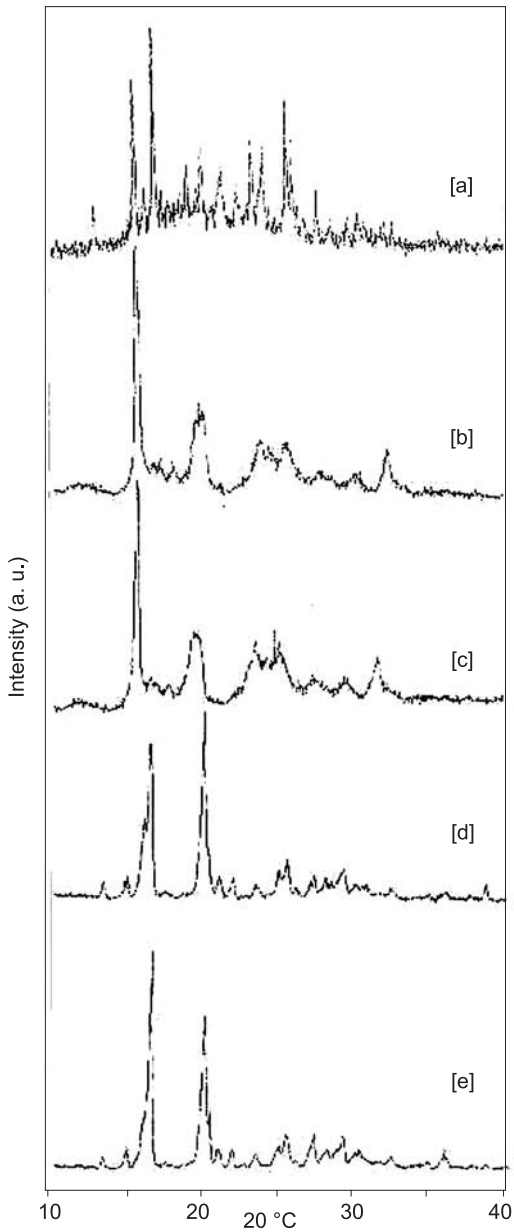

Figure 2. X-ray diffraction (XRD) patterns for: (a) commercial fluconazole-II; (b) fluconazole-I obtained when glassy fluconazole sample was heated above 81°C; (c) fluconazole-I obtained from glassy fluconazole sample aged for 48 h; (d) fluconazole-I obtained by application of uniaxial pressure of 200 kg/cm^2 on fluconazole-II; (e) fluconazole-I obtained by application of uniaxial pressure of 200 kg/ cm^2 on 36 months aged fluconazole-II.

cases of differently processed materials could involve partial transformation of form-I back to form-II prior to melting. It is also possible that the form-I begins to melt prior to its complete transformation to form-II and the peaks corresponding to the melting of form-I and form-II overlap.

The complete transformation of form-I to form-II and subsequent melting would result in two distinct peaks, one due to crystallographic transformation of form-I to form-II and other due to melting

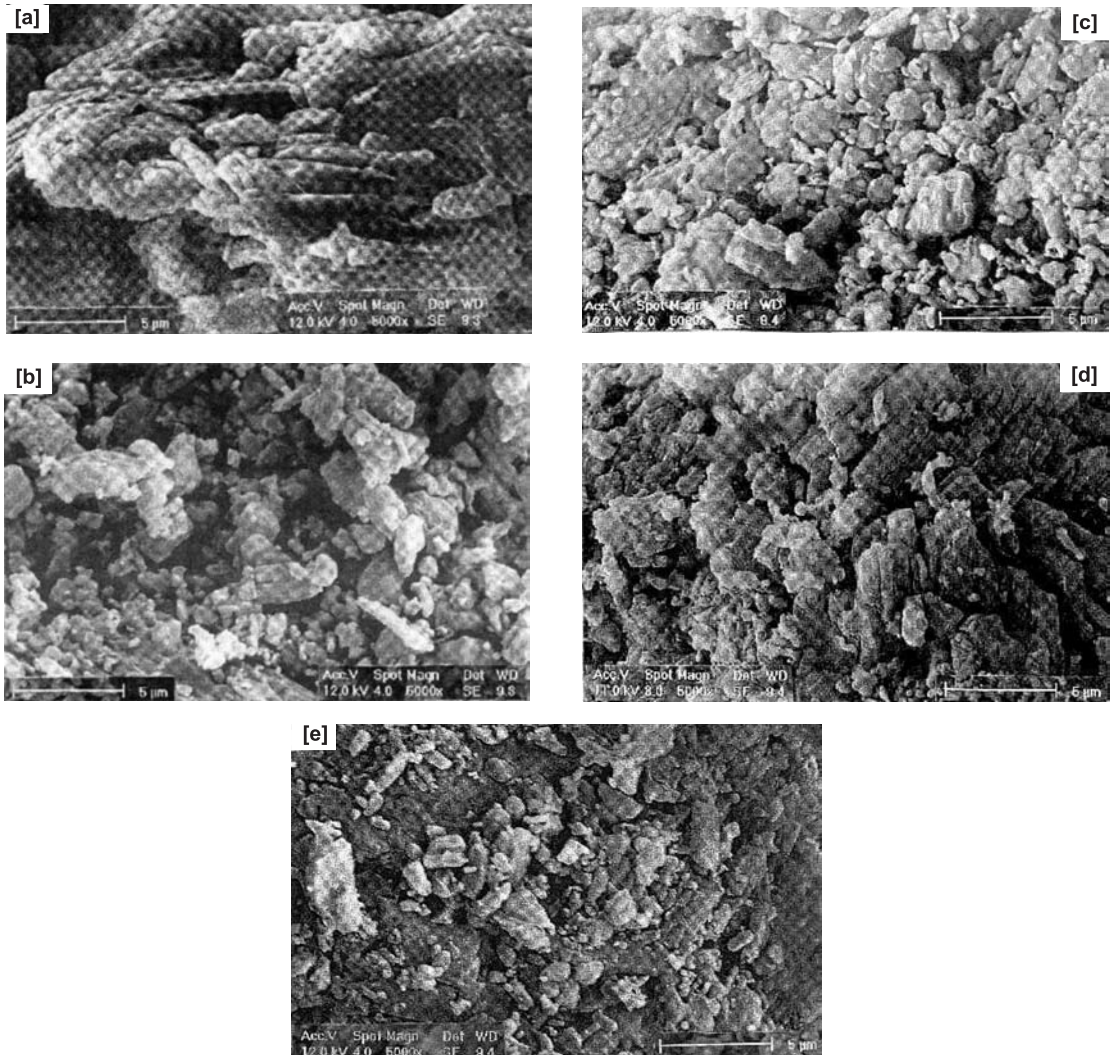

Figure 3. Scanning electron micrographs for: (a) commercial fluconazole-II; (b) fluconazole-I obtained when glassy fluconazole sample was heated above 81° C; (c) fluconazole-I obtained from glassy fluconazole sample aged for 48 h; (d) fluconazole-I obtained by application of uniaxial pressure of 200 kg/cm^2 on fluconazole-II; (e) fluconazole-I obtained by application of uniaxial pressure of 200 kg/cm^2 on 36 months aged fluconazole-II.

of form-II. The enthalpy of crystallographic transformation, in general, is expected to be relatively smaller compared to that for fusion (1). The manual resolution of the composite peak into its components, however, suggests that the magnitude of the enthalpy in the two cases is in the same range (90 to 110 mJ/mg). The schematic of manual resolution of the composite peak is shown in Figure 1c. The major contribution to the enthalpy of the composite peak is therefore, mainly from the melting of the two polymorphs and that from the polymorphic transformation beginning around 135.7°C is relatively much less.

X-ray diffraction results

The X-ray diffraction (XRD) patterns for the commercial drug (form-II) and the polymorphic forms obtained by subjecting the sample to different processing parameters are presented in Figures 2a to 2e. The X-ray pattern for the sample aged for 36 months was identical to that for the original sample and therefore is not included in Figure 2. This observation corroborates our DSC results which indicate the formation of form-I during 36 months ageing in the amount hardly detectable by X-ray diffraction. The XRD patterns presented in Figures 2b to 2e are all identical but distinctly different from that in Figure 2a. These pat-

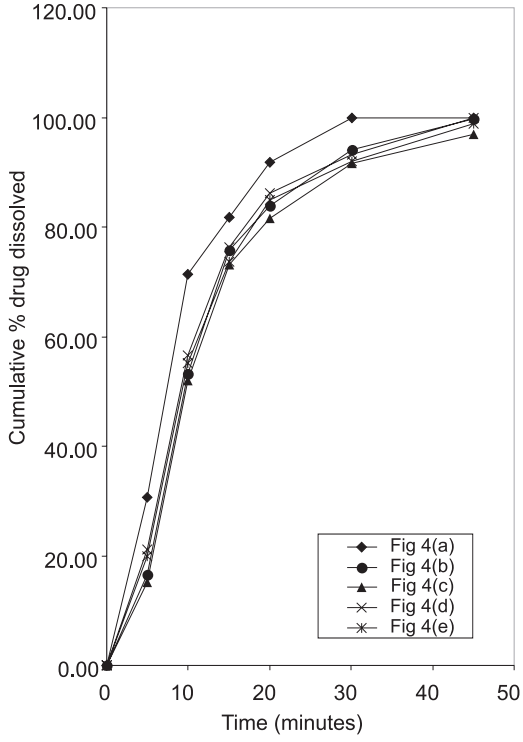

Figure 4. Dissolution profiles: (a) -◆- for commercial fluconazole-II sample; (b) -●- for fluconazole-I obtained when glassy fluconazole sample was heated above 81°C; (c) -▲- for fluconazole-I obtained from glassy fluconazole sample aged for 48 h; (d) -×- for fluconazole-I obtained by application of uniaxial pressure of 200 kg/cm^2 on fluconazole-II; (e) -✳- for fluconazole-I obtained by application of uniaxial pressure of 200 kg cm^{-2} on 36 months aged fluconazole-II.

terns agree closely with that for fluconazole-I reported in reference (10), albeit with few more lines which could be due to untransformed fluconazole-II . This observation indicates that irrespective of different modes of processing of fluconazole-II, (which involves: 1**.** heating glassy fluconazole above 81°C, 2. ageing of this glassy sample at ambient temperature of 30°C for 48 h or 3. application of high pressure on fluconazole-II and on the same sample aged for thirty six months) the polymorphic form obtained is the same. The X-ray diffraction patterns for the products obtained by processing fluconazole-II by different methods are almost similar and presented in Figure 2. Since all the XRD patterns are similar, the representative XRD data for fluconazole-I obtained by heating glassy fluconazole above 81°C are included in Table 2 for comparison with fluconazole-II.

Scanning electron microscopic observations

The scanning electron microscopic (SEM) photographs taken for the commercial sample and the polymorphic forms obtained by different methods outlined above are reproduced in Figures 3a to 3e. It is clear from these Figures that the photographs 3b, 3c, 3d and 3e show identical rod and granular type structures in comparison to the flake-like slabs visible in Figure 3a, suggesting that the polymorphic forms obtained by application of high pressure or by ageing or by heating the glassy mass above 81°C are structurally and morphologically identical but different from the original sample.

In vitro dissolution profile

The plot of cumulative percent of the drug dissolved against time in minutes for fluconazole-II and fluconazole-I obtained by different methods are presented in Figures 4a to 4e. The results show that form-I of fluconazole (Figs. 4b-4e) dissolves relatively slowly compared to fluconazole-II (Fig. 4a) (10). The dissolution profile data for the two forms are presented in Table 3.

CONCLUSION

All the observations presented above lead to the conclusion that the high-pressure form of the fluconazole drug is similar to the form obtained by other methods outlined above and is crystallographically as well as morphologically different from the commercial fluconazole-II drug. The results presented here could be extremely important in the formulation of fluconazole based drugs, since the polymorphic form of the drug, involved in formulations, dictates its solubility and hence its efficacy.

The formulation of the drug in the tablet form is expected to behave differently compared to the drug prepared in the syrup form. The knowledge of influence of pressure on the polymorphic transformation of the drugs is thus very important.

It has now been well established that there is indeed the dependence of the dissolution rate on the polymorphic form of the drug (13-16).

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