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ORIGINAL ARTICLE

Determination of factors affecting kinetics of solid-state transformation of fluconazole polymorph II to polymorph I using diffuse reflectance Fourier transform spectroscopy

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Abstract

Background: It was of interest to investigate the factors affecting kinetics of transformation of fluconazole polymorph II (the metastable form) to fluconazole polymorph I (the stable form) using diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS). *Method*: Fluconazole polymorphs I and II both were prepared by crystallization in dichloromethane. The two forms were characterized using differential scanning calorimetry, thermogravimetric analysis, powder X-ray diffraction, solubility, and DRIFTS. Transformation of polymorph II to polymorph I was also studied under different isothermal temperatures using DRIFTS. Kinetic analyses of the data were done using model-dependent and model-independent methods. Eighteen solid-state reaction models were used to interpret the experimental results. *Results*: Based on statistics, the Prout–Tompkins model provided the best fit for the transformation. The activation energy (E_a) value derived from the rate constants of the Prout–Tompkins model was 329 kJ/mol. Model-independent analysis was also applied to the experimental results. The average values calculated using both methods were not significantly different. Factors affecting kinetics of transformation such as mechanical factors, relative humidity, and the effect of seeding were also studied. Mechanical factors, which included trituration and compression, proved to enhance transformation rate significantly. Relative humidity proved to transform both polymorphs to monohydrate form. The presence of seed crystals of polymorph I was proved not to affect the transformation process of polymorph II to polymorph I. Effect of solvent of crystallization (dichloromethane) was studied. A significant change of the rate of transformation was proved in the presence of solvent vapors, and a change on the mechanism was proposed.

Key words: *Compression; DRIFTS; fluconazole; FTIR; kinetics; metastable; polymorph; relative humidity; seeds effect; trituration*

Introduction

The active ingredient as well as the excipient in solid dosage forms can undergo a variety of physical transformations during pharmaceutical processing and storage[1]. These transformations can affect pharmaceutically important properties such as solubility, stability, powder flow, tableting behavior, and dissolution rate, which ultimately can cause variations in the performance of the product[2]. Therefore, a thorough understanding of the kinetics and mechanisms of solid-state transformations in the early stages of drug development might facilitate the formulation of a stable product with the best physical quality and the best therapeutic effect.

Infrared spectroscopy is a powerful technique to characterize polymorphs and hydrates[3–7]. In its early use, infrared spectroscopy of Nujol mulls[3] or potassium bromide pellets[4,5] was commonly used in quantitative analysis of binary mixtures of polymorphs. Quantification was also commonly done by utilizing infrared absorbance ratio method[3]. These methods had several shortcomings. Combination of Fourier transform infrared

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(FTIR) spectroscopy and diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) provided an alternative to these traditional methods. DRIFTS facilitated sample preparation and avoided exposing the sample to any compression energy that may cause solid-phase transformation[6]. The use of multivariate-calibration techniques such as the partial least squares (PLS) regression method also enabled scientists to perform quantitative analysis on multicomponent mixtures with overlapping bands. DRIFTS was applied by previous investigators to characterize binary mixtures of sulfamethoxazole[6], mixtures of spironolactone[7], and mixtures of Ranitidine–HCl[4].

Fluconazole [2-(2,4-difluorophenyl)-1,3-bis(1H-1,2,4-triazol-1-yl)-propan-2-ol] is a useful drug in the treatment of adults with oropharyngeal and esopharyngeal candidiasis. Its structure is shown in Figure 1. It has desirable pharmacological properties, including a relatively long half-life, the ability to be administered orally or parenterally (intravenous), and the ability to penetrate cerebrospinal fluid[8]. Different polymorphic forms of fluconazole were reported[9–15]. These include three dehydrated forms and one monohydrate and several solvates. The most stable known form is polymorph I[12]. Certain polymorphic transformations were studied and analyzed using thermoanalytical techniques[14]. The kinetics of dehydration of fluconazole monohydrate and the desolvation of fluconazole ethyl acetate were also recently studied using thermogravimetry[15].

The purposes of this investigation were to use DRIFTS to evaluate the kinetics of transformation of fluconazole polymorph II (the metastable form) to fluconazole polymorph I (the stable form) and to study the effect of some of the factors that might affect the kinetics of transformation such as mechanical force, water, solvent, and effect of seeding.

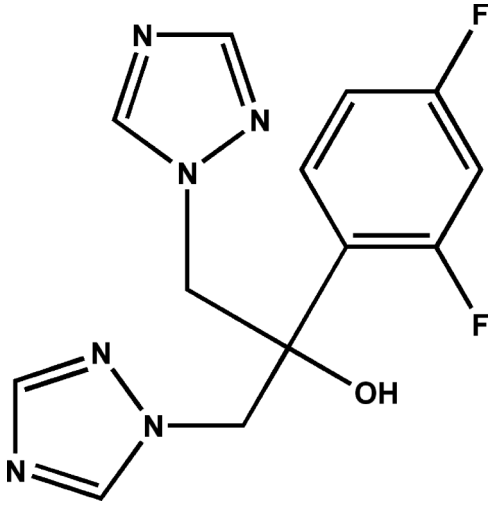

Figure 1. Chemical structure of fluconazole.

Experimental section

Crystal preparation

Fluconazole polymorphs I and II were obtained using the same solvent (dichloromethane) as described previously[12]. Fluconazole monohydrate was also prepared as described previously[13,15].

Powder X-ray diffraction

X-ray patterns were collected at room temperature on a PW 1729 Philips diffractometer using Cu Kα radiation (λ = 1.5418Å) and generator settings of 35 kV and 20 mA. Samples were finely ground (average particle size <180 μm), mounted on a slide, and exposed to the X-ray beam.

Thermal analysis

Differential scanning calorimetry (DSC) traces for different crystal forms were recorded on a Shimadzu DSC-50 differential scanning calorimeter (Shimadzu Corporation, Tokyo, Japan) equipped with Shimadzu software. A heating rate of 1°C/min was used and a nitrogen purge of 20 mL/min was used. The temperature axis was calibrated using ultrapure indium (99.9999%) and open pans were used for the analysis. Thermogravimetric analysis (TGA) was carried out using a Shimadzu TGA-50 equipped with Shimadzu software (Shimadzu Corporation). A heating rate of 10°C/min and a nitrogen purge were used.

FTIR

Infrared spectra were obtained on a Nicolet Avatar 5.1 ESP 360 Spectrometer IR System using Nicolet Omnic software and a diffuse reflectance cell (Nicolet Instrument Corporation, Madison, WI, USA). The particle size of the calibration and the unknown samples was controlled within 100–200 μm. Sieving was used to obtain the appropriate particle size. Particle size effect was discussed by previous investigators[6]. These investigators indicated that decreasing the particle size will aid in the powder packing process and in decreasing the specular reflectance, that is, decrease the radiation that is reflected from the surface of the powder bed without passing through the particles. Analysis of the ground polymorphs by X-ray and DSC indicated that there were no crystalline conversions within 8 hours. For the calibration and the validation samples, different mixtures

of the two polymorphs (I and II) were prepared. Varying amounts of polymorphs I and II were weighed to make a total of 30 mg of fluconazole. Fluconazole was then added to 270 mg of KBr micronized powder to give a total of 300 mg. Fluconazole and KBr were then mixed to obtain a uniform mixture. The mixed powder was then poured into the sample cup and was leveled with a spatula. The spectra of the samples were collected over a wave number range of 400–4000 cm^{-1}. Thirty-two scans were collected at 4 cm^{-1} resolution. Micronized KBr was also poured into the reference cup using the same technique. The constant repacking decreased the standard deviation (SD) remarkably between the readings and gave consistent readings.

PLS method is a statistical technique that examines the specified regions of the calibrating spectra to determine the areas varying statistically as a function of component concentration. The PLS model was applied using the spectral and concentration information from the standards. The method was saved on the computer and on CDs to be used in the future analysis. Daily validation for the FTIR was also performed. The PLS method was also validated. Validation was done using validation standards, linearity, precision, reproducibility, and specificity. FTIR was also used to exclude emergence of degradation products in all the experiments.

Solubility measurement

The solubilities of the different polymorphs in water were determined at 25°C using a sustained release apparatus. Excess amounts of the samples were added to 10-mL screw-top bottles containing 7 mL of water. Teflon tape was placed over the top of each bottle to prevent solution contact with the cap. The bottles were immersed in water inside a water bath, with continuous shaking. Samples were filtered rapidly through teflon membranes, which were installed in stainless steel filter holders. At regular intervals samples were taken and the concentration of each sample was determined using a UV-visible spectrophotometer equipped with a photodiode array detector (Multispec-1501; Shimadzu Corporation). The experiment was run for 7 hours (validated equilibrium stage), and 24-hours samples were taken on the next day. Three runs of the solubility experiment were done on different samples.

Studying thermal transformation of polymorph II to polymorph I

Samples of polymorph II were stored at different isothermal temperatures (60°C, 109°C, 111°C, 113°C, 115°C, 117°C, 120°C, and 130°C) and were analyzed with time. Control samples were kept in silica gel desiccators at 20°C. Analysis was done using DRIFTS. Results from 109°C to 117°C were kinetically analyzed using both model-dependent and model-independent analyses. Out of range temperatures (60°C, 120°C, and 130°C) were used for validation of the analysis.

Studying the effect of mechanical forces on transformation (trituration and compression)

For trituration: Fluconazole polymorph II was triturated using agate pestle and mortar at 20°C. At different time intervals (5 minutes), samples were tested using quantitative DRIFTS method. For compression: The first study confirming compression effect was done on tablets weighing 500 mg each and by applying the maximum force of compression using Pruf system D-S657, type RK-Tronic, Roell and korthrans, Germany. Each tablet was divided into three parts: upper, middle, and lower. Samples were taken from all these parts and analyzed at 20°C.

The same experiment was also conducted on 100 mg tablets with a thickness of 0.71 mm each. The thickness was small, and from technical point of view, it was difficult to divide it into layers. Therefore, each tablet was divided into three separate parts and was then analyzed. Different forces of compression were applied on 100 mg tablets of polymorph II. The pressures reported in this study represented mean values from lower punch. Three tablets, compressed using the maximum compression force, were chosen and were stored at 20°C. Samples of these tablets were taken at different time intervals and were analyzed quantitatively using DRIFTS. Effect of increasing compression time was also studied. The maximum force of compression (43,439.58 Pa) was used to compress tablets, with changing time of compression. The same procedure was also repeated using medium force of compression ($n \geq 3$). All the compression-related experiments were conducted at 20°C.

For humidity-related transformations

Five different relative humidity (RH) desiccators were prepared by dissolving excess salt (extra pure, Scharlau, Spain). They were LiCl RH: 12%; KF RH: 31%; NaI RH: 41%; KBr RH: 84%; and Water RH: 100%. The samples were stored at 20°C in the five environments and were analyzed for moisture content with time using TGA, until there was no further change in samples weights. These samples were also characterized qualitatively using DSC, FTIR, and X-ray diffractometry and the effect of RH on polymorphic transformation was then analyzed.

Effect of presence of seed crystals

Three different amounts of polymorph I were added to polymorph II. These are 5:95, 10:90, and 20:80 as the

ratio of polymorph I:polymorph II. Samples were stored at constant temperature (130°C). Small quantities were taken from each sample and were analyzed quantitatively with time.

Effect of solvent of crystallization

Samples of polymorphs II and I were stored at 20°C in a desiccator, which was filled with dichloromethane solvent. Samples of both polymorphs (I and II) were analyzed quantitatively with time.

Results and discussion

Characterization of polymorphs I and II

The X-ray patterns of polymorph I (Figure 2) match well with the previously published data[9,12], and on the contrary polymorph II X-ray pattern is presented here in its pure form. The DSC endotherms contained single endothermic melting peaks (Figure 3). Polymorph I was proven previously to be the stable polymorph and polymorph II was proven to be the metastable polymorph[12].

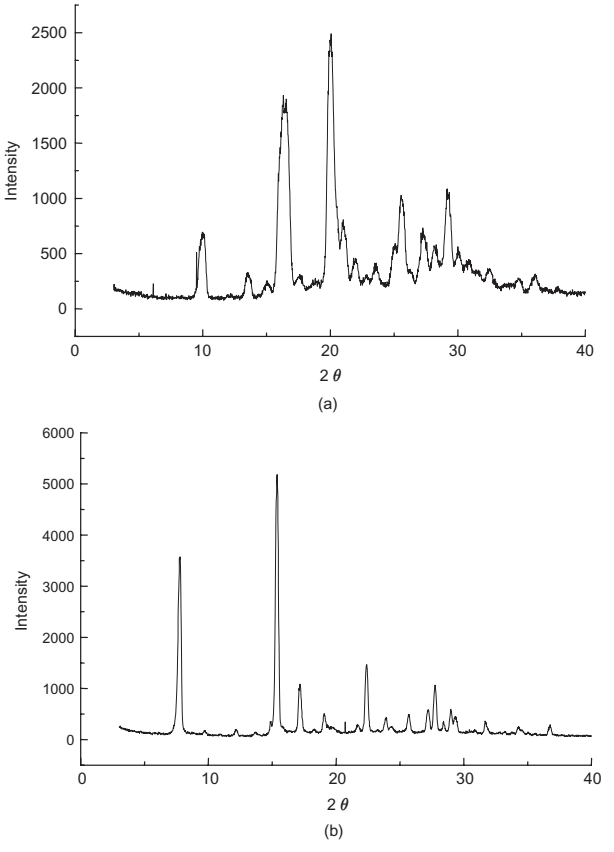

Figure 2. X-ray diffraction of fluconazole: (a) polymorph I and (b) polymorph II.

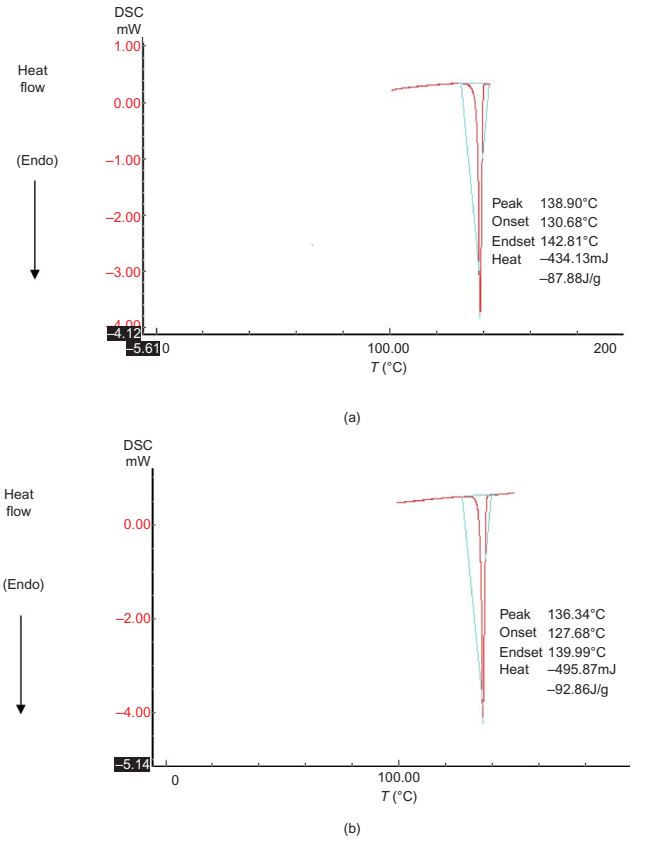

Figure 3. Typical DSC thermograms of fluconazole: (a) polymorph I and (b) polymorph II.

It was also proven previously that polymorphs I and II are monotropic[12]. The two forms were proved to be anhydrous using TGA.

Solubility

The solubility profiles for polymorph I, polymorph II, and the monohydrate (added for comparison) in water are shown in Figure 4. The SD did not exceed 1.5% at all the points. The solubility profile for polymorph I follows the common solubility profiles (primer increase followed by steady maximum solubility value stage). This value for polymorph I is 3.66 ± 0.01 mg/mL. The solubility profile for polymorph II appeared to increase to a maximum (3.84 ± 0.05 mg/mL) followed by a decrease then a steady value (3.57±0.02 mg/mL), which appeared to be less than that of polymorph I. Analysis of the excess drug of polymorphs I and II was done, and the results showed that polymorph I did not change while full transformation to monohydrate occurred during the solubility experiment for polymorph II (data not shown). Monohydrate was characterized previously[13] and solubility was determined. Solubility profile was added for comparison only. The maximum steady

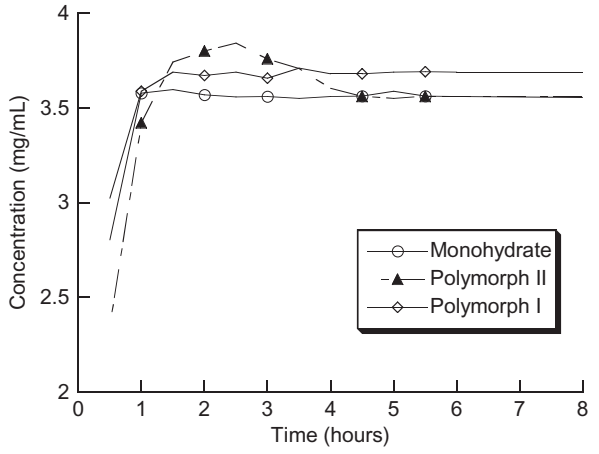

Figure 4. Solubility profiles of polymorph I, II, and monohydrate in water at 25°C.

solubility of the monohydrate (3.56 ± 0.02 mg/mL) agrees very well with the lowest value obtained from polymorph II solubility profile, which also confirms that polymorph II fully transformed to the monohydrate during the solubility experiment.

DRIFTS

Full spectral display is shown in Figure 5 for polymorphs I, II, and (1:1) mixture of both. The FTIR spectra of the two polymorphs match well with the previously published data[12]. Spectral differences and band overlap are apparent in the high-wave number range from 3600 to 2800 cm^{-1}. Different polymorphs of the same drug are expected to have severe interaction in its spectra. This makes PLS method the preferable analysis method in this case. It was used previously in polymorphic analysis[4]. PLS method was tested in applicability in development of a method to quantify binary mixtures of fluconazole polymorphs (I and II). This method was proved to be an excellent method for this type of analysis. The PLS model consists of an optimal number of

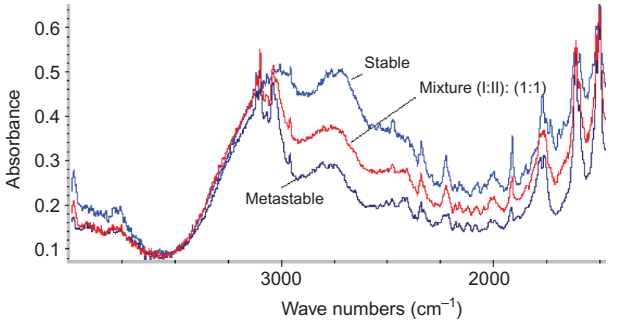

Figure 5. FTIR spectra of forms I, II, and (1:1) mixture of the two. All samples were dispersed as 10% in KBr.

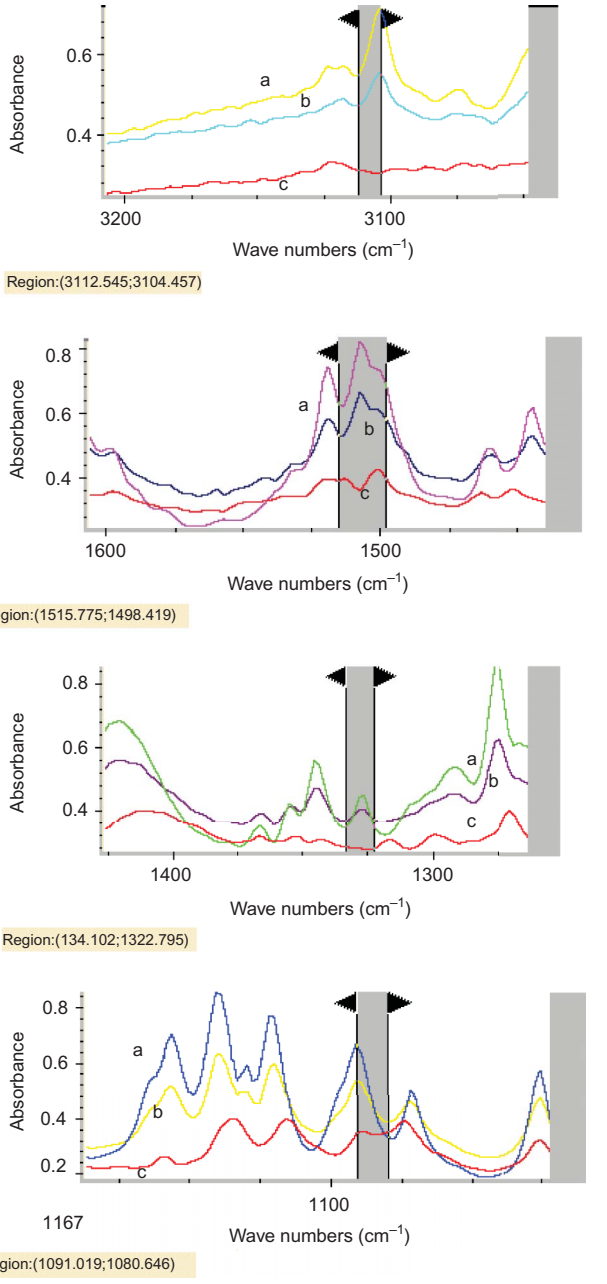

Figure 6. The regions in FTIR spectra which were used in differentiating between fluconazole polymorphs (I and II): (a) polymorph II, (b) mixture of polymorphs (I and II) in ratio (1:1), and (c) polymorph I.

factors or loading vectors to represent the complexity of the system without overfitting the data. Four regions were selected for differentiation between the two polymorphs. These regions are shown in Figure 6. Actual versus calculated concentration values for polymorphs I and II are also shown in Figure 7. Excellent correlations were obtained between the actual and the predicted values for the calibration samples for polymorphs I and II, respectively. The combination of

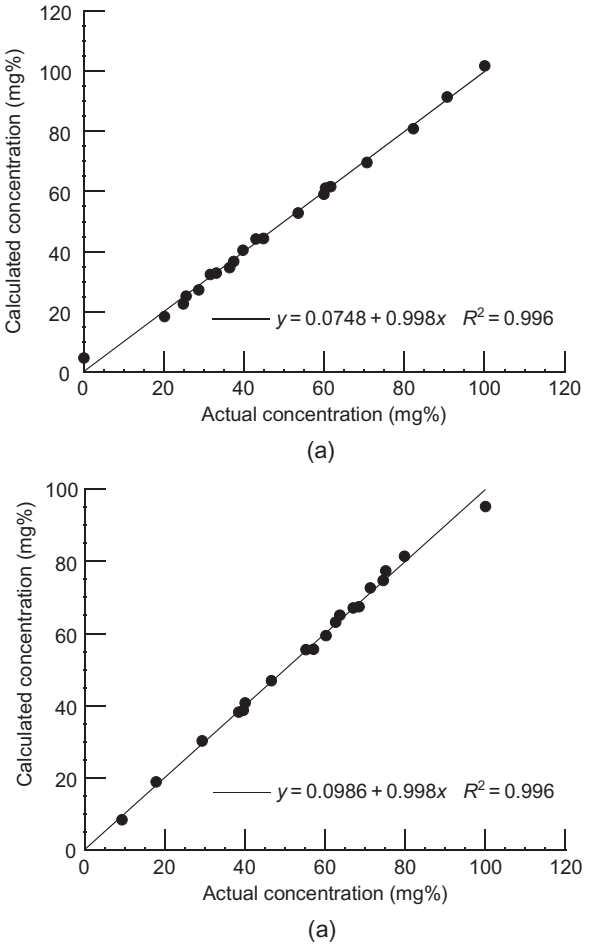

Figure 7. Calculated versus actual concentration values (mg%) for (a) form I and (b) form II fluconazole polymorphs in calibration mixtures.

the four spectral regions and the four factors, utilized in the model, resulted in 99.5% performance index. The linearity study was conducted on 20 binary mixtures, the concentration of which ranges from 0 to 100 mg% in the different mixtures. The precision of the method under the same operating conditions over a short interval of time was excellent. The reproducibility was measured to assure precision in sampling, as consistent sampling procedure is a must in the quantitative work; it was proved to be a reproducible method. The method is specific for the two polymorphs. The model provided a good agreement between the actual and the predicted values for the calibration samples (R^2 = 0.99), with the average absolute error being 0.19 (0.004%). The agreement of the predicted values with the theoretical values demonstrates that, when careful attention is paid to experimental details, diffuse reflectance can be a useful quantitative technique for characterizing fluconazole polymorphs I and II.

Transformation of metastable polymorph II to stable polymorph I

To evaluate thermal transformation kinetics, transformation of polymorph II to polymorph I was studied under different isothermal temperatures. A typical plot of the fraction transformed as a function of time is shown in Figure 8. Data were subjected to two different types of analysis: model dependent and model independent.

Model-dependent analysis

Eighteen solid-state reaction models were applied to the experimental data, and the best fit was obtained using the Prout–Tompkins (PT) equation[16]:

$$\ln\left(\frac{\alpha}{1-\alpha}\right) = kt + c$$

where α is the fraction transformed, k is the reaction rate constant, t is the time, and c is a constant. This model was used to describe most of the sigmoidal curves in solid-state transformations. It assumes that the rate of the solid-state reaction is controlled by linearly growing nuclei that branch into chains and are terminated more rapidly as the number of nuclei increases[16]. A typical plot is shown in Figure 9. The computed activation energy from the Arrhenius plot (Figure 10) was found to be 329 kJ/mol.

An alternative model-free analysis[17] was also applied to the experimental results using the following equation:

$$\ln\left(\frac{\mathrm{d}\alpha}{\mathrm{d}t}\right)_{\alpha} = \ln\left[Af(\alpha)\right]_{\alpha} - \frac{E_a}{RT}$$

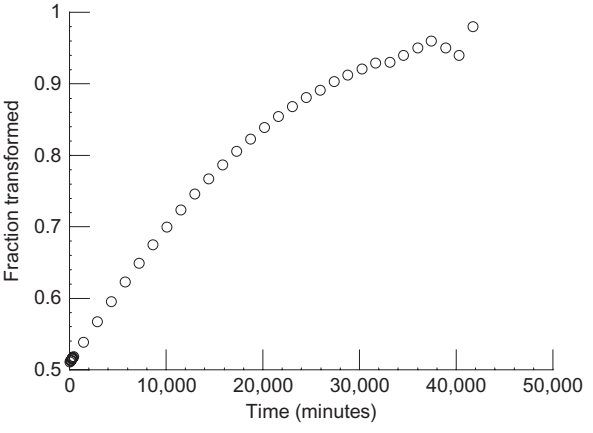

Figure 8. A typical plot of the fraction transformed (α) as a function of time (temperature = 111°C).

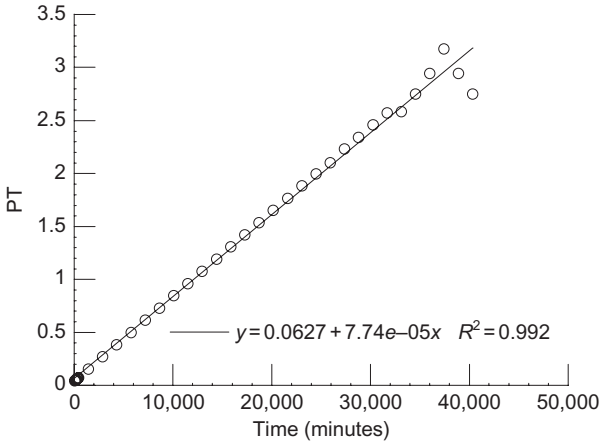

Figure 9. Application of the Prout–Tompkins model to the experimental results (temperature = 111°C).

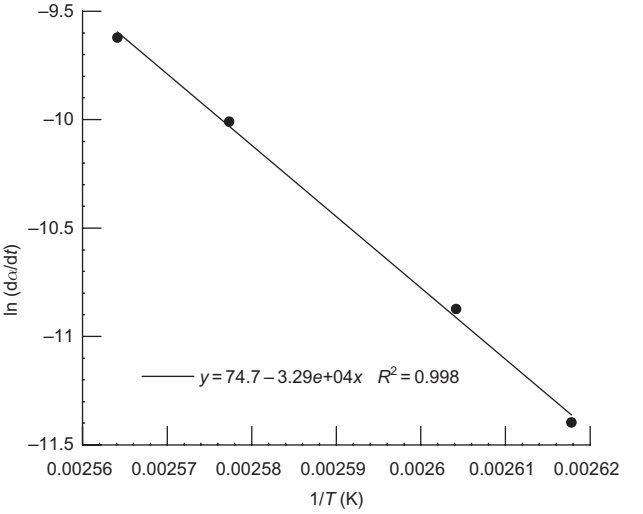

Figure 11. Best linear plot of $\ln(d\alpha/dt)_{\alpha = 0.60}$ versus T^{-1} at $\alpha = 0.60$.

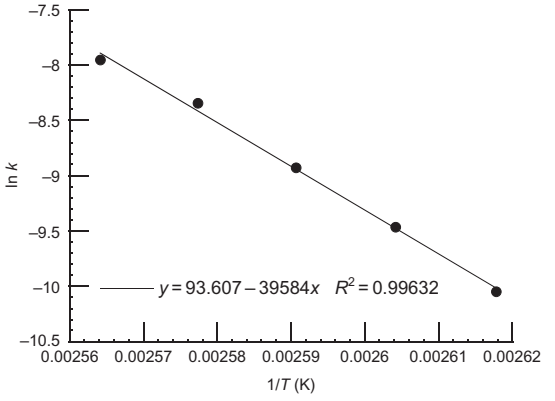

Figure 10. Plot of $\ln(k)$ versus T^{-1} for rate constants derived from fitting to Prout–Tompkins model.

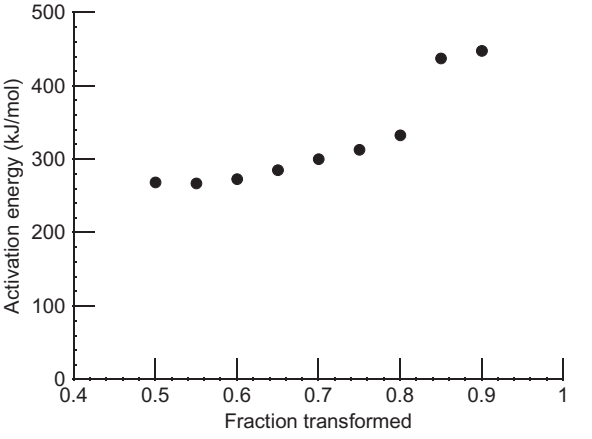

Figure 12. Dependence of the transformation activation energy, E_a, on the transformed fraction, α, analyzed by model-free kinetics.

where α is the fraction transformed, t is the time, A is the frequency factor, E_a is the energy of activation of the reaction, T is the temperature, and R is the gas constant. Plots of $\ln(d\alpha/dt)_\alpha$ against T^{-1} were drawn and were used to calculate the activation energy (E_a). A typical plot of $\ln(d\alpha/dt)_\alpha$ against T^{-1} is shown in Figure 11. The calculated E_a values from all figures are shown in Figure 12. It appears that the activation energy values are approximately the same up to $\alpha = 0.8$. However, the E_a values deviate sharply from the mean at $\alpha > 0.8$. The activation energy calculated up to this point ($\alpha = 0.8$) was 310 ± 25 kJ/mol. It is misleading sometimes to use the model-dependent analysis in which a constant activation energy value is assumed to be constant over the entire α range. It is clear that at $\alpha > 0.8$ extreme deviation occurs, which is expected in most reactions. However, the average value calculated using the model-independent method and the value obtained from the model-dependent method were not significantly different. This assures that the PT model can describe adequately the transformation process of polymorph II to polymorph I.

To validate the analysis method, several samples were also studied experimentally at temperature values outside the analysis range (20°C, 60°C, 120°C, and 130°C). The results obtained experimentally from these studies were compared to the calculated values obtained from the PT model-dependent Arrhenius plot. The experimental and the predicted values were almost identical (Figure 13). This result also indicated that PT model is the proper model to describe the transformation process of polymorph II to polymorph I.

The calculated α-values from the PT model, at 60°C, were also compared to the experimental (actual) values.

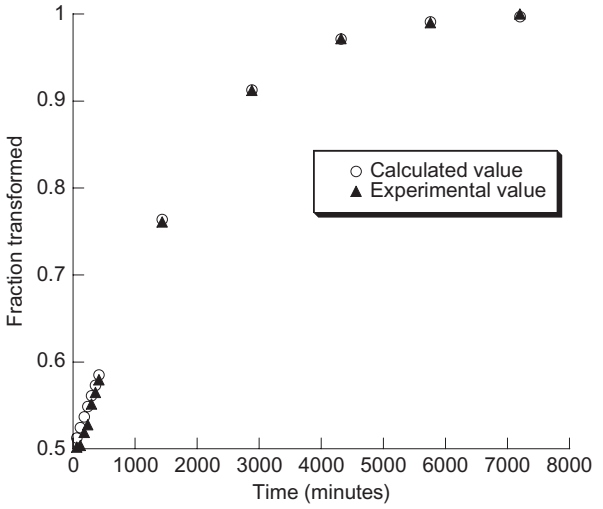

Figure 13. Plot of the fractions transformed (α-values) versus time derived from Prout–Tompkins model, and actual values obtained experimentally at isothermal 120°C.

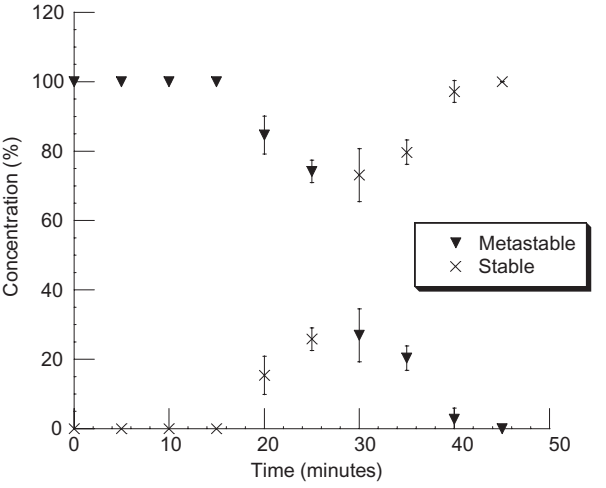

Figure 14. Effect of trituration on polymorphic transformation of fluconazole polymorph II (metastable) to polymorph I (stable).

It is worth noting that this transformation was very slow (not complete). Statistical analysis for the first 30 days showed that there is no significant difference between the two samples (P-value was 0.35, χ^2 = 20.52). The experiment was followed for 8 months. The results confirmed that the transformation process is very slow at the aforementioned temperature and that the model-dependent analysis (PT model) has the ability to predict the rate of transformation process at such temperature. The actual data at 20°C for 12 months showed no transformation or extremely slow transformation (not detectable). Prediction of the transformation process using model-dependent analysis showed that no change in α-value occurred during 2 years. On the contrary, the calculated α started from 0.5. This would be expected because PT model describes the process from this value. From this, it is concluded that model-dependent analysis provides us with the rate of the reaction not values. This may necessitate real experimental data points to calculate the constants in the reaction to describe the reaction sufficiently.

As previously explained[18], trituration can be recognized as the primer-screening test for pressure-induced polymorphic transformations. Each polymorph, in this study, was triturated by pestle and mortar and was quantitatively assayed by DRIFTS. The data obtained suggested that grinding or milling of fluconazole polymorph II is sufficient to induce polymorphic transformation to polymorph I. Trituration is considered advantageous in polymorphic transformations studies because of the following reasons. First, it requires minimal sample. Second, the mortar and pestle have the advantage of assurance that the sample is in constant contact with the stress initiator[19]. The results of three experimental runs are clarified by plotting the concentration (% recovery) versus milling time in Figure 14. Figure 14 shows that transformation (because of trituration by mortar) requires a 20-minute induction period, whereas complete transformation occurs at 45 minutes. The data seem to correlate proportionally after the 20-minute induction period. The obtained r^2-value for a linear fit to this range was 0.91. Although the manual milling force might not be constant all over the experiment period, however, the results obtained from this study indicate that polymorphic transformation occurs because of milling and might suggest a strong evidence for high possibility of compression-related polymorphic transformations.

Compressive stress tends to crush or collapse a body[19]. Data on material's responses influenced by compression are not always given with polymorphic characterization, although it is very important to determine the polymorphic response to the compression process. The energy of tableting process may be dispersed in many directions. One of the most important changes is through crystallographic changes or polymorphic transformation. It seems that most of the compression energy in fluconazole polymorph II compression was spent on consolidation of particles into a tablet and also on polymorphic transformation (polymorph II to polymorph I). The first study confirming compression effect was done on tablets weighing 500 mg with a thickness of 3.39 mm (SD = 0.025 for n = 9). Maximum force (43,439.58 Pa) of compression was applied. Samples taken from lower, middle, and upper sides of the tablets were reduced back into powders and DRIFTS analysis was carried. The lower surface was exposed to, on average, the largest force of compression and it

demonstrated the most rapid and the highest degree of transformation [34.34% (SD ± 4.64)]. The reason for this is that the machine used applies the compression force from the lower punch, and therefore the lower side of the tablet was the primer contact side with the applied force. This explanation can be confirmed by the results that were obtained in the middle region and the upper region. The percentage transformed was lower in the middle region [24.88% (SD ± 4.45)], which in turn was higher than the upper region [11.02% (SD ± 5.68)]. To decrease the material consumption and to get consistent results, the same experiment was done on 100 mg tablets with a thickness of 0.71 mm (SD = 0.024 for n = 20). Technically, it was difficult to divide the tablet into separate layers; therefore the tablets were divided into three equivalent parts, and samples were analyzed. On comparison, higher value of transformation was obtained for the 100 mg tablet. The average transformed product was 40.02% compared to 23.41% in the 500 mg tablets. This may be explained by achieving the maximum packing for the powder and a uniform distribution of force over the tablet that has a small thickness.

Different forces of compression were used to prepare polymorph II tablets to study the polymorphic response of polymorph II versus the force used in compression. The transformed fraction of fluconazole polymorph II to polymorph I showed a sharp increase by increasing the compression force up to 60% of the maximum force used in the experiment. Above 60% a lower increase in the rate of transformation occurred. Results are shown in Figure 15. The results of compression of polymorph II suggested that the compression process induced (39.57%) transformation to the stable form (I) during one compression at the maximum force applied which

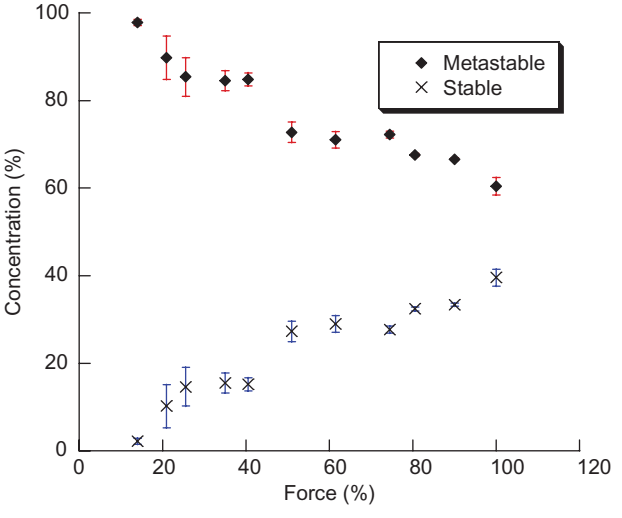

Figure 15. Effect of increasing force of compression on polymorphic transformation of fluconazole polymorph II.

was 43,439.58 (N/m^2) and 60.43% remained as polymorph I. A possible explanation for the transformation process is that the compression energy induces a number of dislocation sites. These are the high-energetic sites that transformation process starts from. The energy of transformation is expected to be lower at these sites. These sites are expected to increase with increasing compaction pressure. This will lead to proportional relation of increasing transformation with increasing force of compression as shown in Figure 15. However, these deformations seemed to be limited by the surface area exposed to compression. This result is assured by reaching full transformation process in the trituration experiments. Manual trituration exposes the maximum surface area of the polymorph to crushing mechanical force. This force is applied to the material from all particle dimensions, causing deformation in all crystal sides. This, in turn, accelerates the transformation process. Compression of fluconazole polymorph I was also investigated and it did not show any transformation when the maximum force of compression was applied.

Effect of compression time (sustained compression) on the solid-state transformation of fluconazole polymorph II to polymorph I was also investigated. This was done by exposing different samples to different times of compression at the maximum force of compression. The results obtained (not shown) indicated that increasing the time of the applied compression force does not cause any increase in the percentage of transformation.

Following the transformation process in stored tablets (20°C), tablets compressed using the maximum compression force showed full transformation within 21 days. However, transformation process (not detectable) for polymorph II without exposure to compression at the same period was extremely slow.

Studying the RH effect on fluconazole polymorphic transformation of forms II and I was done using different RH desiccators at 20°C. Table 1 shows the results obtained for polymorph II. No change in water content was observed in low and medium RHs (12%, 31%, 41%). On the contrary, water content increased at high RHs (84%, 100%), and full transformation occurred to monohydrate polymorph within 4 hours. No significant difference was observed between the two high RHs. The mechanism of transformation is most probably solvent mediated, that is, polymorph II solvation, followed by recrystallization to the monohydrate.

The results for polymorph I transformation in different RHs are shown in Table 2. No transformation occurred at low and medium values (RH < 41%). On the contrary, slow transformation process started at high values (84%, 100%) after 24 hours. The water content increased from 0.5% up to full hydrate content within 72 hours.

Effect of adding polymorph I seeds on the transformation of polymorph II was studied at isothermal

Table 1. Percentage change of the water content of fluconazole polymorph II stored at five different relative humidity desiccators in relation to time measured by TGA at 20°C.

Time (hours)	RH = 12% (%)	RH = 31% (%)	RH = 41% (%)	RH = 84% (%)	RH = 100% (%)
1	0	0	0	1.81 (±0.41)	2.81 (±0.21)
2	0	0	0	3.03 (±0.39)	3.93 (±0.37)
3	0	0	0	3.90 (±0.32)	4.20 (±0.34)
4	0	0	0	5.40 (±0.46)	5.44 (±0.41)
24	0	0	0	5.58 (±0.40)	5.78 (±0.20)
48	0	0	0	5.60 (±0.35)	5.91 (±0.12)
72	0	0	0	5.68 (±0.33)	5.89 (±0.21)
720	0	0	0	5.70 (±0.31)	5.90 (±0.21)

SD was taken for three sample runs.

Table 2. Changes of the water content of the fluconazole polymorph I stored at five different relative humidity desiccators in relation to time.

Time (hours)	RH = 12% (%)	RH = 31% (%)	RH = 41% (%)	RH = 84% (%)	RH = 100% (%)
1	0	0	0	0	0
2	0	0	0	0	0
3	0	0	0	0	0
4	0	0	0	0	0
24	0	0	0	0.52 (±0.28)	0.54 (±0.25)
48	0	0	0	1.12 (±0.15)	1.13 (±0.09)
72	0	0	0	5.60 (±0.41)	5.71 (±0.32)
720	0	0	0	5.77 (±0.39)	5.82 (±0.19)

SD was taken for three sample runs.

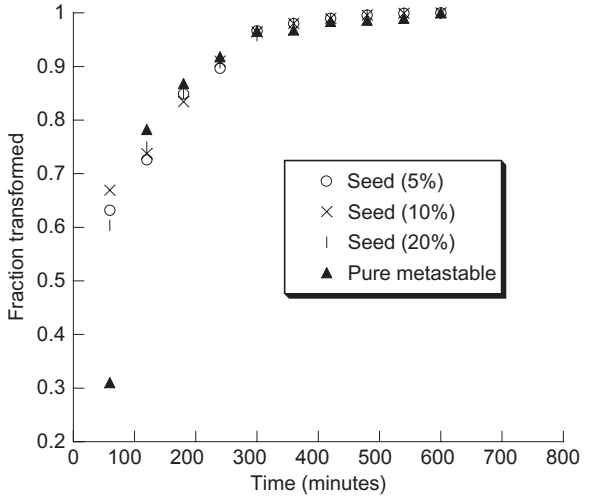

Figure 16. Plot of fraction transformed (α-values) for four different runs, three of which contain different ratios of polymorph I (5%, 10%, 20%) and the pure polymorph II at 130°C.

temperature (130°C). Three different ratios were used. The ratios were 5%, 10%, and 20%. The results were compared to pure polymorph II (metastable). Figure 16 shows the result of the experiments. Statistical analysis proved that there is no significant difference between the four runs (P-values = 0.85, 0.83, and 0.68).

The idea of solvent of crystallization came from RH effect, which enhanced transformation to monohydrate. The mechanism of such transformation is usually explained as solvent-mediated transformation[20]. It involves two steps. These are metastable form dissolution followed by slow recrystallization to the stable polymorph. Dichloromethane was selected because it is the solvent used in the preparation of both polymorphs II and I. Samples were stored in dichloromethane desiccator at 20°C. Quantitative analysis was done in relation to time. Figure 17 shows comparison between polymorph II samples in dichloromethane desiccator to samples in silica gel desiccator. It appears from the results that transformation at 20°C in silica gel desiccators was very slow. The fraction transformed (α-value) did not exceed 0.01. On the contrary, in dichloromethane desiccator the fraction transformed approached 0.2, which means an increase in the transformed fraction. The statistical analysis revealed that there is a significant difference between the two runs (P-value < 0.001). However, the rate of transformation seemed to be very slow in both desiccators for the period of our study (60 days); therefore, it was not possible to follow the complete transformation process in these conditions. Involvement of dichloromethane, in the crystallization process, is most probably going to change the mechanism of transformation from PT to solvent-mediated transformation.

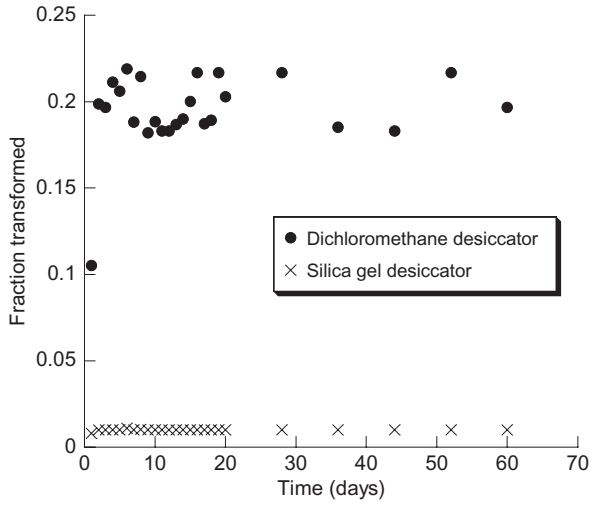

Figure 17. Plots of fraction transformed (α-values) at 20°C in dichloromethane desiccator and in silica gel desiccator for polymorph II.

Conclusion

Preparation and full solid-state characterization were carried out for fluconazole polymorphs I and II. A DRIFTS method was developed to differentiate between the two fluconazole polymorphs (I and II) quantitatively. The analysis was based on PLS analysis. Excellent correlations were obtained between the actual and the calculated values for polymorphs I and II, respectively. This method was used to study the temperature-induced transformation of polymorph II to polymorph I. Based on statistics, the PT model provided the best fit for the transformation.

Mechanical force including manual trituration and compression was proved to enhance transformation process dramatically. Humidity affected both polymorphs (I and II). Polymorph I proved to be more stable and less susceptible to changes than polymorph II, and the transformation to the monohydrate process required more time. The presence of seed crystals of polymorph I was proved not to affect the transformation process of polymorph II to polymorph I. Solvent of crystallization was proved to change the mechanism of transformation to solvent-mediated transformation. However, the rate of transformation was still very slow at the temperature of study. Fluconazole polymorph I showed high stability in high-isothermal temperatures, toward mechanical forces (trituration and compression), and toward solvent of crystallization. The results showed that fluconazole polymorph I is more stable than polymorph II.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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