ELSEVIER

Thermochimica Acta 376 (2001) 175–181

thermochimica acta

www.elsevier.com/locate/tca

Investigation of thermal properties of glassy itraconazole: identification of a monotropic mesophase

Karel Six[a], Geert Verreck[b], Jef Peeters[b], Koen Binnemans[c], Hugo Berghmans[d], Patrick Augustijns[a], Renaat Kinget[a], Guy Van den Mooter[a,*]

[a]*Laboratorium voor Farmacotechnologie en Biofarmacie, Katholieke Universiteit Leuven, Leuven, Belgium*
[b]*Janssen Research Foundation, Beerse, Belgium*
[c]*Laboratorium voor Coördinatiechemie, Katholieke Universiteit Leuven, Leuven, Belgium*
[d]*Laboratorium voor Polymeerchemie, Katholieke Universiteit Leuven, Leuven, Belgium*

Received 4 May 2001; accepted 9 May 2001

Abstract

The purpose of the present work is the elucidation of two endothermic transitions at 74 and 90°C, respectively, observed during differential scanning calorimetry of glassy itraconazole. Modulated temperature DSC (MTDSC), hot-stage microscopy (HSM), HPLC and high temperature X-ray diffraction (HT-X ray) were used to examine the thermal properties of glassy itraconazole. It was found that the preparation mode of the glass does not seem to influence the appearance of both endothermic transitions since they were present during heating of glassy itraconazole which was prepared by cooling the melt or by rapid solvent evaporation of an itraconazole solution. These observations suggest that the appearance of the two endothermic transitions require the liquid state prior to glass formation. The transitions are not due to impurities in the starting material, nor are they caused by thermal decomposition. This was further confirmed by HPLC-analysis. HSM showed structure formation following cooling of the melt, at approximately 87°C; cooling the product further showed a second change in optical contrast. HT-X ray confirmed and identified the formation of a nematic mesophase. The appearance of the two endothermic signals during scanning of glassy itraconazole points to the formation of a mesophase. Due to the nature of itraconazole, it appears as a chiral nematic phase of which the mobility is frozen into a glass upon cooling below 59°C thereby impeding further crystallization.

Keywords: Itraconazole; Liquid crystals; MTDSC; Glassy drugs

1. Introduction

Itraconazole is a potent antifungal drug of the triazole group with activity against histoplasmosis, blastomycosis and onchomycosis. The pharmacological mechanism is the same as that of the structural analogues ketoconazole and miconazole, which interfere with the synthesis of ergosterol of the fungal membrane by inhibition of 14 α-demethylase, a CYP 450 *iso*-enzyme [1]. Because of its very low aqueous solubility ($S < 1$ μg/ml) and poor dissolution rate itraconazole shows a large inter-individual difference in bioavailability after oral administration [2]. It is classified as a class II compound in the Biopharmaceutics Classification System.

* Corresponding author. Tel.: +32-16-345830; fax: +32-16-345996.
E-mail address: guy.vandenmooter@farm.kuleuven.ac.be

0040-6031/01/$ – see front matter
PII: S0040-6031(01)00563-9

The formulation of solid dispersions is generally accepted as a method to enhance the dissolution characteristics of class II drugs. The molecular distribution of the drug in the carrier, together with the enhanced wettability and microenvironment created by the carrier may increase the dissolution rate and solubility. Currently, several formulations are being developed in order to overcome the dissolution rate limited oral absorption of itraconazole. In these formulations, the physical state of the drug is changed from the crystalline to the amorphous state. The presence of the amorphous state also leads to improved dissolution properties because of the absence of a crystalline lattice.

In a previous paper, we reported on the molecular mobility of itraconazole glass below its glass transition, in order to have an estimate of its stability [3]. It was observed that glassy itraconazole ($T_g = 59^\circ C$) shows two endothermic events at 74 and 90°C when scanning the material from $T_g = -40$ to 120°C. The aim of the present study was to explore the origin of these events. Different possibilities were investigated to explain this phenomenon: impurity of the starting material, thermal degradation during the experimental procedure or the appearance of a new structure of pure itraconazole.

2. Experimental

2.1. Materials

Itraconazole (purity more than 99%) was kindly provided by Janssen Pharmaceutica (Beerse, Belgium). Glassy itraconazole was either prepared in the differential scanning calorimeter (DSC) by cooling the melted drug to 40°C at a rate of 20°C/min or by rapidly evaporating dichloromethane from an itraconazole solution.

2.2. Thermal analysis

Modulated temperature DSC (MTDSC) measurements were carried out using a 2920 modulated DSC (TA Instruments, Leatherhead, UK) equipped with a refrigerated cooling system (RCS). Data were treated mathematically using the universal analysis software (TA Instruments, Leatherhead, UK). A flow rate of 40 ml/min of helium was used in the DSC cell while the RCS unit was purged with nitrogen at a rate of 150 ml/min. Samples were scanned using hermetically sealed aluminium pans (TA Instruments, Brussels, Belgium). The amplitude used was ±0.212°C, the period 40 s and the underlying heating rate 2°C/min.

Octadecane, benzoic acid, cyclohexane and indium standards were used to calibrate the DSC temperature scale; enthalpic response was calibrated with indium. The heat capacity signal was calibrated by comparing the response of dry, powdered aluminum oxide to the equivalent literature value in the glass transition region of itraconazole and miconazole. Validation of temperature, enthalpy and heat capacity measurement using the same standard materials showed that deviation of the experimental value from the theoretical one was less than 0.5°C for temperature measurement, while it was less than 0.1% for enthalpy measurement and less than 0.75, 1 and 1.25% for measurement of the heat capacity at 56.85, 26.85 and 6.85°C, respectively.

Hot-stage microscopy (HSM) was performed with an Olympus BX60 polarizing optical microscope equipped with a LINKAM THMS600 hot-stage and a LINKAM TMS93 programmable temperature-controller. Crystalline itraconazole was heated from room temperature to 190°C with a heating rate of 10°C/min and subsequently cooled at the same rate to 45°C. Glassy itraconazole was heated at 2°C/min to 100°C. At different critical temperatures during the heating process photographs were taken.

High-temperature X-ray diffraction was measured on a STOE Transmission Powder Diffractometer System STADI P, with a high-temperature attachment version 0.65.1 (temperature range from room temperature to 1000°C). Monochromatic Cu $K\alpha_1$ radiation ($\lambda = 1.5406$ Å) was obtained with the aid of a curved germanium primary monochromator. Diffracted X-rays were measured by a linear position sensitive detector (PSD). The sample was placed in a quartz glass capillary (outer diameter 0.3 mm, wall thickness 0.01 mm) and spinned during the measurement. Data were collected in the range $2^\circ \leq 2\theta \leq 30^\circ$.

2.3. HPLC analysis with photo diode array (PDA) detection

Glassy and crystalline itraconazole were dissolved in methanol–tetrahydrofuran (MeOH–THF) (50:50

v/v) at a concentration of 0.2 mg/ml. HPLC-analysis was performed using a Waters 600 E system controller, a Waters 77 plus autosampler and a photodiode array detector 996 (Waters, Milford, MA, VS). The column used was Lichrospher 60RP-select B 5 μm (12.5 cm × 0.4 cm) (Merck, Darmstadt, Germany); MeOH — potassium phosphate buffer pH 6.5 (25:75 v/v) was used as mobile phase at a flow rate of 1.0 ml/min.

2.4. Recrystallization procedure of glassy itraconazole

Recrystallized itraconazole was obtained from the glassy material by dissolving it in a mixture of methanol/tetrahydrofurane (50:50 v/v). The solvent was subsequently gently evaporated using a stream of air at ambient temperature inducing recrystallization of itraconazole.

3. Results and discussion

Irrespective of the cooling rate applied, crystalline itraconazole can be transformed to the glassy state by cooling it from the melt [3]. However, in contrast to its structural analogue ketoconazole, the glassy form of itraconazole shows a completely different thermal behavior. Indeed, heating glassy itraconazole showed, besides the glass transition at 59°C, two endothermic transitions at 74 (peak I) and 90°C (peak II), respectively (Fig. 1). The enthalpies for peaks I and II were measured from the total heat flow to be 574 ± 29 J/mol and 916 ± 41 J/mol, respectively. Heating the glassy drug above 90°C followed by cooling to well below T_g showed two exothermic peaks at approximately the same temperatures indicating the reversibility of the transition.

MTDSC provides some major advantages to conventional DSC including the deconvolution into reversing and non-reversing signals. Heat capacity phenomena such as glass transition are able to follow the fast temperature modulation and will be resolved in the reversing heat flow while kinetically hindered phenomena, which are time and temperature dependent appear in the non-reversing heat flow. This separation of signals gives us more specific information of the thermal properties of the occurring phenomena. Fig. 1 shows the deconvolution of the total heat flow of glassy itraconazole during a heating run. The endothermic phenomena appear in the reversing as well as in the non-reversing signal. This observation suggests that the two transitions can be correlated to melting phenomena which can be indeed resolved in both signals [4–7]. Since the first step of melting occurs very fast it is, therefore, able to follow the temperature modulation, while the rest of the melting is kinetically hindered and will appear in the non-reversing signal. By changing the modulation program we were able to increase or decrease the percentage of the enthalpy that was resolved in the reversing or non-reversing heat flow (data not shown).

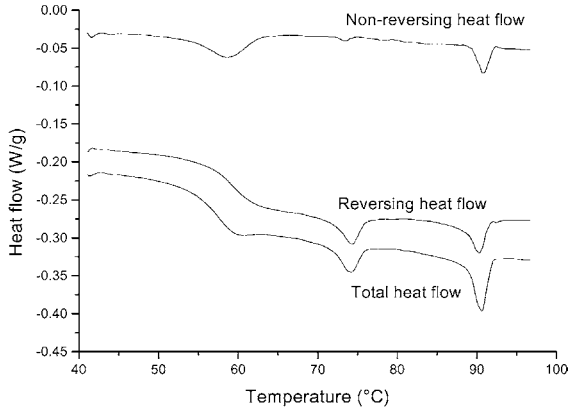

Fig. 1. The total, reversing and non-reversing heat flow of glassy itraconazole. The underlaying heating rate was 2°C/min, the amplitude 0.212°C and the period 40 s.

Glassy itraconazole, which was prepared by cooling from the melt, did not recrystallize, even not when it was heated to the theoretical melting point. However, mechanical stimulation by scratching the glass induced recrystallization. Fig. 2 shows a DSC heating profile of glassy itraconazole, which was partially recrystallized. Heating transforms the remaining glass completely to the crystalline state.

In order to explain the observed transitions, two hypotheses were tested. (1) The presence of an impurity in the material, either caused by thermal degradation or present in the starting (crystalline) material, or (2) the presence of a new spatially ordered arrangement of itraconazole molecules in the glassy state. The presence of impurities in the starting (crystalline) material could be ruled out since both transitions were

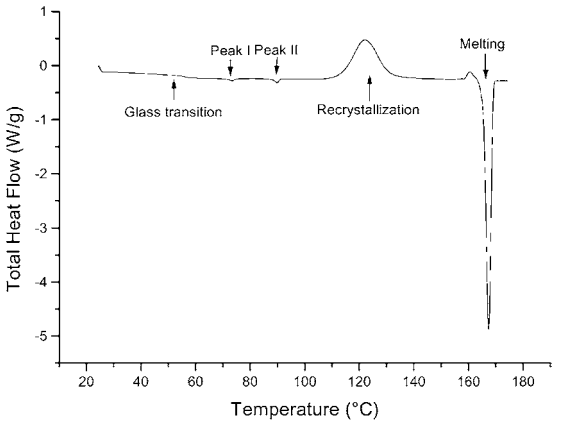

Fig. 2. Total heat flow curve of glassy itraconazole containing mechanically induced crystals.

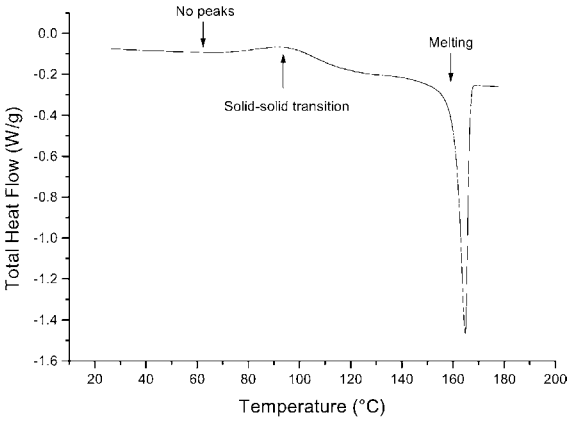

Fig. 3. Thermogram of itraconazole recrystallised from MeOH–THF (50:50).

absent. It is, however, possible that itraconazole decomposes during melting resulting in the observed transitions. In this respect, it was very interesting to observe that scanning itraconazole which was transformed to the glassy state by rapid solvent evaporation (at 140°C) of a solution of the drug in dichloromethane also showed the two transitions at the same temperatures and with the same enthalpies, indicating that the liquid state is a prerequisite for the observed phenomena. If, on the other hand, not all itraconazole was dissolved before the solvent was evaporated, the peaks and the glass transition did not appear but the drug remained crystalline.

When glassy itraconazole was dissolved and recrystallized from MeOH–THF (50/50), and subsequently analyzed with MTDSC, the two unknown peaks disappeared completely and a melting transition at 168°C could be observed (Fig. 3). Melting and subsequent cooling of itraconazole again induced the two transitions. This indicates that the peaks were probably not caused by the presence of an impurity. As shown in Fig. 3, an exothermic peak appeared at 90°C. Given the fact that this transition was irreversible and decreased in enthalpy as a function of time (data not shown), it is suggested that a monotropic solid–solid transition takes place.

In order to find further evidence for thermal degradation, itraconazole was stored at 250°C, which is 80°C above the melting temperature of the crystalline product, followed by cooling to $T_g = -25$°C. Rescanning resulted in the same two endothermic transitions at the same temperature with the same enthalpies. Storing the product for 1 or 8 h at 250°C did not change this result.

In a next set of experiments the chromatographic purity of glassy itraconazole prepared by the melt–cooling procedure was compared to that of the crystalline starting material. As can be seen from Fig. 4, both spectra show the same picture. Analysis of the UV spectrum (Rt = 5.61 min) of the main peak of both products at three different time points indicated that the difference in the ratio of the absorbance at

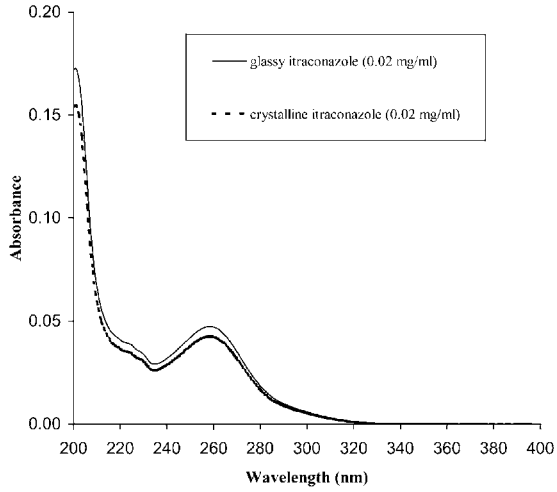

Fig. 4. UV absorption spectra of glassy and crystalline itraconazole.

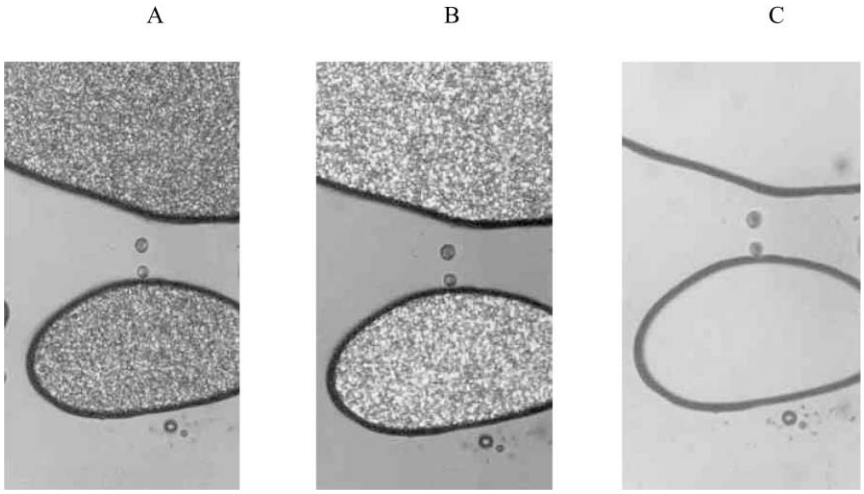

Fig. 5. Hot-stage microscopy of glassy itraconazole. Magnification is 100%. (A) Glassy itraconazole at 45°C; (B) glassy itraconazole at 83°C; (C) isotropic itraconazole at 94°C.

$\lambda = 202$ nm and $\lambda = 257$ nm (two maxima in the spectrum) between the two products was less than 1%. The correlation between the ratio was 0.996.

From the above-described experiments, thermal degradation can be excluded and other phenomena must be responsible for the observed phenomena.

In order to visualize the thermal behavior of itraconazole, hot-stage microscopy was performed. The crystalline starting material melts at 168°C; above this temperature the material is completely isotropic. The material remains isotropic upon cooling until 90°C. At this temperature, which corresponds to the endothermic peak observed in the DSC experiments (heating run), the formation of an optical texture is clearly visible. Cooling the material further shows a second structural change at 74°C, corresponding to the first peak in the DSC experiments (heating run). Beneath 74°C, the viscosity of the compounds increases markedly. Further cooling did not show additional changes. The heating sequence is given in Fig. 5. Consecutive heating–cooling sequences between T_g and 100°C showed the reversibility of both transitions. The fact that this structured material remains liquid at 90°C indicates the formation of a monotropic mesophase. This observation was quite surprising since the molecular structure of itraconazole (Fig. 6) does not show the structural features typical for mesogenic molecules. In general, mesophases can be expected for either rod-

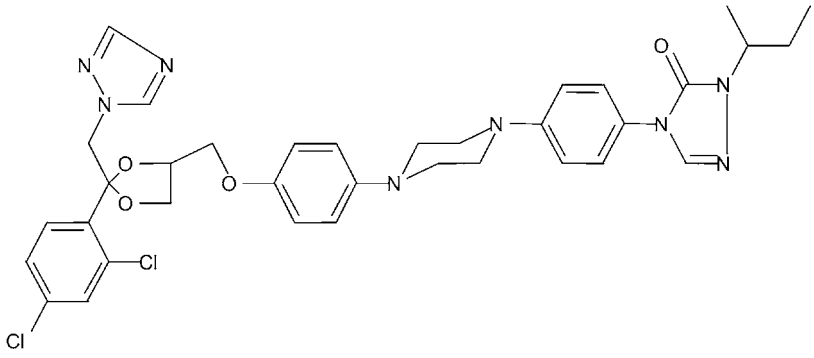

Fig. 6. Molecular structure of itraconazole.

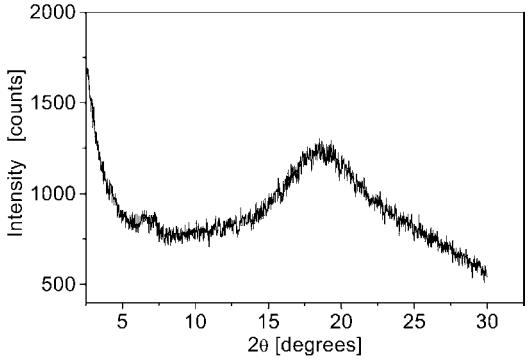

Fig. 7. X-ray diffractogram of itraconazole at 82°C.

like (calamitic) or disk-like (discotic) molecules [8]. Although itraconazole has some structural anisotropy, it contains no terminal linear aliphatic chain(s).

In order to determine the nature of the mesophase, high-temperature X-ray diffractograms of itraconazole were recorded. At room temperature itraconazole is crystalline, which is evident from the many diffraction peaks in the wide-angle region of the X-ray diffractogram. All these diffraction peaks disappear at the melting point (168°C). In the X-ray diffractogram of the melt cooled to 82°C two diffuse maxima are seen; one in the low angle region at $2\theta = 7^\circ$ and one in the wide angle region at $2\theta = 18^\circ$ (Fig. 7). This picture is typical for a nematic phase [9]. No changes could be observed in the X-ray diffractograms upon further cooling to room temperature not even at 74°C (peak in the DSC thermogram). This indicates that itraconazole does not crystallize when cooled from the melt, but that in fact the mesophase is frozen into a glassy state. Moreover, when the glassy mesophase is heated again, total clearing of the texture is observed at 90°C (the temperature at which the mesophase is formed upon cooling). Although the X-ray diffractogram is typical for the nematic phase, the optical texture is pointing to a smectic mesophase: no nematic droplets are visible when the mesophase is formed from the isotropic state, no flash-effect could be observed when pressing with a needle on the coverglass, and there is no indication for Brownian motion of the molecules. The texture could be described as the ''blurred Schlieren'' texture of the smectic C phase [10]. No homeotropic regions are present. Although these findings contradict each other at first sight, they can be rationalized by the fact that itraconazole is a chiral molecule. In this case the mesophase is a chiral nematic phase (N^*), which gives the same diffraction pattern as a nematic phase, but of which the texture sometimes resembles the smectic textures [9,10]. The thermal phenomenon at 74°C (with a marked increase in viscosity) can be interpreted as a rotational restriction of the molecules. Consequently, all the mobility of the ordered molecules is frozen at the glass transition (59°C).

Full evidence for the assignment of the mesophase as N^* is only possible when high-temperature SAXS would be available. Although unlikely, there is always the possibility that the compound forms a smectic phase with a layer thickness of the length of more than one molecule (through dipole–dipole interactions between the chlorine and nitrogen atoms at both ends of the molecules). In this case, the (0 0 1) quasi Bragg peak would be outside the measurement range of the XRD equipment we used. On the other hand, we do not observe higher order peaks.

4. Conclusions

The results from the present investigation provide evidence for the formation of a mesophase during cooling of liquid itraconazole. Two reversible transitions are observed: the first at 90°C represents the transition of the isotropic liquid to the formation of a chiral nematic mesophase; the second transition is probably caused by rotational restriction of the molecules. Further cooling of the monotropic mesophase does not result in crystallization, but to freezing of the mesophase order into a glass. The glass transition temperature is located at 59°C.

Acknowledgements

The authors acknowledge the financial support from Janssen Research Foundation (Beerse, Belgium) and FWO–Vlaanderen. K. Binnemans is postdoctoral fellow of the FWO–Flanders (Belgium). The authors wish to thank Prof. G. Meyer and Dr. D. Hinz (University of Cologne, Germany) for access to high temperature XRD equipment and recording the X-ray diffractograms.

References

[1] J.A. Barone, J.G. Koh, R.H. Bierman, J.J. Colaizzi, K.A. Swanson, M.C. Gaffar, B.L. Moskovitz, W. Mechlinski, V. Van De Velde, Antimicrol. Agents Chemother. 37 (1993) 778.
[2] S.M. Grant, S.P. Clissold, Drugs 37 (1989) 310.
[3] K. Six, G. Verreck, J. Peeters, P. Augustijns, R. Kinget, G. Van den Mooter, Int. J. Pharm. 213 (2001) 163–173.
[4] M. Reading, D. Elliott, V. Hill., in: Proceedings of the 21st NATAS Conference, Atlanta, GA, USA, 1992.
[5] K. Ishikiriyama, B. Wunderlich, Melting of PET analyzed by temperature modulated calorimetry. Macromolecules 30 (14) (1997) 4126–4132.
[6] J.E.K. Schawe, E. Bergmann, Investigation of polymer melting by temperature modulated differential scanning calorimetry and its description using kinetic models, Thermochim. Acta 305 (1998) 179–186.
[7] B. Wunderlich, I. Okazaki, K. Ishikiriyama, A. Boller, Melting by temperature modulated calorimetry. in: Proceedings of the 25th NATAS Conference, McLean, VA, USA, 1997, pp. 49–56.
[8] P.J. Collings, H. Hird, Introduction to Liquid Crystals, Taylor and Francis, London, 1997.
[9] J.M. Seddon, in: D. Demus, J. Goodby, G.W. Gray, H.-W. Spiess, V. Vill (Eds.), Handbook of Liquid Crystals Vol. 1, Wiley-VCH, Weinheim, 1998, pp. 635–679.
[10] D. Demus, L. Richter, Verslag Chemie, Wiley-VCH, Weinheim, 1978.