



HEATING EFFECT ON THE SOLID CHARACTERISTICS AND SOLUBILITY OF ANTI-HIV DIDANOSINE

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Accepted on: 26-12-2011; Finalized on: 20-02-2012.

ABSTRACT

Didanosine (2',3'-dideoxyinosine) is a synthetic analogue of dideoxy purine nucleoside inosine has been reported to inhibit the replication of human immunodeficiency virus (HIV). Heating effect on the solid characteristics of anti-HIV didanosine has been studied by various methods. The aim of this work was to investigate the solid-state properties and the solubility in water of didanosine due to heating. Crystal habit of didanosine was heated and allowed to melt. The whole change was observed under a polarizing microscope equipped with a camera and heater. Didanosine were stored in oven at 60°, 150° and 200°C for 1 hour. The resulting samples were characterized by powder x-ray diffractometer (PXRD), differential scanning calorimeter (DSC), Fourier transform infrared (FTIR), and scanning electron microscopy (SEM). Solid characterization of didanosine after heating at 60°C and 150°C for 1 hour by PXRD, DSC, FTIR, and SEM showed that there were not significant differences with didanosine without heating. Solubility experiments showed that solubility of didanosine in water increase after heating at 60°C and 150°C for 1 hour. Didanosine after heating at 200°C showed the lower solubility. The PXRD, DSC, FTIR, and SEM showed that heating of didanosine at 200°C causes the formation of a new crystal structure which allegedly is its main degradation product, hypoxanthine.

Keywords: Didanosine, solid characterization, heating, PXRD, DSC.

INTRODUCTION

Didanosine (2',3'-dideoxyinosine) is a synthetic analogue of dideoxy purine nucleoside inosine has been reported to inhibit the replication of human immunodeficiency virus (HIV) (Fig. 1). Didanosine act as competitive inhibitors of reverse transcriptase. The drug is approved by the U.S. Food and Drug Administration for the treatment of adults and children over age above 6 months¹. Pharmaceutical formulation containing the didanosine is still in development by one of the domestic pharmaceutical industry. Chemical and physical stability data for didanosine indicate that the drug is stable for at least 24 months at 30°C. Hypoxanthine, didanosine's major degradation product, forms in only very small amounts when didanosine is exposed to 25°C under high intensity light (400 foot-candles) or 87% relative humidity².

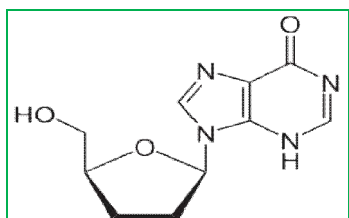


Figure 1: Structure of didanosine²

Some didanosine properties are unfavorable from a pharmacokinetics point of view. These include the tendency of didanosine to hydrolyze at the stomach pH, its low oral bioavailability and very short biological half-life³⁻⁴. To improve these functional properties, a conceivable strategy consists of addressing solid-state

properties of the drug, as for instance preparing a crystal form with a desirable solubility and dissolution rate⁵.

Solid-state properties of active ingredients are crucial in pharmaceutical development owing to their significant clinical and economic implications. Differences in physical properties of various solid forms and variations in the degree of crystallinity have an important effect on the processing of drug substances into drug products, while differences in solubility may have implications on the absorption of the active drug from its dosage form, by affecting the dissolution rate and possibly the mass transport of the molecules⁶⁻⁹. Transfer of heat energy to the substance cannot be avoided in many processes in pharmaceutical manufacture. Influence of heating on solid state transformation have been carried out on some drugs^{10,11}.

Thus far, no reports are currently found in the literature on the solid-state characterization of didanosine, although its use as an active ingredient of pharmaceutical oral solid dosage forms comes from 1991. Moreover, few studies dealing with didanosine polymorphism are reported¹².

Based on the above, it is very important to perform characterization of solid state transformation in drug raw material didanosine due to heating process, so it can be avoided processes in the manufacture of pharmaceutical preparations which can lead to decrease bioavailability of pharmaceutical preparations. The aim of this work was to investigate the solid-state properties and the solubility in water of didanosine due to heating.



MATERIALS AND METHODS

Chemical and reagents

Didanosine commercial material with purity of >99% was obtained from PT. Kimia Farma, Tbk, Indonesia. Methanol and other reagents were purchased from Merck Chemicals Indonesia without any purification.

Observation of didanosine habit due to heating

A drop of didanosine solution in methanol was placed on object glass and covered, then allowed to recrystallize. Crystal habit of didanosine were heated and allowed to melt. The whole change was observed under a polarizing microscope Olympus BX-50 equipped with an Olympus SC30 camera and heater.

Preparation of samples

Each 2 g didanosine were placed in vial. The vials were stored in oven at 60°, 150° and 200°C for 1 hour. The resulting samples were characterized by PXRD, DSC, FTIR, and SEM.

Characterization by PXRD

PXRD data were collected with Shimadzu XRD-7000 X-ray powder diffractometer. The sample was scanned within the scan range of $2\theta = 5^\circ$ to 35° continuous scan, at a scan rate of $2^\circ/\text{min}$. Relative index of crystallinity of didanosine was calculated by compare of area under peak of didanosine after heating with didanosine without heating.

Thermal Analysis by DSC

Differential scanning calorimetry (DSC) was performed with a Perkin Elmer DSC-6. 2-5 mg of each sample was placed in crimped sample pan. The sample was heated from 30° to 350°C at a heating rate of $10^\circ\text{C}/\text{min}$. The samples were purged with a stream of flowing nitrogen at 20 mL/min.

Characterization by FTIR

Infrared spectra of samples were collected by using a Perkin-Elmer FTIR equipped with a deuterium triglycine sulfate detector (DTGS). The spectrum obtained in transmission mode on KBr pellets in the wave number range $4000\text{-}450\text{cm}^{-1}$.

Characterization by SEM

The electron microscopy measurements were performed at a Carl Zeiss SEM EVO, Germany. Specimens were mounted on the metal sample holder with a diameter of 12 mm using a double-side adhesive tape and coated with gold-palladium under vacuum.

Determination of didanosine solubility in water

The solubility of samples in water were determined at ambient temperature using an orbital shaker on each sample. Excess amounts of compound were added to 5 mL of the media, mix it continuously and then filtered after 48 hours of equilibration. The bulk solutions were diluted and measured spectrophotometrically using

Shimadzu 1601-PC spectrophotometer at 249.1 nm. The calibration curve for didanosine ($y=0.0538x-0.0169$) was linear from 0.2 to 16.0 $\mu\text{g}/\text{ml}$ ($r=0.9999$). The experiments were carried out in triplicate to provide mean values and standard deviations (S.D.)

RESULTS AND DISCUSSION

Didanosine Habit Transformation on Heating

Didanosine is a very important drug in the treatment of AIDS, but until now there are only two articles^{12,13} studied its solid-state properties. The combination of traditional hot-stage microscopy with new technologies such as high-resolution micrography, image capture, storage manipulation, and presentation has permitted more comprehensive physical property characterizations to be conducted¹⁴. Optical polarized microscope photos showed that needle-shaped crystal habit of didanosine recrystallized in methanol (Fig. 2a). The needle-shaped crystals melt in the range of temperature of $178^\circ\text{-}180^\circ\text{C}$ (Fig. 2b) and quickly recrystallized to prism-shaped crystals in the range of temperature of $181^\circ\text{-}183^\circ\text{C}$ followed by the color change to brown (Figs. 2c and 2d). Prism-shaped crystals melted in the range of temperature of $270^\circ\text{-}290^\circ\text{C}$ followed by decomposition (Fig. 2e). The change of needle-shaped crystals didanosine after melting into the prism-shaped crystals was not due to polymorphic transformation, but the decomposition of didanosine into its main degradation product, hypoxanthine. This is supported by the fact that the melting point of the prism at temperatures $> 250^\circ\text{C}$ corresponding to the melting point of hypoxanthine¹⁵.

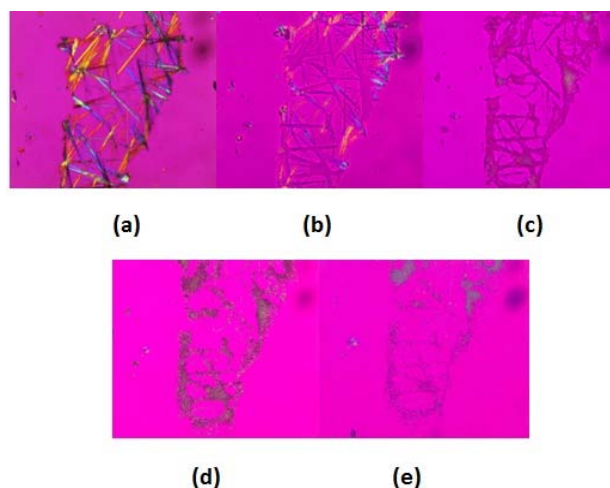


Figure 2: Crystals habit of didanosine, (a) didanosine crystal habit before melting, (b) didanosine crystals began to melt (c) began to recrystallization into new crystals (d) completed recrystallization into new crystals (e) new crystals began to melt

PXRD Pattern

The X-ray diffraction patterns of pure didanosine and after heating at 60° , 150° , and 200°C for 1 hour are given in Figure 3. The PXRD-spectra patterns showed that didanosine has many peaks with the main peaks at $2\theta = 6.0^\circ$, 10.6° , 12.0° , 14.9° and 20.2° . The highest intensity was shown at 6.0° , which is then used to calculate the

relative index of crystallinity. There was no change in the location of the main peaks of didanosine after heating at 60° and 150°C for 1 hour, but changes the intensity of these peaks. Relative index of crystallinity of didanosine after heating at 60° and 150°C for 1 hour were 32.9% and 31.1%, respectively. In Fig. 3d, significant changes occurred in didanosine diffractogram after heating at 200°C for 1 hour. Didanosine main peaks disappear and only appear two new main peaks at 13.4° and 27.5°. This suggests the formation of new crystal structure that are similar to hypoxanthine diffractogram (Fig. 3e).

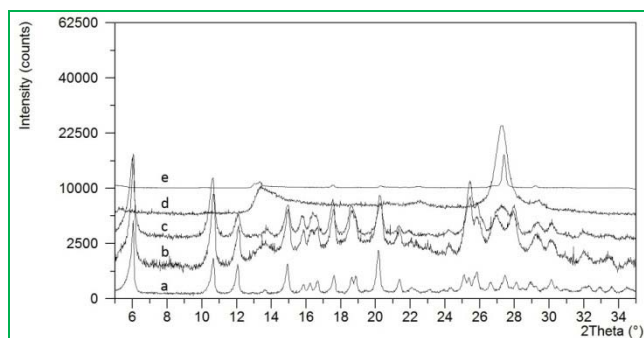


Figure 3: XRD-spectra intensity (arbitrary units) as a function of 2θ (a) didanosine, (b) didanosine after heating 60°C, 1 hour, (c) didanosine after heating 150°C, 1 hour, (d) didanosine after heating 200°C, 1 hour, and (e) hypoxanthine.

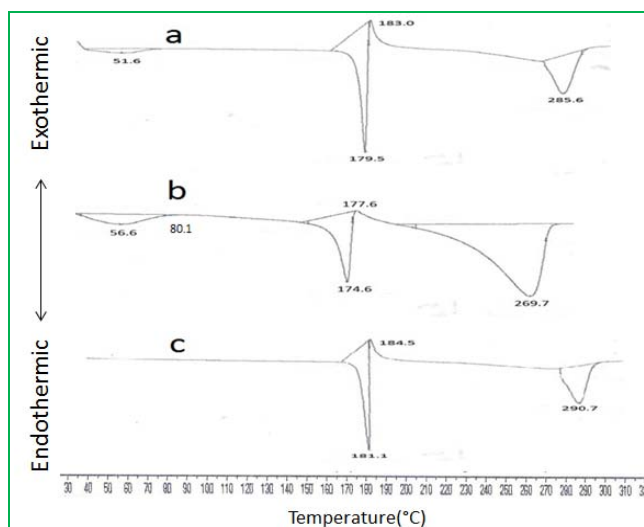


Figure 4: DSC-thermogram of (a) didanosine, (b) didanosine after heating 60°C, 1 hour, and (c) didanosine after heating 150°C, 1 hour.

DSC Thermogram

DSC-curve of didanosine and didanosine after heating are shown in Fig. 5. A broad endotherm peak at 51-56°C and ends at 80°C in DSC-thermogram of didanosine and didanosine after heating at 60°C for 1 hour, indicates of water losses. That condition due to the extremely hygroscopic properties of commercial didanosine. The broad endotherm peak was not observed in didanosine after heating at 150°C for 1 hour, because the water has been lost before characterization by DSC. The presence of water in commercial didanosine had been observed by Felipe¹³. DSC-thermogram shows there are two other endothermic peaks at 179.5°C which is the melting point

of the didanosine and the 285.0°C which is a new crystalline melting point. The exothermic peak appeared at 183°C due to recrystallization of the didanosine melt. This is agreement with optical polarization microscopy results. That exothermic peak was probably caused by the degradation of didanosine into hypoxanthine.

FTIR Spectra

Common techniques used to characterize drugs and excipients are infrared (IR) and Raman spectroscopy, which are sensitive to the structure, conformation and environment of organic compounds. Because of this sensitivity, they are useful characterization tools for pharmaceutical crystal forms. Figures 5a, 5b, and, 5c showed that there is no significant shift in the O-H group vibration after heating at 60°C and 150°C for 1 hour. Thus, didanosine does not decompose when heated at temperatures up to 150°C. In Fig. 5d, vibration shift of the O-H group from 3271 cm⁻¹ to 3436 cm⁻¹ occurred after didanosine was heated at 200°C for 1 hour. This is agreement with PXRD results which showed formation of a new crystal structure.

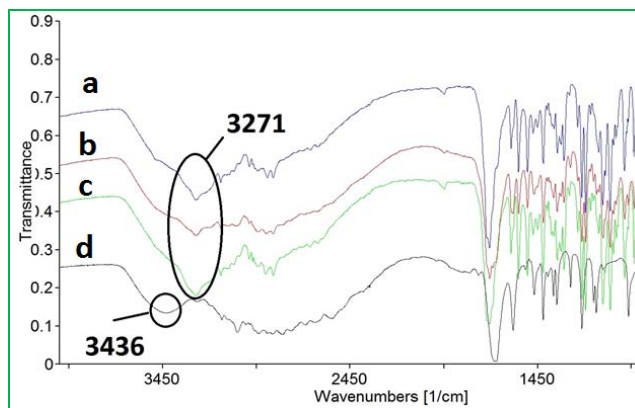


Figure 5: FTIR spectra of (A) didanosine, (B) didanosine after heating 60°C, 1 hour, (C) didanosine after heating 150°C, 1 hour, (D) didanosine after heating 200°C, 1 hour.

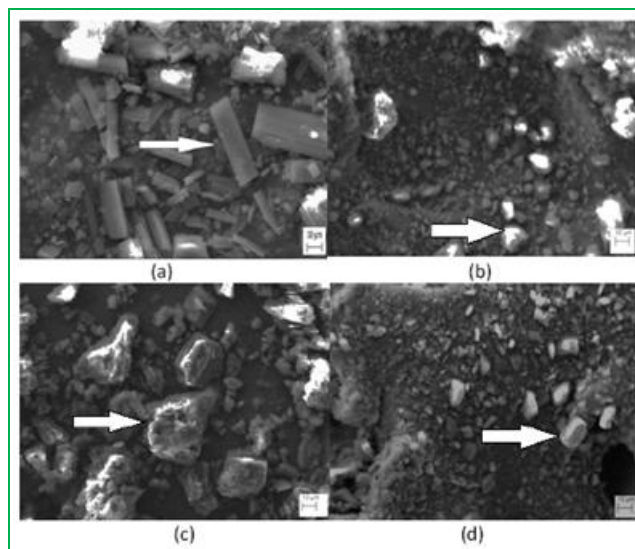


Figure 6: Scanning electron micrograph photos with magnification 1000x of (a) didanosine, (b) didanosine after heating 60°C, 1 hour, (c) didanosine after heating 150°C, 1 hour, (d) didanosine after heating 200°C, 1 hour

SEM Photomicrographs

Fig. 6 show representative SEM photomicrographs of some of the samples. Fig. 3a showed needle-like habit crystal of didanosine commercial without heating. SEM photomicrographs in Figs. 3b and 3c showed that didanosine crystal slightly changed, i.e. it became more amorphous after heating 60°C for 1 hour and form larger solids after heating at 150°C for 1 hour. SEM photomicrographs didanosine after heating at 200°C for 1 hour showed the formation of prism-shaped crystals. The larger solids formation caused by loss of water from the crystal surface of didanosine.

Solubility

Solubility experiments showed that solubility of didanosine in water increased after heating at 60°C and 150°C for 1 hour, but decreased after heating at 200°C for 1 hour (Table 1). There was significant decreasing of solubility of didanosine after heating at 200°C for 1 hour. The solution of didanosine after heating at 200°C for 1 hour is more turbid than didanosine and didanosine after heating 60°C and 150°C for 1 hour. This is probably due to at 200°C didanosine began to decomposed into hypoxanthine. Hypoxanthine has a very low solubility in water.

Table 1: Solubility of Didanosine in water at ambient temperature

| Materials | Solubility (mg/mL) |
|---|--------------------|
| Didanosine | 24.83±0.73 |
| Didanosine after heating at 60°C for 1 h | 27.77±0.98 |
| Didanosine after heating at 150°C for 1 h | 29.61±0.55 |
| Didanosine after heating at 200°C for 1 h | 1.31±0.03 |

Mean±S.D., n=3

CONCLUSION

Solid characterization of didanosine due to heating at 60°C and 150°C for 1 hour by PXRD, DSC, FTIR, and SEM showed that there were not significant differences with didanosine without heating. Solubility experiments showed that solubility of didanosine in water increased after heating at 60°C and 150°C for 1 hour. Didanosine after heating at 200°C showed the lower solubility. The PXRD, DSC, FTIR, and SEM showed that heating of didanosine at 200°C causes the formation of a new crystal structure, which allegedly is its main degradation product, hypoxanthine.

Acknowledgment: We thank Agung Kisworo (Research and Development Unit of PT. Kimia Farma, Tbk) for the gift of didanosine commercial material.

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