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Studies on Crystal Modifications of Ganciclovir

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Ganciclovir is an acyclic guanine nucleoside analog and is a well-known anti-viral agent that inhibits replication of herpes virus. Ganciclovir is reported to exhibit polymorphism. However, based on the previous studies on the polymorphs it was found that a thorough investigation for the polymorphs of Ganciclovir was required. We report the solid-state characteristics of four polymorphic forms, viz. form I, form II, which are anhydrous, and form III, Form IV, which are hydrates. These hydrates exist as hemihydrates and monohydrates. The physicochemical characteristics for the forms of ganciclovir have been performed using techniques such as powder X-ray diffraction, thermoanalytical methods, infrared, near-infrared spectroscopy, and variable-humidity and temperature powder X-ray diffraction.

Keywords Ganciclovir; polymorphs; powder X-ray diffractometry

Introduction

Ganciclovir, or 9-(1,3-dihydroxy-2-propoxymethyl) guanine, is an acyclic guanine nucleoside analog and is a well-known anti-viral agent [1]. It is an acyclic nucleoside analogue of 2′-deoxyguanosine that inhibits replication of herpes virus. Clinical studies reveal ganciclovir to be active against cytomegalovirus (CMV) and herpes simplex virus.

Ganciclovir is available as a U.S. Pharmacopeia (USP) reference standard but the polymorphic form of the USP standard is not known [2]. Solid-state characterization for the USP standard is not reported, hence we name the polymorphic form of USP form I. According to the patent [3], ganciclovir exists as an anhydrate as well as a hydrate. The anhydrous form has been briefly characterized by Powder X-ray diffraction (PXRD) and differential scanning calorimetry (DSC) and is reported to be stable and nonhygroscopic [3]. Kawamura and Hirayama recently published the crystallographic structure for a form of ganciclovir [4]. The form has been reported to exist in a monoclinic system having a space group of $P2_1$ and the unit cell parameters as a = 4.380 Å, b = 10.909 Å, c = 11.601 Å, and $\beta = 99.11°$. This form, which is reported as anhydrous with the crystallographic data available, correlates to our form II. The hydrate mentioned in the patent is reported as hygroscopic above a humidity of 76%.

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It maybe concluded from the literature review that ganciclovir exhibits polymorphism and exists as anhydrous and hydrous forms. However, based on the previous studies on the forms it was found that a thorough investigation for the forms of ganciclovir was required. Although information regarding the forms of ganciclovir is available, the documents lack the scientific basis required to understand the relationship between the various forms.

Characterization of polymorphs in pharmaceuticals is a very important aspect of drug development and manufacturing [5–8]. According to International Conference on Harmonization (ICH) guidelines, active pharmaceutical ingredients (APIs) must be screened for polymorphism [9].

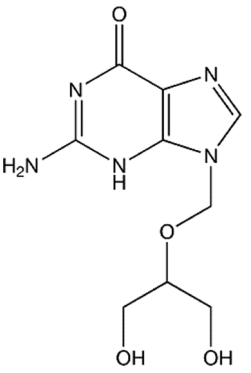
In this article we aim at a comprehensive characterization of the previously reported forms; that is, the U.S. Pharmacopeia reference standard (form I) of ganciclovir and the anhydrate (form II). This study also relates to the crystallization studies of ganciclovir in search of novel forms as well as the evaluation of these forms using different analytical techniques. During our investigation of the ganciclovir forms, we came across two novel forms (form III and form IV), which we have investigated and reported in this article.

In this article we aim to study the physicochemical characteristics for the forms of ganciclovir using techniques such as the (XRD, DSC), thermogravimetric analysis (TGA), Fourier transform infrared (FTIR) and near-infrared (NIR) spectroscopy, microscopy, as well as variable-humidity and -temperature powder X-ray diffraction (VTPXRD).

Experimental

Materials

Ganciclovir, USP reference standard, was analyzed without any further purification. Ganciclovir USP is called form I. Ganciclovir was obtained from the Chemical Research Division of Matrix Laboratories Limited, India. The received sample of Ganciclovir was distilled in dimethylformamide (analytical grade, Merck) and the dry powder of form II obtained. Ganciclovir was also slow crystallized from water (Millipore), a water–methanol mixture, ethanol (analytical grade, Merck), and isopropyl alcohol (laboratory grade, Merck). Fine needles of ganciclovir crystallized from water are called as Form III. Thin flakes of ganciclovir obtained when crystallized from a mixed solvent of water and methanol (1:1) and dried at 50°C–60°C is called as form IV.



Structure of Ganciclovir

Methodology and Instruments

Powder X-Ray Diffractometry. The PXRD patterns were obtained with a PANalytical, Philips X'Pert PRO diffractometer equipped with a θ/θ goniometer using Cu-anode, automatic divergence slit, and X'celerator detector. Data were collected at a tube voltage of 40 kV and a tube current of 30 mA, at a step size of 0.03° in the angular range of 2θ of 2–50° for a scan time of 50 s.

Variable Temperature X-Ray Powder Diffractometry. Samples were measured using a variable temperature PXRD, Bruker axs D8, Discover. The VT-PXRD experiments were performed with Cu Kα1 radiation using a Varioα1 monochromator and Lynx Eye detector. The angular range was 2–40° with a step size of 0.03°. The humidity and temperature were controlled by an *ANSYCO Sycos H-Hot*. The samples were measured at different temperatures up to 240°C with a heating rate of 0.2°C/s. The sample holder was placed in an air tight, thermally insulated chamber provided with an inlet and outlet for nitrogen purge gas at a controlled humidity.

Fourier Transform Infrared (FTIR) Spectroscopy. FTIR spectra were obtained with Perkin Elmer Spectrum One spectrometer. The samples were prepared on KBr disks and the spectra were collected over a spectral range of 4000 to 500 cm^{-1}, resolution of 4 cm^{-1}, and 16 scans.

Near-Infrared (NIR) Spectroscopy. Spectra were collected using a Bruker MPA spectrometer. The samples were scanned in the range of 4000 to 12,500 cm^{-1} and the data processed using OPUS software.

Differential Scanning Calorimetry. DSC thermograms were recorded with a Q1000 (TA Instruments). Approximately 1–3 mg of the sample was weighed into standard aluminum pans with a lid. Dry nitrogen was used as purge gas at a flow rate of 50 mL/min. Data were collected at a heating rate of 10°C/min over a temperature range of 30°C to 300°C. The temperature and enthalpy calibration for the instrument was performed with pure indium (melting point 156.6°C, heat of fusion 28.45 J/g).

Thermogravimetric Analysis. TGA was performed with a Q5000IR (TA Instruments). Samples of approximately 3–5 mg were placed on a preweighed aluminum pan. Temperature calibration of the instrument was performed using a ferromagnetic material such as nickel. The Curie-point temperature was measured and the instrument was calibrated. Dry nitrogen was used as purge gas at a flow rate of 25 mL/min. Data were collected at a heating rate of 10°C/min over a temperature range of 30°C to 300°C.

Results and Discussion

Powder X-Ray Diffractometry

The powder pattern for all the forms are shown in Figure 1. The patterns exhibit characteristic patterns with prominent peaks at the following angular positions (Table 1). The powder pattern for ganciclovir form II was found to be comparable to the anhydrous form disclosed in the patented literature [3]. The powder pattern was also found to be comparable to the simulated powder pattern for ganciclovir

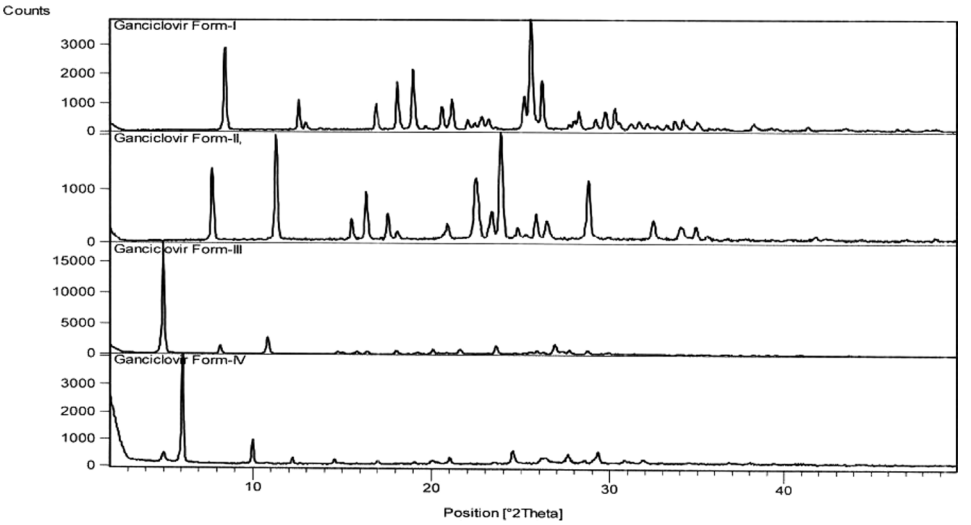


Figure 1. Powder X-ray diffraction pattern of four forms of ganciclovir.

obtained from the crystallographic data [4], thereby indicating that the crystallographic data collected for ganciclovir are those of form II, the anhydrous form (Figure 2). Form III crystallized at room temperature, whereas crystals of form IV were obtained when dried at elevated temperature of 50°C–60°C, indicating that the forms had preferential crystallizations based on the drying temperatures. Form IV was found to show a slight contamination of form III with a peak at 5.0°2θ. Data for form IV were collected by gas chromatography (GC) to detect the presence of any methanol. The methanol content was found to be less than 1000 ppm by GC, indicating form IV to be substantially free of methanol.

Growing of single crystals was attempted for both form III and form IV, but crystals suitable for data collection could not be obtained.

Variable Temperature / humidity X-Ray Powder Diffractometry

The stability of the forms was studied using VTPXRD and humidity-controlled PXRD (Scheme 1). Ganciclovir form I, form III, and form IV were subjected to temperature ranging from ambient to 240°C. Ganciclovir form I is stable upto 230°C

Table 1. Characteristic 2θ values for the forms of Ganciclovir

Form	2θ values
Form I	8.4, 12.5, 16.9, 18.1, 19.0, 21.1, 25.5, and 26.1
Form II	7.8, 11.3, 16.4, 22.5, 23.9, and 28.8
Form III	5.1, 8.2, 10.9, 18.1, 20.1, 21.7, 23.7, and 27.0
Form IV	5.0, 6.1, 10.0, 12.2, 14.6, 17.0, 19.1, 20.1, 21.1, 24.5, 26.4, 27.6, and 29.4

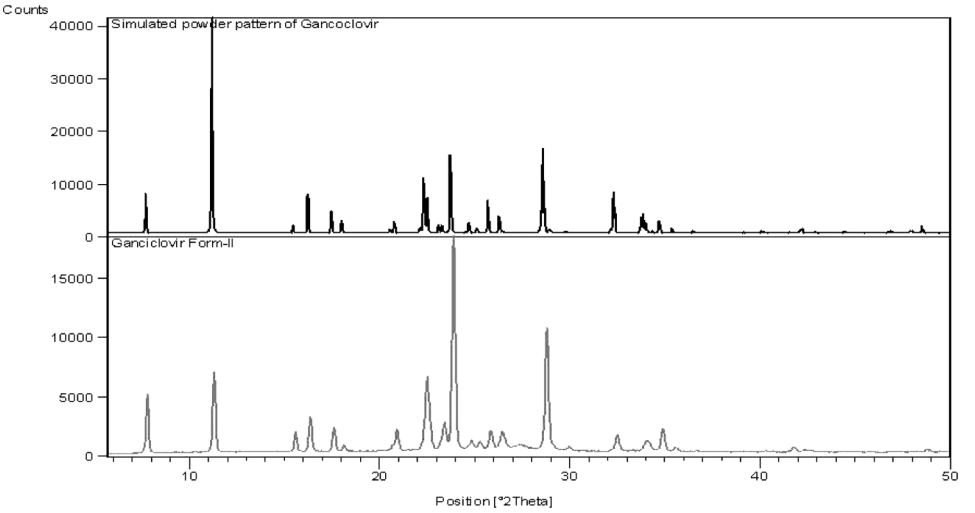
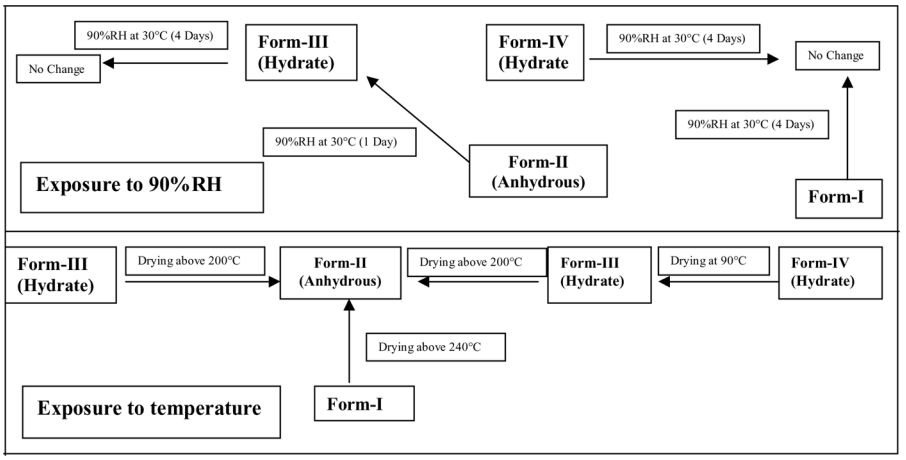


Figure 2. PXRD overlay of ganciclovir form II with reported simulated powder pattern [4].

and it was found to convert to form II when heated above 240°C (Figure 3). Form III was found to undergo conversion to form II at a temperature at around 210°C (Figure 4). Ganciclovir form IV is stable up to 70°C; after this temperature it undergoes two polymorphic transitions. The first transition between 90°C and 110°C indicates that the polymorph is converted to form III and the second transition is above 210°C, where it converts to form II (Figure 5).

Because ganciclovir is reported to be hygroscopic in nature [2], the effect of high humidity of 90% was studied. Ganciclovir form I, form II, form III, and form IV were exposed to high humidity conditions of 90% at 30°C (Figures 6–9). Form II converted to form III upon exposure to high humidity of 90%. The rest of the forms did not undergo any significant change.



Scheme 1. Summary of experiments involving exposure to high humidity and drying.

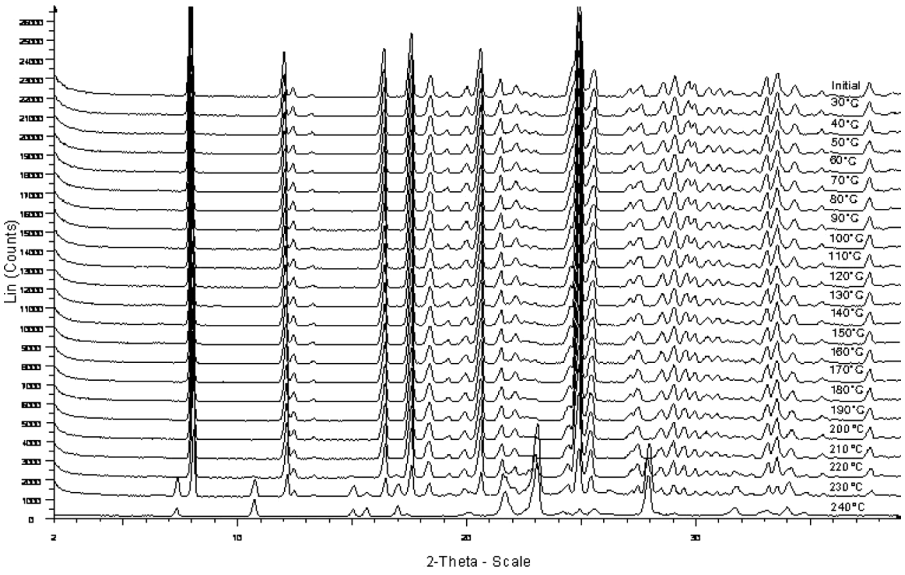


Figure 3. VTPXRD of ganciclovir form I.

FTIR Spectroscopy

The FTIR spectra were collected for form I, form II, form III, and form IV. The spectra for each form were different, with characteristic absorption bands as shown in Table 2. Except in the –NH region, form I and form II show similar spectral bands. In form I the NH stretching is sharp, whereas in Form II it is broad. This can be explained by the inter molecular hydrogen bonding in form II, which is derived from the X-ray crystal structure. The NH/OH and C=O bonds are involved in four intermolecular hydrogen bonds in form II. Form III and form IV show similar

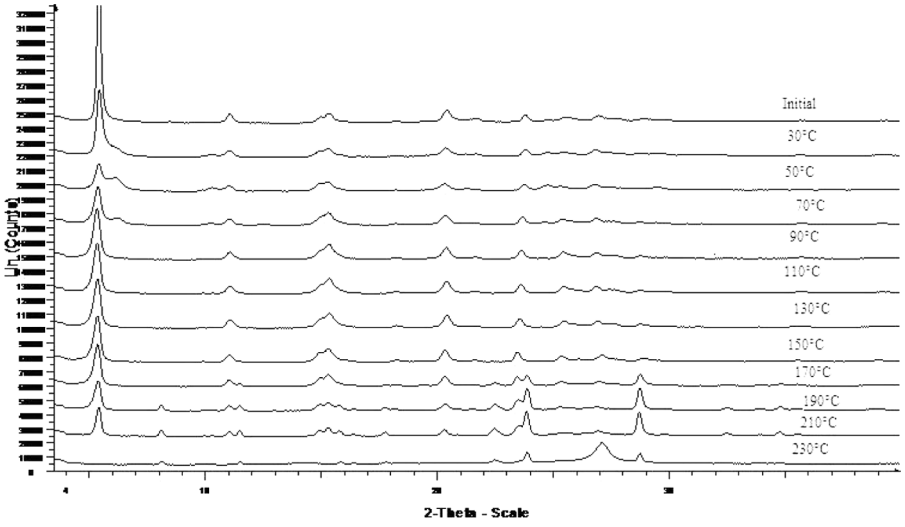


Figure 4. VTPXRD of ganciclovir form III.

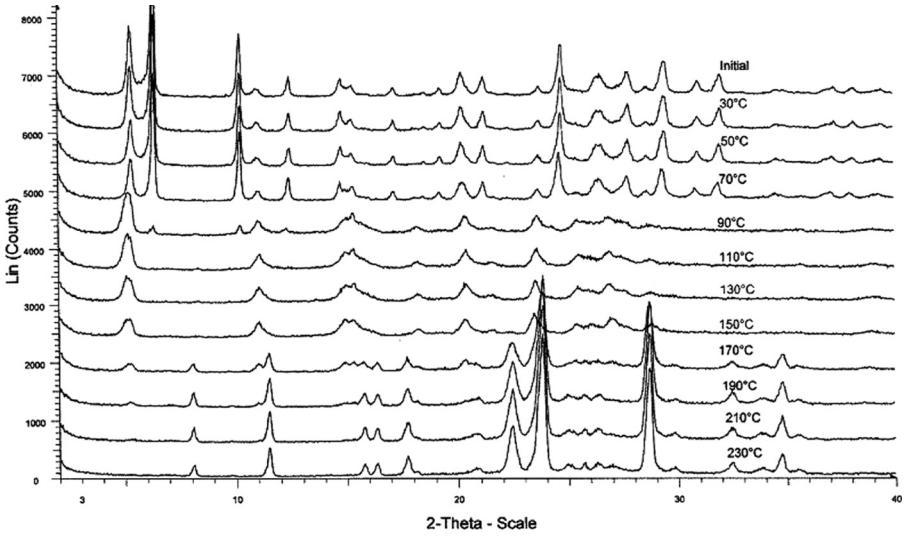


Figure 5. VTPXRD of ganciclovir form IV.

IR stretchings compared to form I and form II. Major stretchings are shifted to higher wavenumbers in form III and form IV as shown in Table 2. It may be assumed that the molecular interaction in these two forms is different from form I and form II (Figure 10) [10].

Near-IR Spectroscopy

The four forms of Ganciclovir show different patterns in the near-IR region. The full NIR spectrum extends from 14,285 to 4000 cm^{-1}. The short-wavelength NIR ranges

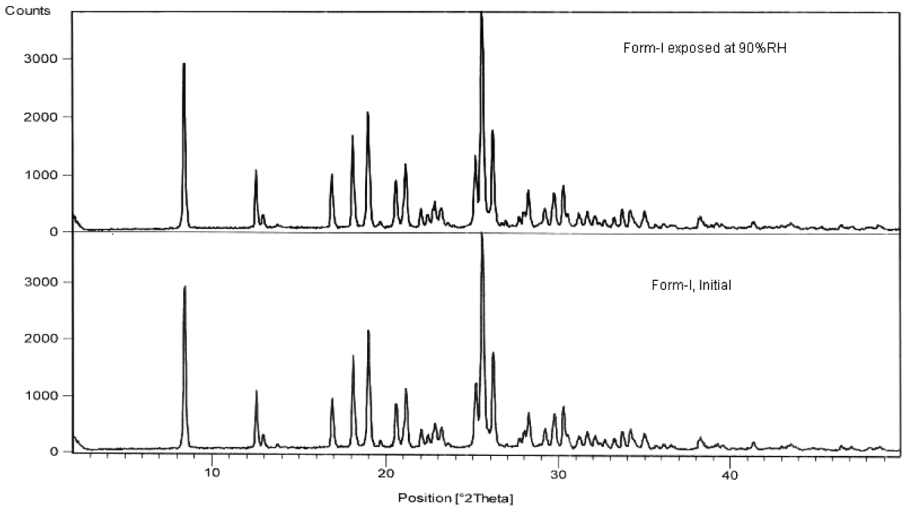


Figure 6. Effect of high humidity on ganciclovir form I.

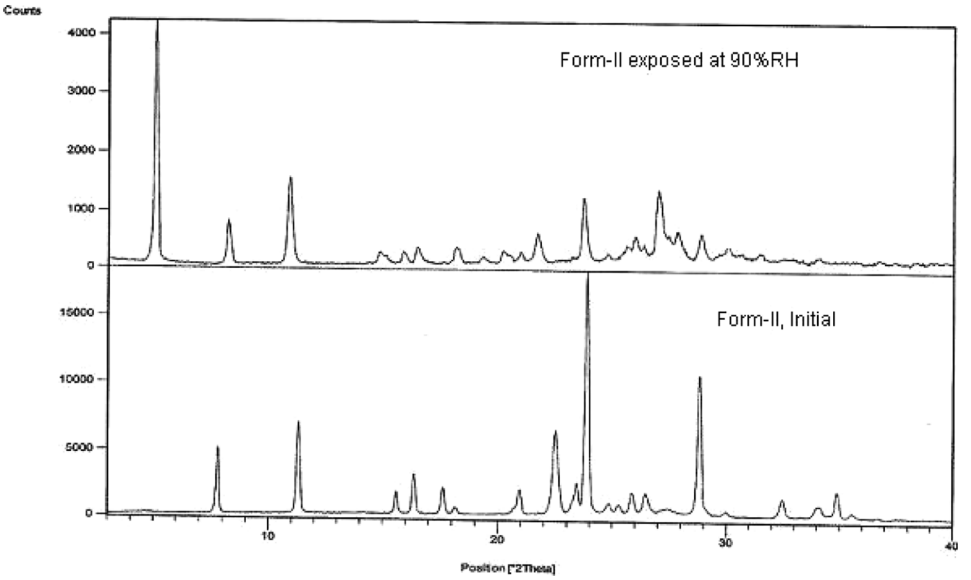


Figure 7. Effect of high humidity on ganciclovir form II.

from 12,500 to 8600 cm^{-1}. The first overtone spectra and combination spectra range from 6500 to 5500 cm^{-1} and 5000 to 4000 cm^{-1} respectively [11–13]. Water absorption bands are observed at 8600, 6940, and 5200 cm^{-1} [12,13]. Characteristic peaks due to water were observed at around 7000–6900 and 5200–5100 cm^{-1} for form III and form IV. Form II showed mainly N-H absorption bands around 6800 and 6500 $cm^{-1,}$ indicating the form as anhydrous (Figure 11).

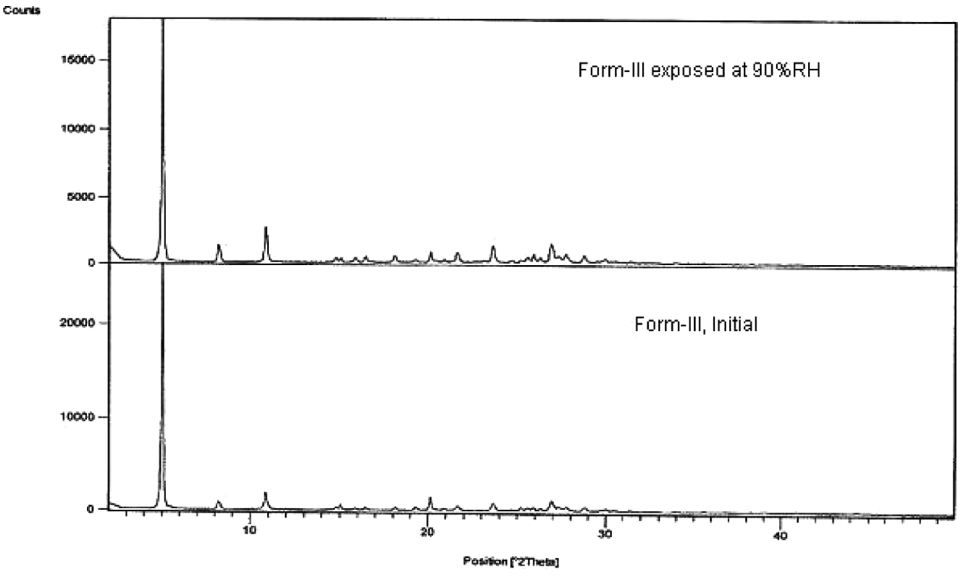


Figure 8. Effect of high humidity on ganciclovir form III.

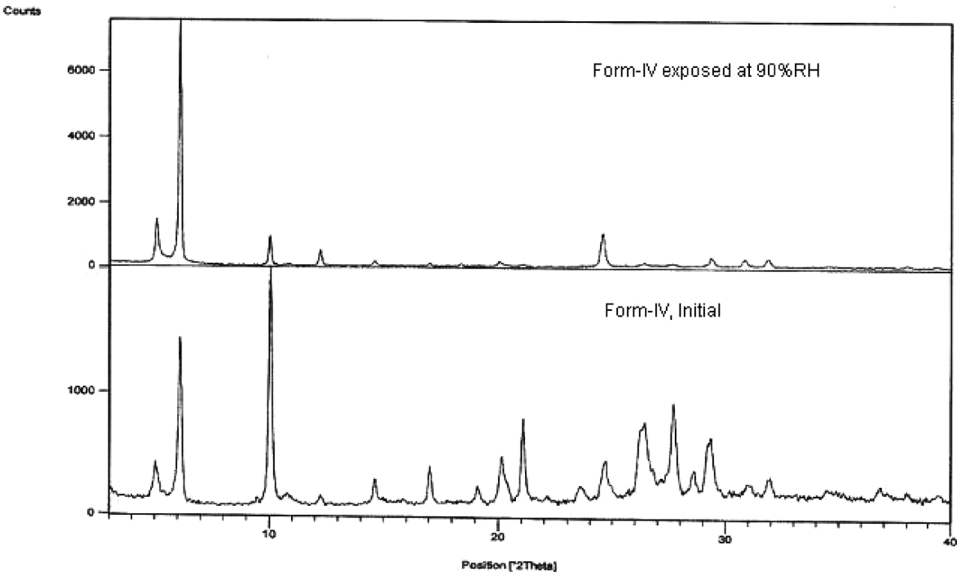


Figure 9. Effect of high humidity on ganciclovir form IV.

Thermal Analysis

Ganciclovir forms I, II, III, and IV were measured by DSC and TGA. According to Burger and Ramberger [14], in the case of enantiotropism, the transition of two

Table 2. Characteristic absorption bands of FTIR

Assignments, mode of vibration	Characteristic absorption bands			
	Form I	Form II	Form III	Form IV
N-H/O-H, stretching	3420, 3320	3432, 3318	3319	3430, 3308
Aromatic C-H, stretching	3147	3168	3172	3179
Aliphatic C-H, stretching	2942, 2893	2907, 2853, 2709	2950, 2877, 2738	2951, 2879, 2719
C=O, stretching	1687, 1659	1690, 1634	1733, 1694	1732, 1695
Aromatic C=C/ C=N, stretching	1611, 1574, 1543	1609, 1578, 1541	1610, 1582, 1542	1609, 1579, 1542
Aliphatic C-H, bending	1493, 1370	1473, 1391	1488, 1388	1488, 1393
C-N, stretching	1304	1302	1307	1307
C-O-C, asymmetric stretching	1246, 1171	1282, 1183	1225, 1182	1226, 1182
C-O-C, symmetric stretching	1097, 1065, 1045	1109, 1091, 1056	1100, 1060	1102, 1072
Aromatic C-H	782, 769	784, 772	778, 756	780, 758

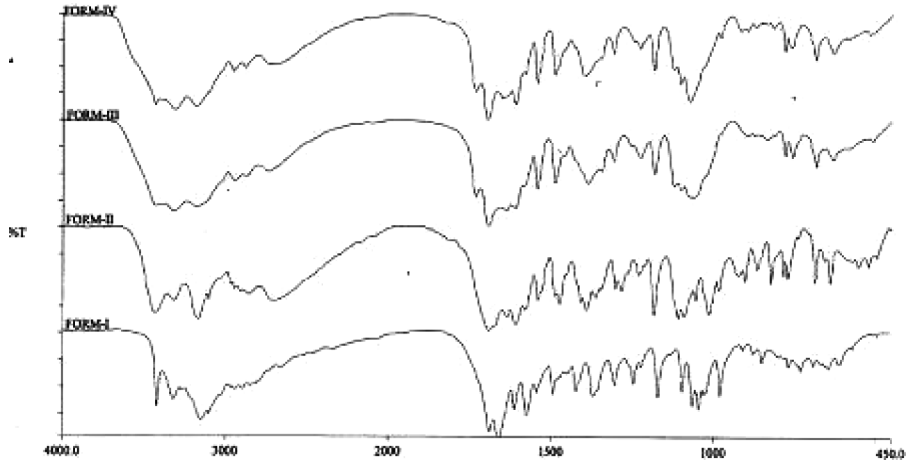


Figure 10. FTIR of four forms of ganciclovir.

polymorphic transitions is endothermic, whereas for monotropism the transition is exothermic. Ganciclovir form I shows an enantiotropic relationship between form I and form II. A solid–solid transition is observed due to the melting of form I at 228°C. Form I converts to form II at 252°C without any weight loss, indicating the form to be anhydrous (Figure 12a). Similar transitions were observed from the VTPXRD data. A single endotherm was observed for ganciclovir form II with a melting at 252°C without any weight loss, indicating the polymorph to be anhydrous (Figure 12b). The DSC thermograms for ganciclovir form III and form IV are similar with endothermic loss up to 110°C–130°C. Both the polymorphs undergo solid–solid transition and finally convert to form II. It may be observed from the DSC data that form III and form IV share a monotropic type of relationship with form II.

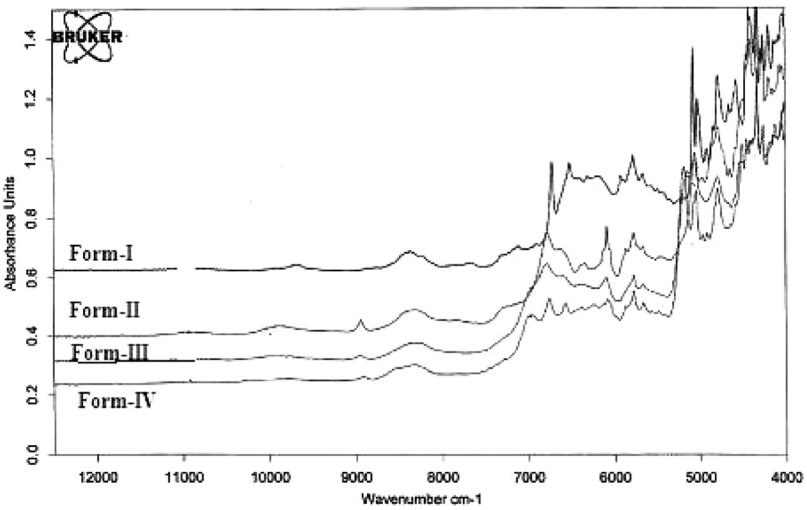
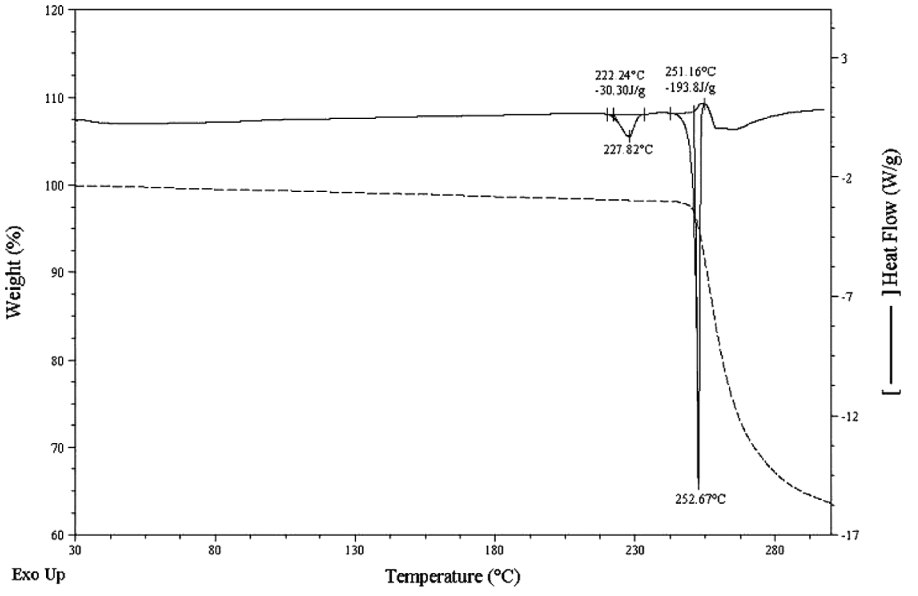
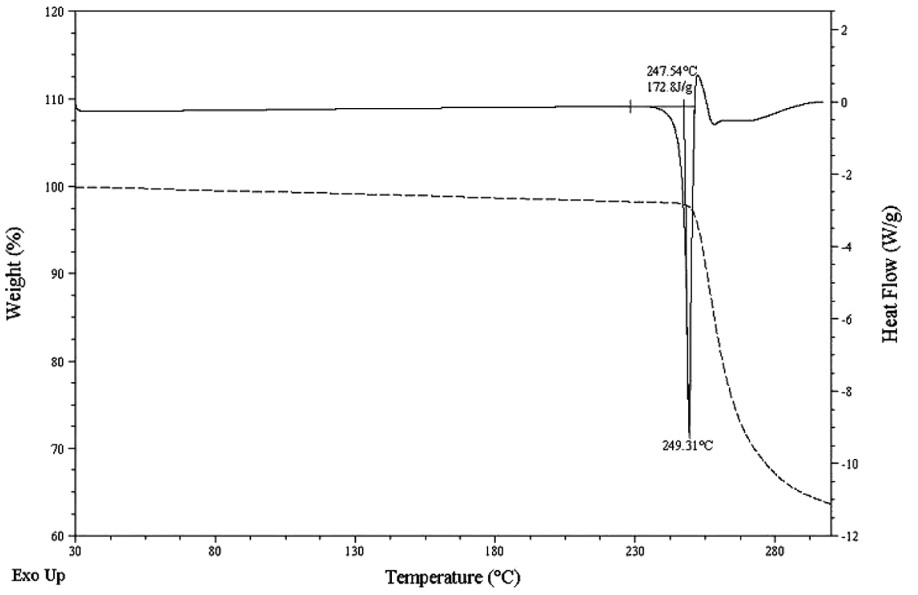


Figure 11. NIR of four forms of ganciclovir.

Form III has a melting at around 159°C, whereas Form IV shows a melting endotherm at around 149°C. It may be noted that ganciclovir melts and decomposes simultaneously at around 252°C. According to the literature [15–18], hydrates are

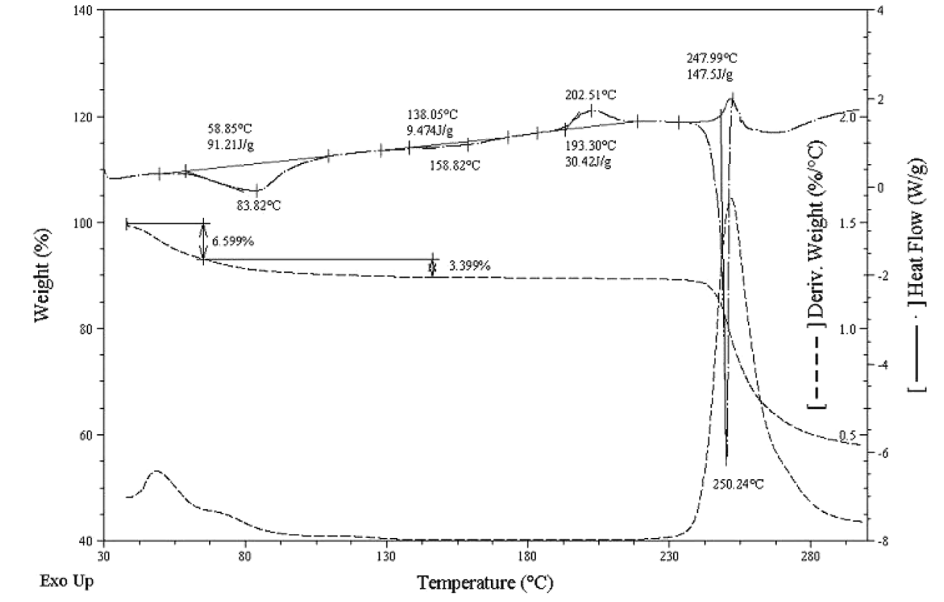


(a)

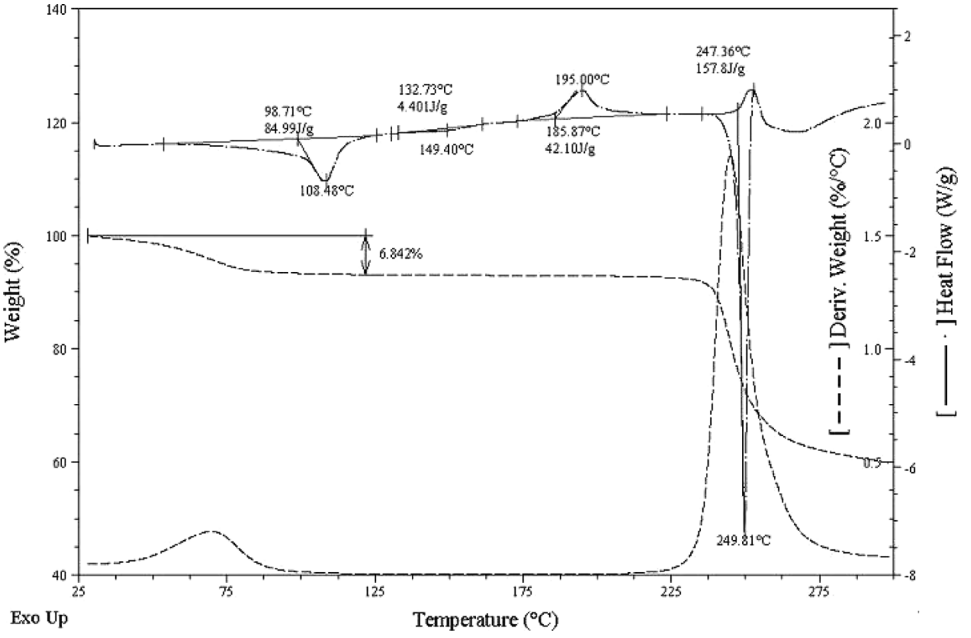


(b)

Figure 12. (a) DSC-TGA overlay of ganciclovir form I; (b) DSC-TGA overlay of ganciclovir form II; (c) DSC-TGA overlay of ganciclovir form III; (d) DSC-TGA overlay of ganciclovir form IV.



(c)



(d)

Figure 12 Continued.

crystalline materials with water incorporated into the crystal lattice. Hydrates can be of three types: channel, isolated site hydrates, and ion-associated hydrates (involving a metals ion). Weight loss due to dehydration below 100°C is usually associated with

channel-type hydrates. Channel water exists in channels or tunnels of the crystal lattice from which solvent molecules are mobile. The water molecules come out and enter freely into the crystal lattice. In lattice hydrates the water is located inside the crystal lattice and can be removed only by destroying the crystal lattice.

The TGA thermograms for ganciclovir form III and form IV indicate the forms to be hydrates. From the TGA data, Ganciclovir form III shows a total weight loss of 10.0% with a two-step loss. The first loss occurring below 55°C can be attributed to the surface or free moisture (weight loss by TGA: 6.6%), whereas the second weight loss of 3.4% is due to the stoichiometric amount of a half mole of water or hemihydrate (theoretical water content: 3.4%). The slow removal of water over a broad temperature range in form III indicates a channel-type hydrate (Figure 12c). The nature of the form III hydrate is also confirmed from the VTPXRD data where the pattern remains unchanged until 170°C.

Ganciclovir form IV shows a sharp single-step loss of 6.8%, which is comparable to the theoretical water content of ganciclovir, monohydrate (theoretical water content: 6.6%). The dehydration occurs as a single step up to 110°C. As observed from the VTPXRD, form-IV undergoes a structural change around the same temperature, indicating ganciclovir form IV to be a monohydrate as well as a lattice hydrate (Figure 12d).

Conclusion

The physicochemical properties of the four forms of ganciclovir have been extensively studied with the USP standard called form I. Form I could not be obtained by crystallization methods. The study demonstrates that ganciclovir reported in the previous literature is form II. Based on the studies carried out, ganciclovir form II was found to be thermodynamically stable. Forms I, III, and IV were found to be stable at high humidity of 90%. Crystallization of ganciclovir from dimethylformamide, water, and mixed solvents (water–methanol) resulted in the formation of stable forms—one anhydrate (form II) and two hydrates (form III and form IV). The two hydrates were proven to be hydrates, with form III existing as a hemihydrate and a channel hydrate and form IV as a monohydrate and a lattice hydrate.

The present study on the characterization of the forms of ganciclovir exemplifies the value of complementary analytical approaches to gain an understanding of the solid-state forms.

Acknowledgment

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